Review

Mendel, 150 years on

T.H. Noel Ellis[1], Julie M.I. Hofer[1], Gail M. Timmerman-Vaughan[2], Clarice J. Coyne[3] and Roger P. Hellens[4]

[1] Institute of Biological, Environmental & Rural Sciences, Aberystwyth University, Gogerddan Campus, Aberystwyth, Ceredigion, SY23 3EB, UK
[2] The New Zealand Institute for Plant & Food Research Ltd, Christchurch 8140, New Zealand
[3] USDA-ARS Western Regional Plant Introduction Station, Washington State University, Pullman, Washington, USA
[4] The New Zealand Institute for Plant & Food Research Ltd, Auckland, New Zealand

Mendel's paper 'Versuche über Pflanzen-Hybriden' is the best known in a series of studies published in the late 18th and 19th centuries that built our understanding of the mechanism of inheritance. Mendel investigated the segregation of seven gene characters of pea (*Pisum sativum*), of which four have been identified. Here, we review what is known about the molecular nature of these genes, which encode enzymes (*R* and *Le*), a biochemical regulator (*I*) and a transcription factor (*A*). The mutations are: a transposon insertion (*r*), an amino acid insertion (*i*), a splice variant (*a*) and a missense mutation (*le-1*). The nature of the three remaining uncharacterized characters (green versus yellow pods, inflated versus constricted pods, and axial versus terminal flowers) is discussed.

Mendel's studies: species, traits and genes

Mendel's paper 'Versuche über Pflanzen-Hybriden' [1] is the best known in a series of studies published in the late 18th and 19th centuries [2–4] that built our understanding of the mechanism of inheritance [5]. The title of Mendel's paper is usually mistranslated in English as 'Experiments in Plant Hybridisation' rather than 'Experiments on Plant Hybrids', reflecting the impact of his work on the science of genetics rather than Mendel's own concern with the nature of hybrids and their implications for the 'Umwandlung einer Art in eine andere' - transformation of one species into another. There is also a misconception, as a result of R.A. Fisher's attack on Mendelism [6], that Mendel's results and experimentation were in some way suspect. These defamatory criticisms include imputations on the scope of his experimental work, his understanding of what he wrote and statistical interpretations of his results; although they have been roundly debunked [7,8], they remain embedded in common opinion.

In his paper, Mendel described eight single gene characters of pea, of which he investigated the segregation of seven. The eighth is the 'purple podded' character determined by the gene *Pur* on linkage group I. He also discussed the segregation of three traits (tall versus short, green versus yellow pods and inflated versus constricted pods) in common bean (*Phaseolus vulgaris*) that are likely orthologues of the corresponding characters he studied in pea. For both species Mendel used additional species names (such as *Phaseolus nanus* or *Pisum saccharatum*). These names are no longer used and we would consider these types as variants – Mendel commented that there is no 'sharp line between the hybrids of species and varieties as between species and varieties themselves'.

From a biological perspective Mendel's genes appear to be an unrelated set of genes that are uninformative about a single process; but they did elucidate the process of genetic inheritance itself. They are therefore important from an historical perspective and they illustrate a diversity of gene functions and types of mutation. Uncovering the molecular basis of these mutations solves a longstanding mystery in genetics.

This review focuses on the identification of four of Mendel's genes (*R/r*, round versus wrinkled seed; *I/i*, yellow versus green cotyledons; *A/a*, coloured versus unpigmented seed coats and flowers; and *Le/le*, long versus short internode length). In addition, the possible natures of three other characters studied by Mendel (*Gp/gp*, green versus yellow pods; *P/p* or *V/v*, inflated versus constricted pods; and *Fa/fa* or *Fas/fas*, axial versus terminal flowers) are discussed.

Linkage

A major conclusion from Mendel's work was that the factors determining individual traits segregated independently of one another. We now know that this is not always the case. The associated segregation of parental allelic combinations, known as genetic linkage, is well established. Fortunately Mendel studied segregation at multiple unlinked loci. This meant his results were not confounded by linkage, which would have been much more difficult to interpret. The issue of linkage is sometimes egregiously combined with the criticism of the quality of Mendel's data to imply falsely that he somehow suppressed inconvenient data [7]. Unfortunately these discussions suffered from confusion in the literature regarding chromosome numbers, linkage data and their combination [9]. Our current view of the position of the genetic loci Mendel studied is presented in Figure 1. As discussed below, there is some uncertainty about the identity of the genes for the fasciated (terminal) flowers (*Fa* or *Fas*) or the constricted pod phenotypes (*P* or *V*); therefore, the map locations of all are indicated. From this distribution of genetic loci it is clear that there are two possible cases where linkage could have confounded Mendel's results: these are *R–Gp* and *Le–V*.

The wrinkled seed character that Mendel studied was *R* versus *r* on linkage group V [10]. The character 'green versus

Corresponding author: Hellens, R.P. (roger.hellens@plantandfood.co.nz).

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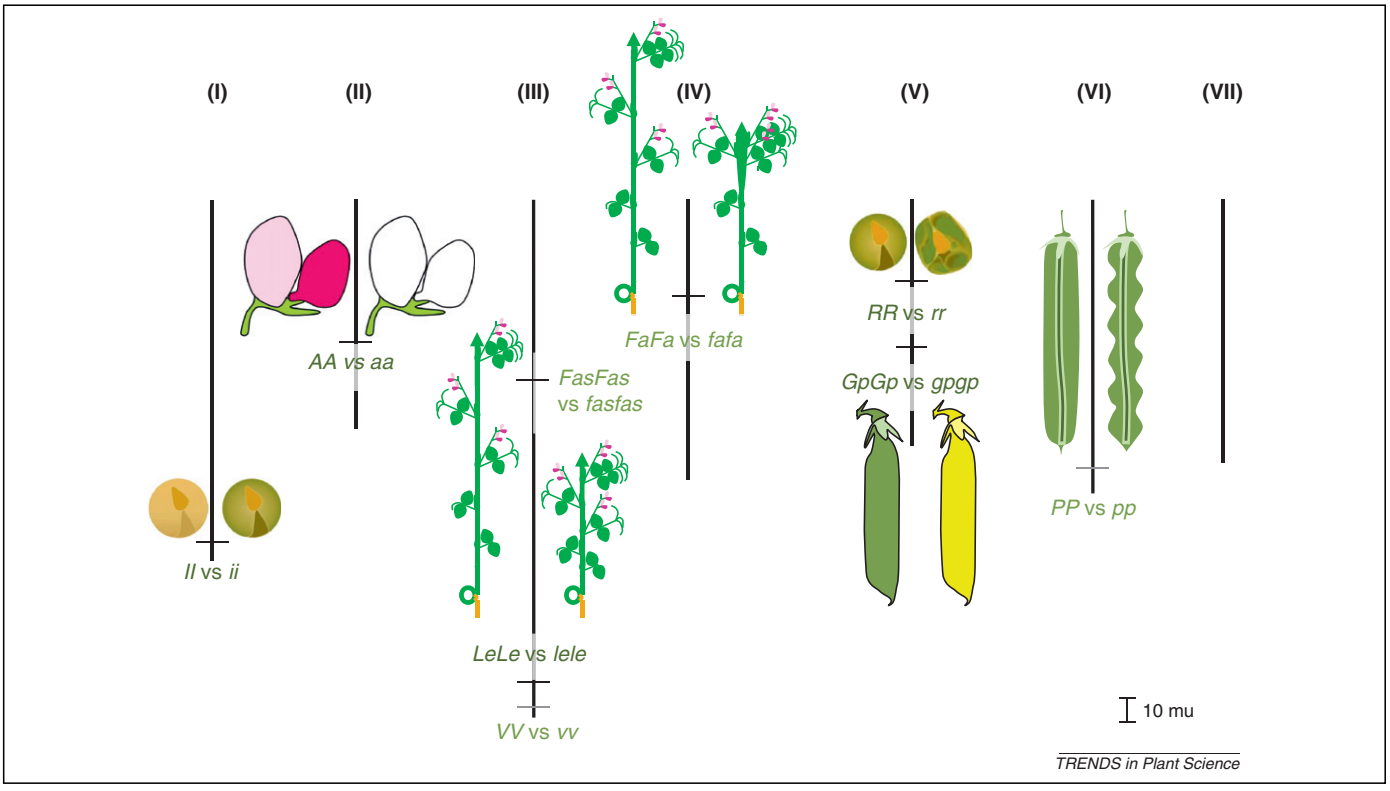

Figure 1. Genetic location of Mendel's seven characters on pea linkage groups. Yellow versus green cotyledons *II*/*ii* on linkage group **(I)**; seed coat (and flower) colour *AA*/*aa* on linkage group **(II)**; tall versus dwarf plants (*LeLe*/*lele*) on linkage group **(III)**; difference in the form of the ripe pods (*PP*/*pp* or *VV*/*vv*) on linkage groups **(III)** and **(VI)**, respectively; difference in the position of the flower (*FasFas*/*fasfas* or *FaFa*/*fafa*) on linkage groups **(III)** or **(IV)**, respectively; round versus wrinkled (*RR*/*rr*) on linkage group **(V)**; and colour of unripe pod (*GpGp*/*gpgp*) on linkage group **(V)**.

yellow pod' is unambiguously *Gp* versus *gp*, also on linkage group V. Linkage between these two loci can be detected [11]. In Mendel's study of two- and three-factor crosses he used approximately 600 F_2 individuals. He did not present data on the combination of *RR GpGp* crossed with *rr gpgp*, but some F_2 plants derived from this cross were probably grown as implied by the text, 'further experiments were made with a smaller number of experimental plants in which the remaining characters by twos and threes were united as hybrids' [1]. In one recombinant inbred population derived from the cross between the inbred John Innes Germplasm lines JI15 and JI399 [12], the recombination fraction between the *R* locus (genotyped using a molecular marker assay) and *Gp* is 36%, resulting in an expected segregation ratio of 9.6:2.4:2.4:1.6 rather than 9:3:3:1. Mendel would have needed about 200 plants in the 'smaller number' to have a 5% statistically significant deviation from independent assortment. Furthermore, linkage group V in pea, most likely corresponding to chromosome 3, behaves unusually in this cross because the number of chiasmata is never greater than one [12]; usually two or three occur. The recombination fraction calculated above is therefore the smallest that Mendel could have encountered, so it is unlikely that genetic linkage would have been discernable in any of the crosses that Mendel examined.

Genes and their mutant alleles

Round versus wrinkled (R *versus* r)

The wrinkled phenotype is striking because plants that appear completely normal bear seeds that are irregular in shape (Figure 1). The immature seeds do not appear unusual, but by maturity there are many differences between the wild-type and mutant seeds. These include diverse features such as subcellular arrangement of organelles, the ratio of the two major types of storage protein, the shape of starch granules, the amylose to amylopectin ratio of the starch polymers and sugar content [13]. There are several genes in pea that confer a wrinkled (rugosus) phenotype and all are lesions in enzymes involved in starch biosynthesis [14–17]. However, only the *r* mutant is known to have been available to Mendel [10].

A biochemical approach was taken to identify the gene encoded by *R* [10]. It was known that *rr* lines were distinguished from wild-type by their reaction to an antibody raised against the starch branching enzyme, so this antibody was used to identify cDNA clones. These cDNAs provided the route to isolating the structural gene encoding a starch branching enzyme (EC 2.4.1.18). Subsequent analysis showed that this gene co-segregated with the *R* locus and that wrinkled (*r*) mutants were disrupted in this gene by the insertion of a non-autonomous type II transposon (called *Ips-r*) related to the Ac/Ds family [10] (Figure 2). Thus the first of Mendel's mutants to be characterized corresponded to a mutation in a gene encoding a biosynthetic enzyme and it was potentially associated with an active transposon. No systematic search for other alleles at the *R* locus has been undertaken and the active and autonomous form of the transposon has not been identified.

Yellow versus green cotyledons (I *versus* i)

Ripe wild-type *II* seeds are yellow because the chlorophyll is lost as the seeds mature, whereas *ii* seeds remain green

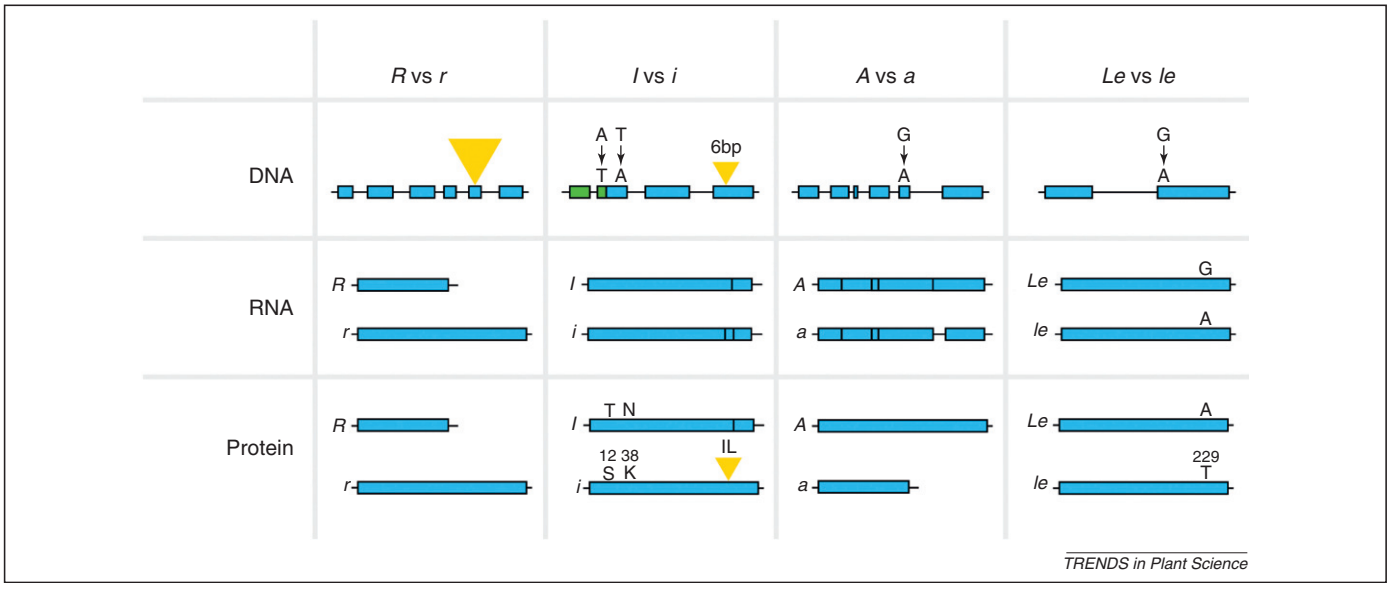

Figure 2. Mutations in Mendel's genes. Round versus wrinkled (*R* vs *r*): encoding starch branching enzyme I (SBEI). In the mutant allele, a transposon is inserted into the open reading frame (large triangle), disrupting both transcription (larger transcript) and translation in mutant lines. Yellow versus green cotyledons (*I* vs *i*): encoding a stay-green protein (SGR). In the mutant allele, a six nucleotide insertion in the coding sequence leads to a two amino acid insertion in the translated protein, disrupting gene function. Other amino acid changes in the signal peptide are not thought to disrupt function. Seed coat (and flower) colour (*A* vs *a*): encoding a basic helix–loop–helix transcription factor (bHLH). In the most common mutant allele, a single nucleotide change at an intron junction disrupts RNA processing leading to a transcript with an additional eight nucleotides and a truncated protein. Tall versus dwarf plants (*Le* vs *le*): encoding gibberellic acid 3-oxidase. A single nucleotide substitution in the coding sequence leads to an alanine (A) to threonine (T) substitution at position 229 that reduces the activity of the enzyme.

(Figure 1). This difference can be seen through the seed coat, but is clearest if the testa is removed. The phenotype is somewhat variable: wild-type seeds that dry out early sometimes retain green colour, whereas green *ii* seeds can sometimes bleach. Chlorophyll is a central component of the plant photosynthetic machinery and the compound responsible for the green colour in plants. A dynamic pathway of chlorophyll biosynthesis and degradation [18] maintains the amount of chlorophyll in photosynthetic tissues and reduces it in low light, during senescence, or other specific phases of plant development.

As green cotyledons are the recessive phenotype, a mutation in the chlorophyll degradation pathway best explains the molecular nature of this trait. Studies in species such as rye grass (*Festuca pratensis*) [19], rice (*Oryza sativa*) [20], maize (*Zea mays*) [21], pepper (*Capsicum annuum*) [22] and *Arabidopsis* (*Arabidopsis thaliana*) [23,24], have identified several genes with 'stay-green' phenotypes, such as: *PAO*, which encodes pheophorbide *a* oxygenase that converts pheophorbide *a* to red chlorophyll catabolite [21–24]; *CBR*, which encodes chlorophyll *b* reductase that converts chlorophyll *b* to chlorophyll *a* [20]; *Stay-Green* (*SGR*), which encodes a protein that is thought to aid the disassembly of light harvesting complex II, allowing chlorophyll to enter the degradation pathway [22,25]; and *PPH*, which encodes pheophytin pheophorbide hydrolase that converts pheophytin *a* to pheophorbide *a* [26].

The first indications that a mutation in a *SGR* gene might be responsible for the *i* mutation were the observations of genetic linkage between a pea orthologue of *SGR* from rice and the *I* locus together with a reduction in the accumulation of *SGR* transcripts in *ii* pea lines [27]. The molecular nature of this lesion was later described [28], and several sequence differences were observed. Two nucleotide differences in the region predicted to function as a signal peptide were initially considered as explanations for the stay-green phenotype because they lead to amino acid substitutions. However, when bombarded into onion (*Allium cepa*) epithelial cells, both the *I* and *i* sequences fused with green fluorescent protein (GFP) were able to target fluorescence into the plastid compartment, indicating that the function of the signal peptide was not compromised by these substitutions. A third sequence difference in *ii* lines consisted of a six-nucleotide (two amino acid) insertion (Figure 2). To assess the consequence of this insertion, a modified form of the rice *SGR2* gene containing the same insertion was transformed into the *sgr-2* mutant. This construct was unable to complement the *sgr-2* mutant rice line, whereas the rice *SGR2* gene was able to complement the mutant. Neither *SGR* allele from pea was able to complement the rice *sgr-2* mutation [28].

SGR appears to direct chlorophyll to the degradation pathway [25]. Although the mechanism by which the protein achieves this is unclear, it seems that a small modification of the protein sequence, as seen in the green cotyledon pea lines, might be sufficient to disrupt the function of the protein.

*Seed coat (and flower) colour (*A *versus* a*)*

The *a* mutation abolishes anthocyanin pigmentation throughout the plant. In pea, as in many other plants, the appearance of red, purple or blue pigments is due to the accumulation of anthocyanin compounds. The different shades of red, purple and blue pigmentation are due to the chemical makeup of the individual anthocyanin compounds, in particular the presence of hydroxyl groups and sugar moieties, together with the pH of the vacuole where they accumulate and other compounds that

complex with the anthocyanins [29–31]. Anthocyanin pigmentation is patterned in space and in response to environmental stimuli such as high light or cold temperatures. Mutants that affect the pattern of pigmentation (such as *Pur* q.v.) are well represented in *Pisum* germplasm but in *a* mutants there is no accumulation of anthocyanin in any part of the plant.

A gene that encodes a basic helix–loop–helix (bHLH) transcription factor was identified as a candidate gene for the *A* locus through comparative genomics [32]. The genetic map of pea was aligned to genomic sequences of *Medicago* (*Medicago truncatula*) using the sequences of cDNA probes known to flank the *A* locus. Annotated genes within about a 10 Mb region of the medicago genome were then scrutinized to identify candidate genes with predicted functions known to influence anthocyanin accumulation. No putative biosynthetic genes were identified in this region. Only one potential regulatory gene, a *bHLH* gene similar to *Arabidopsis TT8* was identified. Degenerate primers designed to the medicago gene were used to isolate the pea orthologue, which was then mapped to linkage group II and shown to co-segregate with the *A* locus (Figure 1). Gene models for this *bHLH* gene were derived from BAC DNA sequences from both coloured and white-flowered lines [32].

Of the 16 single nucleotide polymorphisms (SNPs) identified between the two gene models, the majority (13/16) were silent mutations. Two SNPs predicting amino acid changes were subsequently found in both coloured and white-flowered lines, excluding them as candidates for the causal mutation. The remaining SNP, a G-to-A transition at the splice donor site of intron six of the gene model, occurred only in white-flowered lines. This change interferes with RNA splicing such that eight nucleotides of intron sequence are retained in the processed mRNA, corresponding to a truncated peptide on translation. To confirm that this SNP determined the mutant phenotype, the white-petal phenotype was complemented by transient transformation using biolistics. BAC DNA from both coloured and white-flowered lines was shot into pea petals and coloured foci were observed after introduction of the wild-type but not the mutated gene. Finally, the *A* gene was sequenced from a range of pea germplasm. In this selection, all 60 pea lines with coloured flowers had an intact intron junction (Figure 2), and most but not all (78/88) white-flowered lines had the mutated intron junction. Of the ten remaining white-flowered lines, seven exotic lines carried a different mutation, a single nucleotide insertion in exon six that is predicted to introduce a frameshift and lead to truncation of the protein on translation. No significant deviation from the wild-type sequence has been found in the three other white-flowered lines; however, it is not certain that these three lines are *a* mutants, and the entirety of the gene has not been sequenced [32].

*Tall versus short (*Le *versus* le*)*

Many pea genes are now known to be involved in the synthesis of (*Lh*, *Ls*, *Na*, *Sln* and *Le*) [33–38], or in the response to (*LaI* and *Cry*), the plant hormone gibberellin (GA) [39]. On the basis of its phenotype and distribution among varieties the *Le* gene is considered to be the one studied by Mendel [33,40–42]. *LeLe* plants are tall, *lele* plants are dwarf (Figure 1); this difference is due to internode length rather than the number of nodes.

The *Le* gene product was implicated in GA biosynthesis in early experiments that showed that stem elongation in dwarf seedlings was stimulated by application of GA_3 [43,44]. The activity of the *Le* gene product was established because the conversion of GA_{20} to GA_1 (one of the active forms of GA) was much greater for *LeLe* than for *lele* plants [36], and GA_1 levels were higher in the shoots of *LeLe* versus *lele* plants, whereas GA_{20} amounts were elevated in *lele* plants [45,46]. As a consequence of these studies, it was hypothesized that *Le* encodes a GA 3-oxidase (GA 3β-hydroxylase). GA 3-oxidase activity was shown to be reduced in *lele* plants [45] and subsequent identification of the *Le* gene demonstrated that it encodes a GA 3-oxidase (EC 1.14.11.15) [40,41].

A partial *Le* sequence was obtained by screening a cDNA library at low stringency with *Arabidopsis GA 3-oxidase* (*AtGA4*) probe. This enabled the isolation of full length *le* and *Le* genomic sequences [40]. Sequence alignment revealed a G-to-A transition conferring an alanine-to-threonine substitution at position 229 in the *le-1* gene product (Figure 2). Although this residue is not invariant among plant 2-oxoglutarate-dependent dioxygenases, the class of enzymes to which GA 3-oxidase belongs, it nevertheless lies within a highly conserved region of the protein. Linkage analysis demonstrated co-segregation of the pea *GA3ox* sequence and *Le*. GA 3-oxidase enzymatic activity was demonstrated following recombinant expression of the cDNAs from *Le* and *le-1* plants in *Escherichia coli*. The GA_{20} substrate was converted to GA_1 by both cDNA expression products but the enzyme encoded by *le-1* showed approximately 5% of the activity of the wild-type. The identity of *Le* as *GA3ox* was further supported by the characterization of two additional induced alleles; *le-2* (formerly known as le^d) and *le-3* [41]. The *le-2* mutant was found to carry both the alanine-to-threonine substitution at position 229 found in *le-1*, and a second mutation; a single base deletion of G376, which was inferred to confer a frameshift and premature termination of translation. This mutation at a second site confirms that the *le-2* allele is derived from Mendel's *le-1* allele [47]. The *le-3* line contains a C-to-T transition resulting in a histidine-276 to tyrosine amino acid substitution. The gene products from *Le*, *le-1*, *le-2* and *le-3* GA 3-oxidase clones were assayed following expression in *E. coli*. The relative activities of the recombinant enzymes for two substrates, GA_4 (converted to GA_9) and GA_{20} (converted to GA_1), were $Le > le\text{-}1 \approx le\text{-}3 > le\text{-}2$ [41,47].

The *le-2* allele is likely to be a null allele because the recombinant protein exhibits no activity when GA_{20} is used as a substrate and GA_1 product is measured [47]. Until recently, this was difficult to equate with the *le-2* plant phenotype, which is not an extreme dwarf, and is capable of limited GA_{20} to GA_1 conversion [45]. A second pea *GA 3-oxidase* gene (*GA3ox2*) that is expressed primarily in roots but also in shoots might be responsible for the low level of GA 3-oxidase activity in *le-2* plants [39].

Uncharacterized genes

*Inflated versus constricted pods (*P *versus* p *or* V *versus* v*)*

The inflated versus constricted pod phenotype refers to the presence or absence of a layer of lignified cells (sclerenchyma) adjoining the epidermis of the pod wall and is referred to as parchment (Figure 1). Pods without 'that rough skinny membrane' are described in Gerard's 1597 *Herball* [48], and in general this cell layer is absent in vegetable pea types where the whole pod is eaten (mangetout). Absence of this cell layer leads to a pod that is constricted around the seeds at maturity. There are two sub-types of this class of cultivar, one with a thickened pod wall (*nn*) and no 'string' along the ventral suture (*sin2sin2*) called 'snap' or 'sugar snap' peas. The second type, sometimes called 'snow pea' has thin pod walls (*NN*) and usually has a stringy pod (*Sin2Sin2*). Although breeders commonly combine *NN* with a wrinkled seed character (*rr*) it is difficult to recover vigorous stringless plants with wrinkled seeds [49]. Mendel referred to peas with this pod characteristic as '*P. saccharatum*' suggesting that he used a 'sugar snap' type (probably *rr Sin2Sin2 NN* with either *vv* or *pp*).

It is difficult to be sure which locus Mendel was studying because homozygous individuals carrying mutations in either of the two genes *P* or *V* lack this cell layer [42]. However, we can make some deductions. Mendel studied the segregation of multiple factors in single crosses and although he did not report studies of the joint segregation of 'stem length' and 'pod form' he did report that small-scale experiments combining characters 'by twos and threes' were undertaken. The *V* and *Le* loci are linked, about 15 cM apart on linkage group III [50], so the combination of these two characters in a single cross would have deviated from his expectation for independent segregation. There are therefore two likely possibilities: (i) Mendel studied *vv* in small populations where the deviation from expectation due to linkage was not seen, or (ii) the character state he studied was determined by *pp*; the *P* locus is located on linkage group VI [51] and so would have segregated independently of *Le*.

The 'parchments' of *PP* and *VV* genotypes are secondary cell walls deposited after the cessation of cell growth and are composites of cellulose, hemicelluloses and lignins [52]. Secondary wall biosynthesis has been characterized biochemically and genetically, and studies on transcription factors in *Arabidopsis* indicate that a group of NAC domain proteins and their downstream targets act as regulators [53]. Three homologues of these transcription factor genes are located on chromosome 2 of medicago in regions syntenic with *P* and *V* and are under investigation as candidates for *P* or *V* in pea.

*Green versus yellow pods (*Gp *versus* gp*)*

The *gp* mutation conveys another striking phenotype. *GpGp* plants have green pods whereas *gpgp* plants have yellow pods (Figure 1). Young stems and buds at flowering are also noticeably yellow, whereas leaflets are green as normal. As with the cotyledon colour locus *I*, the green pod/yellow pod *Gp* locus appears as a difference in the accumulation of chlorophyll. In contrast to the *I* locus where the wild-type dominant form is yellow and the recessive mutant form is green, for the *Gp* locus the wild-type dominant form is green and the recessive mutant form is yellow. This suggests that the mutant form *i* represents a failure of chlorophyll degradation, whereas the mutant form *gp* fails to develop a normal chlorophyll complex in the pods [54,55]. Interestingly the region of the medicago genome syntenic to *Gp* contains a gene *Medtr7g080590* annotated as 'chloroplast lumenal protein related' which has similarity to the *Arabidopsis LCD1* gene, mutants of which have a pale phenotype under standard growth conditions and bleach in response to ozone [56]. This phenotype has some similarities to *gp* suggesting that it is a candidate worth investigating further.

*Axial versus terminal flowers (*Fa *versus* fa *or* Fas *versus* fas*)*

The position of flowers, and hence the seeds, on a crop plant is of great importance in agriculture. Several genes determine flower location in pea. Homozygous mutants carrying the *det* gene are determinate with a terminal inflorescence. This mutation has been characterized at the molecular level [57]; however, it is most unlikely that this was the gene studied by Mendel because he described the mutant form as having 'a false umbel', implying a fasciated type with a broadened stem and a 'crown' of many flowers. Alleles of fasciation genes have been widely used in pea breeding, particularly in conjunction with a mutation that confers synchronicity in flowering time [58].

In pea, mutations at several different loci are known to confer a fasciated phenotype; of these, the genes *Fa* (linkage group IV) and *Fas* (linkage group III) are two that are not also defective in nodulation [59]. Mendel would most likely have noticed the yellowness of plants defective in nodulation, thus *Fa* and *Fas* are contenders for the 'difference in the position of the flowers' character. The *Fa* locus has been conventionally assigned to Mendel's trait, but the evidence for this is not definitive. The distance between *Fas* and *Le* on linkage group III is sufficiently large that linkage would have been difficult to detect without intervening markers.

In general, stem fasciation is thought to result from failure of cellular organization within the shoot apical meristem. In *Arabidopsis*, several classes of genes are known to contribute to this organization and to show loss-of-function phenotypes that include shoot fasciation. These include small secreted peptides encoded by *CLAVATA3 (CLV3)/ENDOSPERM SURROUNDING REGION (ESR)*-related genes, which are known to act as ligands for transmembrane proteins such as CLV1 in the shoot apical meristem [60]. These interactions transmit a signal that keeps the stem cell population in check. A failure in the CLV signalling pathway leads to increased stem cell accumulation, as seen in the fasciated phenotypes of *clv1* and *clv3* mutants [61–63]. *CLV*-related genes are therefore obvious candidates for *Fa* and *Fas* in pea, as well as genes that affect the cell cycle. Although many cell cycle mutants are embryo-lethal, several have been characterized in *Arabidopsis* that are viable and have a fasciated shoot phenotype. Among these are the *atbrca2* mutants, which carry lesions in a homologue of the breast tumour susceptibility factor BRCA2 [64], and the *fasciata1* (*fas1*)

and *fas2* mutants [65], which are affected in the genes encoding the p150 and p60 subunits of chromatin assembly factor 1, respectively. A BLAST search of the region of the medicago genome syntenic with *Fa* shows that it contains a homologue of *CLV1* whereas the region syntenic with *Fas* contains a *CLV1* homologue and a *BRCA2* homologue.

Conclusion

As 150 years have elapsed since Mendel's experiments [6,32], it is difficult to state with certainty that the alleles he studied have been identified. In this respect, the diversity of mutant alleles can be informative: lines studied by Mendel must have carried spontaneous mutations. We should also bear in mind that multiple independent spontaneous mutations are unlikely because spontaneous mutation rates are very low with respect to the time since domestication. We do not know the number of different spontaneous alleles for *r* in pea germplasm, but for *Le* the *le-2* allele appears to be derived from *le-1* (and *le-3* is an induced mutation) so there has been a single introduction of this dwarf trait into cultivars. The diversity of mutant *a* alleles has been studied and one is predominant in cultivated lines. A second rare allele restricted to a small subset of landraces is known. This again suggests a single introduction of this character into modern cultivars, or cultivars available in Mendel's time. In contrast, several spontaneous *i* alleles exist, suggesting independent introductions of this trait, which seems remarkable. The types of lesion in Mendel's mutants are various: transposon insertion (*r*), missense mutation (*le-1*), splice variant (*a*) and amino acid insertion (*i*). The mutations affect diverse biological processes; two genes encode enzymes (*R*, *Le*), one is a regulator of a biochemical pathway (*I*) and the most recently described (*A*) is a transcription factor of a family first known for its role in cancer biology. So far, a range of different approaches has been used to identify four of Mendel's seven loci; new comparative genomic tools have identified candidates for the three remaining loci.

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