

Mendel's Genes: Toward a Full Molecular Characterization

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ABSTRACT The discipline of classical genetics is founded on the hereditary behavior of the seven genes studied by Gregor Mendel. The advent of molecular techniques has unveiled much about the identity of these genes. To date, four genes have been sequenced: *A* (flower color), *LE* (stem length), *I* (cotyledon color), and *R* (seed shape). Two of the other three genes, *GP* (pod color) and *FA* (fasciation), are amenable to candidate gene approaches on the basis of their function, linkage relationships, and synteny between the pea and *Medicago* genomes. However, even the gene (locus) identity is not known for certain for the seventh character, the pod form, although it is probably *V*. While the nature of the mutations used by Mendel cannot be determined with certainty, on the basis of the varieties available in Europe in the 1850s, we can speculate on their nature. It turns out that these mutations are attributable to a range of causes—from simple base substitutions and changes to splice sites to the insertion of a transposon-like element. These findings provide a fascinating connection between Mendelian genetics and molecular biology that can be used very effectively in teaching new generations of geneticists. Mendel's characters also provide novel insights into the nature of the genes responsible for characteristics of agronomic and consumer importance.

MODERN genetics is founded on the classical study of seven genes by Gregor Mendel, even though until relatively recently little was known about their function. Recent advances in molecular biology have dramatically changed this situation and unveiled the identity of four of Mendel's genes. Mendel started his experiments by carefully selecting seven qualitative characters in peas (*Pisum sativum* L.) in 1856, and his results were published in 1866. His work then went largely unnoticed for the next 34 years before its rediscovery by three independent researchers—Hugo de Vries, Carl Correns, and Erich von Tschermak—in 1900 (Bateson 1900). However, as soon as the work was rediscovered, it created controversy (e.g., Weldon 1902). The closeness of Mendel's experimental observations to those predicted by his theories have led to numerous articles and ongoing debate about whether the data could have been obtained in the published form without some modification. There have been many plausible arguments made for and against this view by a range of eminent statisticians and geneticists (e.g., Fisher 1936; Fairbanks and Rytting 2001; Hartl and Fairbanks 2007). Some have gone so far as to

suggest that the theories behind Mendel's two laws were not even correctly articulated in his original paper (Monaghan and Corcos 1993). However, given the lack of suitable terminology at the time, this seems a very harsh judgment and one with which we do not concur. Fisher (1936) certainly saw the significance of Mendel's contribution to the field even though he was the one who raised several concerns about the data. Some of these concerns still exist, and the controversy has not been resolved, despite recent implications to the contrary (Franklin 2008; Stigler 2008). We do not intend to pursue the matter here, but we believe that rational explanations have been given to potentially explain some of the problems with the data and their interpretation (e.g., Hartl and Fairbanks 2007; Franklin 2008). However, these issues cannot be definitively resolved, and it is probably time to concentrate on the remarkable understandings that arise from Mendel's insightful paper.

It is fascinating to trace the fate of Mendel's genes over the past 145 years. In some cases, we can do this with considerable certainty, tracing it through to the molecular cloning of the gene concerned and the establishment of its function, but for other genes the history is somewhat murkier. For at least one of the characters, we cannot even be sure which locus Mendel studied, as modern molecular biology has, over the last few decades, highlighted the existence of

Table 1 Seven characters of *P. sativum* examined by Mendel and a summary of the genes, phenotypes, and presumed mutations involved

Trait	Dominant phenotype	Recessive phenotype	Symbol group	Linkage group	Cloned	Gene function	Molecular nature of mutation
Seed shape	Round	Wrinkled	<i>R</i>	V	Yes	Starch branching enzyme 1	0.8-kb insertion
Stem length	Tall	Dwarf	<i>LE</i>	III	Yes	GA 3-oxidase1	G-to-A substitution
Cotyledon color	Yellow	Green	<i>I</i>	I	Yes	Stay-green gene	6-bp insertion
Seed coat/flower color	Purple	White	<i>A</i>	II	Yes	bHLH transcription factor	G-to-A at splice site
Pod color	Green	Yellow	<i>GP</i>	V	No	Chloroplast structure in pod wall	Unknown
Pod form	Inflated	Constricted	<i>V?</i>	III	No	Sclerenchyma formation in pods	Unknown
Position of flowers	Axial	Terminal	<i>FA</i>	IV	No	Meristem function	Unknown

References are given in the text.

multi-gene families for some of his characters. In this sense, it is ironic that a discipline that owes so much to Mendel's work has somewhat muddied the waters regarding the genes/characters that he studied. Unfortunately, the lines of peas that Mendel used are not known with certainty (Bhattacharyya *et al.* 1990; Hellens *et al.* 2010). However, given that his research built on the previous work of others and that we have some historical accounts of what mutants were available at the outset of his experiments in the 1850s (*e.g.*, Knight 1799), we can make an educated guess in most instances.

Even harder than defining which seven loci Mendel studied is the question of which mutations he used. Theoretically, it is now quite clear that there are many possible mutations in each gene that could produce the same phenotype, especially if Mendel's original mutation was a null. Again, the only way of determining this is to see what material would have been available in central Europe at the time that Mendel performed the studies. Many of the mutations that he examined were being used in the cultivars grown at that time. Indeed, several of them had been exploited for agricultural purposes for many hundreds of years (*e.g.*, white flowers, dwarf habit, sugar pods), and some cultivars possessing these mutations that predate his work are still available in collections (*e.g.*, the John Innes *Pisum* germplasm collection).

Our main interest here is to provide an overview and some personal perspectives on what we now know about Mendel's genes at the physiological, biochemical, and especially the molecular level. Table 1 summarizes this information for each of his seven characters. To date, four of the seven genes—*R*, *LE*, *A*, and *I*—have been cloned and their function explored in detail. Much less is known about the other three genes—*GP*, *FA*, and a gene controlling pod sclerification (probably *V*).

Seed Shape

The first character that Mendel considered was the form of the dry seed. He described these seeds as either round (sometimes with depressions) or irregular and wrinkled. White (1917) gave the symbol *R* for round seeds and *r* for wrinkled seeds. While there are several other genes described that can control the round/wrinkled phenotype

[*e.g.*, *rb* (Hadfield and Calder 1933; Kooistra 1962; Wang *et al.* 1998)], it seems clear that the only mutant available in Europe at the time of Mendel's work was at the *R* locus (Bhattacharyya *et al.* 1990). This character also appears to be the first for which a detailed anatomical and physiological explanation was sought. As early as 1903 (Gregory 1903), it became clear that round and wrinkled seeds differed in the quantity and shape of the starch grains that occurred in storage cells in the cotyledons. This gene difference also influenced the sugar content and the fresh weight of the developing seeds (Stickland and Wilson 1983). Wrinkled seeds possess elevated sucrose, fructose, and glucose levels, and this appears to result in a higher water content in immature seeds due to increased osmotic pressure and hence water uptake. In addition, the wrinkled seeds contain a higher percentage of lipids (Coxon and Davies 1982) and a reduced percentage of some storage proteins such as legumin (Davies 1980; Domoney and Casey 1985). The importance of seed storage products to nutrition has resulted in a large number of excellent studies on the round/wrinkled difference from the early 1900s to the 1990s. Detailed efforts in the 1980s to produce good isogenic material (Hedley *et al.* 1986) ultimately led to a better understanding of the nature of the difference at the biochemical level.

Given the wide range of pleiotropic characteristics that result from a difference at the *R* locus, it seemed possible that *R* is a regulatory gene that controls multiple structural genes, leading to the wide range of different characteristics. However, considerable biochemical evidence accumulated to suggest that the primary lesion in *r* embryos was in starch biosynthesis (*e.g.*, Matters and Boyer 1982; Edwards *et al.* 1988; Smith 1988). It is easy to see how a limitation in starch biosynthesis may lead to an increase in the accumulation of sugars and a change in the osmotic pressure in the developing seeds. A major breakthrough came with the demonstration (Smith 1988) that one of the major isoforms of a starch-branching enzyme, *SBE1*, was missing from wrinkled (*r*) seeds. This difference subsequently led to the *R* gene being the first of Mendel's genes to be cloned (Bhattacharyya *et al.* 1990). These authors showed a complete cosegregation between a polymorphism in the *SBE1* gene and the seed-shape difference at the *R/r* locus. They then went on to demonstrate that, in the *r* line, the *SBE1* gene is interrupted

by a 0.8-kb insertion. This insertion appears to be very similar to the *Ac/Ds* family of transposable elements from maize. They further showed that this failure by wrinkled seeds to produce one form of SBE1 led to complex metabolic changes in starch, lipid, and protein biosynthesis in the seed. Bhattacharyya *et al.* (1990) assumed that the mutation that they sequenced was the one used by Mendel (1866).

Thus the first of Mendel's genes to be cloned turned out to be a structural gene, and the effects of the mutation that he studied show the importance of this step for normal seed development.

Stem Length

The gene controlling stem length used by Mendel is assumed to be *LE*. This assumption appears to be reasonable since there is no evidence that other dwarfing mutations were available at this time, even though dwarf plants had been used agriculturally since at least the 1500s (Blixt 1972). It was White (1917) who gave the dwarf trait the gene symbol *le*.

Between the 1950s and 1980s there were various functions suggested for the *LE* gene. These included a role not only in controlling gibberellin levels but also in determining the sensitivity (Kende and Lang 1964) or turnover (Kohler 1970) of gibberellins, or the level of various growth inhibitors (Kohler and Lang 1963; Chailakhyan 1979; Smith 1992). However, during the 1980s it became increasingly clear that *LE* was involved with regulating the level of the bioactive gibberellin GA₁ by influencing gibberellin biosynthesis. The primary reason for success at this time was the availability of suitable genetic material and the examination of hormone levels in the tissue actually undergoing elongation (Potts *et al.* 1982) rather than in tissues rich in gibberellins (*e.g.*, developing seeds). Subsequently, the availability of appropriately labeled intermediates in the gibberellin biosynthetic pathway and the development of sensitive physicochemical methods allowed it to be shown that the GA₁ level is ~10-fold higher in the tall, wild-type *LE* plants than in mutant *le* plants (Ingram *et al.* 1984; Ross *et al.* 1989). A combination of genetic, physiological, and analytical techniques suggested that *LE* might code for a 3 β -hydroxylase enzyme that converts GA₂₀ to GA₁ (Ingram *et al.* 1983, 1984), although at this stage the possibility that the *LE* gene was a regulatory gene could not be excluded. The *le-1* mutation (presumed to be Mendel's dwarf mutation) was shown to be leaky when a more severe allele was found (Ross and Reid 1987). This was supported by the trace amounts of GA₁ found after feeds of GA₂₀ to *le-1* plants (Ingram *et al.* 1986).

LE was the second of Mendel's genes to be cloned when in 1997 two groups working independently reported the successful isolation of this gene (Lester *et al.* 1997; Martin *et al.* 1997). This was made possible by the previous isolation (by transposon tagging) of a putative GA 3 β -hydroxylase gene from *Arabidopsis*, *GA4* (Chiang *et al.* 1995). (The term

3 β -hydroxylase was later changed to 3-oxidase.) Heterologous screening of a pea cDNA library with *Arabidopsis GA4* yielded a partial clone that was 61% identical to the probe (Lester *et al.* 1997). DNA gel blot analysis revealed a restriction fragment length polymorphism between pea isolines differing at Mendel's *LE* locus, which cosegregated with the tall/dwarf difference. Recombinant expression in *Escherichia coli* of the cDNA open reading frames from *LE* and *le-1* plants showed that the *LE* clone encodes a protein capable of converting an inactive precursor, GA₂₀, to the bioactive GA₁. The *le-1* mutation dramatically reduced the GA 3-oxidase activity of the expression product. This reduction is associated with an alanine-to-threonine substitution in the predicted amino acid sequence of the enzyme, near its proposed active site. This results from a single base substitution, from G to A, in Mendel's *le-1* allele (Lester *et al.* 1997). Virtually concurrently, Martin *et al.* (1997) used a PCR-based strategy to clone *LE* and also reported on two further mutant alleles, *le-3* and *le^d* (later renamed *le-2*). Mutation *le-3* also involves a single base substitution that results in a single amino acid substitution while *le-2* was a single base deletion in Mendel's *le-1* allele and appeared to be a null allele. Lester *et al.* (1999) confirmed that *le-2* results in a truncated protein with no GA 3-oxidase activity.

Thus the second of Mendel's genes to be cloned also turned out to encode a structural gene. Fortunately, Mendel's *le-1* mutation disrupts the conversion of an inactive GA into one with potent biological activity. Establishing this site of action of *LE* in the GA biosynthetic pathway was essential for showing that the inactive precursor did not possess activity in its own right (Ingram *et al.* 1986). If the mutation affected an earlier step in the somewhat long pathway, then the mutation would have been much less instructive. It turns out also that Mendel's *LE* gene is regulated by auxin (O'Neill *et al.* 2010; Ross and Reid 2010), another hormone of historical interest, and arguably the most studied of the plant growth substances. Interestingly, even though gibberellins are implicated in a wide range of processes critical for plant yield, including root growth, seed development, and nodulation (*e.g.*, Ferguson *et al.* 2011; Ross *et al.* 2011), Mendel's *le-1* mutation has been used in agriculture for many centuries. This appears to have been possible because the *LE* gene is only one of a family of GA 3-oxidase genes, with at least one other member having greater expression in the roots and seeds and compensating for any loss of 3-oxidase activity in *le-1* plants (Weston *et al.* 2009).

Cotyledon Color

The third of Mendel's genes to be sequenced was the gene responsible for cotyledon color. This gene was given the symbol *I* by White (1917), but received little attention at the biochemical or physiological level until the early 2000s, when a concerted effort was made to understand genes controlling plant senescence, and chlorophyll degradation in particular. Stay-green mutants were identified in

many species including rice (*sgr*), *Arabidopsis*, and *Festuca* (Armstead *et al.* 2007), and it was shown that Mendel's *i* mutant had a similar phenotype. It was shown that not only do the cotyledons in pea exhibit a green color in the mature, dry seed as reported by Mendel (1866), but also senescing leaves remain green, as do detached leaves placed in the dark (Armstead *et al.* 2007; Sato *et al.* 2007; Aubry *et al.* 2008). This was the result of reduced chlorophyll breakdown during dark incubation (Sato *et al.* 2007). Armstead *et al.* (2007) showed cosegregation between cotyledon color (*I/i*) and a stay-green (*sgr*) homolog in pea, suggesting that Mendel's cotyledon color character may reflect allelic variation in a *sgr* homolog responsible for the stay-green phenotype in both dicots and monocots.

Sato *et al.* (2007) also showed the association between the *I* gene and the pea homolog of the stay-green gene originally isolated from rice, *SGR*. Molecular analysis of three independent *i* alleles confirmed that the *I* gene encodes the *SGR* homolog in peas. One of the *i* mutants possessed a two-amino-acid insertion in a conserved region of the *SGR* protein. When a construct was made in *OsSGR* c-DNA with a 6-bp insertion at this point, coding for these two amino acids, it did not complement the *sgr* mutant in rice, in contrast to the wild-type construct. The other two *i* alleles resulted in no *SGR* expression due to the production of a truncated protein attributable to incorrect splicing in one case and a transcriptional defect in the other (Sato *et al.* 2007). Aubry *et al.* (2008) confirmed the nature of the two-amino-acid insertion *i* mutation and concluded that this was responsible for Mendel's green cotyledon characteristic. However, they provide no evidence that this was indeed the specific mutation that Mendel had used. Given that Sato *et al.* (2007) had found three distinct *i* alleles among the nine lines possessing green cotyledons that they examined, and that all three alleles may have been present in cultivated European material from that period (M. Ambrose, personal communication), this question requires further examination.

The exact function of the *SGR* protein has been debated by many workers. While it is clear that it is involved with the chlorophyll catabolic pathway, the absence of known domains has made it difficult to deduce a function from the gene sequence (Aubry *et al.* 2008; Sato *et al.* 2009).

Seed Coat and Flower Color

Mendel noted that colored seed coats were always associated with colored (purple) flowers. He also noted that these colored varieties possessed pigmentation in the leaf axils. On the other hand, a clear or colorless testa was always associated with white flowers and the absence of pigmentation in the leaf axils, suggesting that these were pleiotropic effects of a single gene. Subsequently, most analyses have emphasized the color of the flowers and use this as the basis for a dominant wild-type characteristic, *A* (von Tschermak 1912; White 1917). A second locus conferring white flowers, *a2*, has been identified from a mutagenesis study (Marx

et al. 1989). Statham *et al.* (1972) indicated that *A* is necessary for general flavonoid production in the plant, including anthocyanin production in the flowers and leaf axils. This was confirmed by Harker *et al.* (1990) when they showed that a key enzyme in flavonoid biosynthesis, chalcone synthase (*CHS*), is produced by a three-gene family. They showed that both *A* and *A2* are required for the expression of *CHS1* and for wild-type levels of the expression of *CHS3* in petal tissue. They showed that the *A* and *A2* genes appear to spatially regulate the expression of the *CHS* genes. They went on to suggest that the selective transcription of the *CHS* genes may be mediated by a combination of different *trans*-acting genes interacting with multiple *cis*-acting elements, allowing the regulation of expression of individual members of a multi-gene family (Harker *et al.* 1990). The discovery that *A* was potentially a regulatory gene controlling the spatial expression of different members of a structural multi-gene family, at the time, was an exciting finding. Recently, the *A* gene has been identified and shown to encode a basic helix-loop-helix (bHLH) transcription factor (Hellens *et al.* 2010), confirming its regulatory function.

This gene was isolated using the synteny between the *Medicago* and pea genomes and the linkage relationships of *A* to candidate genes (Hellens *et al.* 2010) known to regulate anthocyanin biosynthesis in other species (Koes *et al.* 2005). After examining many white-flowered varieties, Hellens *et al.* (2010) identified two distinct *a* alleles in which the *bHLH* gene was disrupted. They were able to rescue pigment formation by bombardment of white petals with the wild-type gene and an ortholog from *Petunia*, confirming the identity of the *A* gene. Uimari and Strommer (1998) had earlier shown that maize *bHLH* genes could complement the *a* mutation in petals of peas, but they had not been able to determine the precise action of the *A* gene.

Examination of the geographic distribution of the two *a* alleles identified by Hellens *et al.* (2010) indicated that one is common and probably of Eurasian origin. It was present in material that was known to be in Europe at the time of Mendel's work since this mutation is carried by two of Knight's cultivars, Knight's Marrow and Knight's Dwarf White. Indeed, white-flowered varieties of peas appear to have been grown since at least the 13th century [De Crescenzi (*ca.* 1300) in Hellens *et al.* 2010] although the mutation could have occurred much earlier during domestication. Mendel was probably aware of the work of Knight (1799) since he gives the same reasons, and in the same order, as Knight for choosing pea as an experimental organism (Hellens *et al.* 2010). The *a* mutation results from a G-to-A transition at the GT splice donor site of intron 6, leading to the spliceosome identifying the next available GT motif which is eight nucleotides downstream. This leads to a frameshift and a premature stop codon. The second *a* allele was present in a number of accessions (Hellens *et al.* 2010), and it was concluded that it was probably of African origin and involves an additional nucleotide in exon 6 that would cause a frameshift and a premature stop codon. Thus the

fourth of Mendel's genes to be cloned was shown to encode a regulatory gene.

Pod Color

Of Mendel's three genes that have not been sequenced, the color of the immature pods has probably received the most attention. During the 1980s there were detailed studies on the action of the gene *GP* (White 1917), which controls the green/yellow color of the pods. Price *et al.* (1988) studied the structural and physical basis of this difference and showed that the yellow pod (*gp*) mutation resulted in the mesocarp containing plastids with an internal membrane system restricted to single and paired membranes. Unlike the plastids of green pods (*GP*), the mutant form lacked grana and contained only 5% of the chlorophyll of the wild-type green pods. However, the *gp* mutation did not change the chloroplasts in the endocarp of the pods (Price *et al.* 1988), although it is clear from Mendel's paper (1866) that this mutation also influenced the color and presumably the chloroplasts in some other tissues, including the calyx, leaf veins, and peduncles. These changes in chloroplast structure lead to reduced photosynthetic activity in the pods and hence reduced uptake of CO₂ (Price and Hedley 1988).

Given our knowledge of the linkage arrangements for the *GP* locus (linkage group V), the synteny between the pea and *Medicago* genomes, and the identification of genes in other species that are known to result in tissue-specific regulation of chloroplast development [*e.g.*, the nuclear transcription factor gene *GLK* (Waters *et al.* 2008)], it may now be possible to identify candidate genes that may control the green/yellow pod color difference. We trust that it will not be long before the use of this type of information results in the last three of Mendel's classical genes being fully identified at the molecular level.

Pod Form

Mendel (1866) referred to the form of the ripe pod as either inflated or deeply constricted (with the pod being quite wrinkled in appearance). Wild-type pods are inflated, with a complete layer of sclerenchyma on the inside of the pod wall. There are two different single-gene recessive mutants, *p* and *v*, that lack a complete layer of sclerenchyma in the endocarp of the mature pod, and their pods are deeply constricted because they are inflated only in those areas where the seeds have filled. These pods are edible while immature and are referred to as sugar pods. Varieties with this characteristic have been grown for many hundreds of years [Ruel (1537) in Lamprecht and Svensson 1963; Blixt 1972] but have become more popular in the past 20–30 years. The mature pods of *P v* plants do possess small patches of sclerenchyma in the endocarp while the pods of *p V* plants possess a strip of sclerenchyma along each side of the suture. Double-mutant plants totally lack sclerified cells on the inner side of the pod. Unfortunately, we cannot be

certain which mutant Mendel used in his crosses. It has normally been assumed that Mendel was working with alleles at the *v* locus since the *v* mutant more adequately matches the phenotype described for this trait by Mendel (Blixt 1975b). This is supported by anecdotal evidence from our mentor, Ian Murfet, who in 1965 asked Herbert Lamprecht about this matter. He replied that, although the names of the varieties used by Mendel remain a mystery, all the common sugar pod peas of that time had genotype *P v* with the possible exception of one variety recommended for forcing in the greenhouse. Lamprecht said that many years earlier he had asked his old relative Erich von Tschermak if he could clear up the question of which varieties Mendel had used but was told that the original papers were not to be found. Lamprecht's response provides a fairly direct connection to the work of the early 1900s.

If Mendel was indeed working with a difference at the *v* locus, he might have been expected to find linkage between *V* and *LE* as they are 12.6 cM apart in linkage group III (Rassmusson 1927). However, he did not work extensively on this gene combination and may not have even made the bifactorial cross (Blixt 1975b), or if he did, he may have checked only to find that all four combinations of the characters occurred (I. C. Murfet, personal communication). If Mendel had worked with alleles at the *p* locus, linkage would not have been an issue. The question about why Mendel did not find linkage has often been raised as a potential criticism of his work. However, the information above indicates that there are at least two legitimate reasons why he may not have observed linkage in this case, the only one in which two of the seven characters that he studied might possibly have been strongly linked.

The cause of the reduced sclerification in the pods of *p* or *v* plants does not appear to have been studied in any detail. This trait has clearly received less attention than any of the other seven traits of Mendel, making the prediction of putative candidate genes difficult. A detailed anatomical, physiological, and biochemical analysis of the trait may, however, suggest the nature of the primary lesion in pod development and hence allow the molecular nature of the mutant to be postulated.

Position of the Flowers

The last of Mendel's characters concerns the positioning of the flowers. Mendel noted that the flowers were either axial and distributed along the main stem or terminal and "bunched at the top of the stem and arranged almost in a false umbel," with the upper part of the stem being "more or less widened in section" (Mendel 1866, page 5). Interestingly, according to English translations, Mendel did not use the term "fasciation" with regard to this character. However, there seems little doubt that Mendel was referring to fasciation even though other mutations (*e.g.*, those affecting flowering time) can also affect flower positioning. Furthermore, in this case we can be reasonably confident about the

actual type of mutant pea that Mendel used because an early and authoritative translator, William Bateson, was clearly convinced that Mendel had worked with a type known as “Mummy Pea” (Bateson 1909).

Today the type line for Mendel’s fasciated character is JI 5 and is held at the John Innes Institute *Pisum* collection (M. Ambrose; <http://data.jic.bbsrc.ac.uk/cgi-bin/germplasm/pisum/>). JI 5 is also known as Mummy Pea, indicating a continuum from the early 1900s, although it should be noted that five other lines in the JI collection are also termed Mummy Pea. White (1917) was another to use the name Mummy in relation to the fasciated character, pointing out that “Irish Mummy of H. Eckford, Wem, England” was “the common fasciated variety.” As for several of the other characters, White (1917) was the first to ascribe a gene symbol, in this case *Fa* for the wild-type form. Since then, another gene for fasciation (*FAS*) has also been documented (see Sinjushin and Gostimskii 2008).

The *FA* gene has not yet been cloned although fasciation has been linked with *clavata* genes in other species (Leyser and Furner 1992). *FA* in peas is in linkage group IV, which is syntenic with *Medicago* chromosome 8. BLASTing suggests that there may be a *clavata*-like candidate in this region, which could be a candidate for *FA*. This type of information has been used successfully to clone other genes in peas (e.g., Weller *et al.* 2009; Hellens *et al.* 2010). Interestingly, there may also be a link between fasciation and nodulation in peas (Krusell *et al.* 2011), and detailed work will be required to examine these possibilities.

Conclusions

The cloning of four of Mendel’s genes has confirmed, with a few nuances, the Mendelian implication that there are fundamental units of inheritance that are passed from generation to generation. Indeed, if he were still with us, Mendel would probably be well pleased by the 20th century discoveries from the disciplines of cytology, biochemistry, and molecular biology. However, he would probably have also been surprised by some of the findings, for example, the finding that there can be intragenic recombination and other “large-scale” disruption of the inheritance units, which he envisaged to be transmitted intact from parent to offspring. However, such discoveries have added to and have refined the Mendelian model, rather than undermined it, in much the same way that 20th-century findings confirmed the concept of the atom while at the same time defining even more fundamental particles. Indeed, it is not inappropriate to draw this parallel between the development of Mendelian genetics and particle physics during the latter part of the 19th century and the 20th century. In general, it is fair to say that, although incomplete, the molecular characterization of Mendel’s genes thoroughly vindicates his original conclusions, helping to place arguments about whether his primary data were modified in some way in the purely academic basket. As suggested recently by Franklin

(2008), it is time to end that controversy and to focus on the important and fundamental implications that derive from Mendel’s genes.

Certainly Mendel would be surprised by the phenomenon of linkage. It appears that an element of luck was involved with his choice of characters, which are either not linked or, if linked (as may have been the case with the stem length and pod form characters), possibly not subject to a detailed dihybrid analysis by Mendel. It should be noted that during the 1950s and 1960s it became common for textbooks to indicate that Mendel’s seven characters were each located on separate chromosomes. In 1970 Ian Murfet pointed out this mistake, and it was subsequently corrected by a brief article in *Nature* by Stig Blixt (1975a). Indeed, with the improved linkage maps available today the seven traits in fact are thought to occupy only five of the seven linkage groups (Table 1), with only *le* and *v* showing strong linkage [*r* and *gp* may show weak linkage in some crosses (Murfet 1990)].

Our above gene-by-gene analysis shows that Mendel examined a broad range of types of genes and mutations (Table 1). The results obtained seem typical of molecular genetic analyses published over the last two decades and therefore provide a very useful group of genes for demonstrating the basis of molecular genetics, and its relation to Mendelian genetics, to students. For example, of the four genes that have been cloned, *LE* is a structural gene that acts late in a well-understood biochemical pathway leading to the synthesis of a key hormone (Lester *et al.* 1997), while another gene, *A*, encodes a transcription factor that spatially regulates a gene family catalyzing an early step in the synthesis of flavonoids, a broad group of secondary compounds (Harker *et al.* 1990; Hellens *et al.* 2010).

It turns out also that Mendel’s mutations are due to a range of changes at the molecular level (Table 1). While we cannot be certain in some instances of the exact mutations that he used, the range appears to include single base substitutions causing a single amino acid substitution [e.g., *le-1* (Lester *et al.* 1997)], disruptions to splice sites [e.g., *a* (Hellens *et al.* 2010)], small insertions [e.g., *i* (Sato *et al.* 2007)], and large transposon-like insertions [e.g., *r* (Bhattacharyya *et al.* 1990)]. These are typical of the causes found for spontaneous mutations in many species and for a wide range of processes. The mutations result in leaky alleles in some cases (e.g., *le-1*) but in null alleles in others (e.g., *a* and *r*). Several have been of enormous practical benefit to the development of pea as an agricultural species. For example, dwarfism was used to reduce lodging (falling over) well before its widespread introduction into cereal crops during the “green revolution,” while loss of pod sclerenchyma to produce an edible pod and reduced starch production to improve sweetness were selected to improve product quality well before consumer satisfaction surveys came to the fore.

The genes have also been isolated using a range of different molecular techniques based on heterologous screening, synteny, mapping, candidate gene analysis, and allelic diversity. In fact, it would be possible to teach a well-balanced and modern

genetics course based almost entirely on the use and understandings derived from Mendel's seven characters.

Acknowledgments

We thank our mentor Ian Murfet for his comments on the manuscript, his unpublished communications, and his teaching and enthusiasm for Mendelian genetics. We also thank Jim Weller for comments and Laura Quittenden for technical assistance.

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