Pea (*Pisum sativum* L.) in Biology prior and after Mendel's Discovery

Petr SMÝKAL

Department of Botany, Faculty of Science, Palacký University Olomouc, Olomouc, Czech Republic

Abstract

Smýkal P. (2014): **Pea (*Pisum sativum* L.) in biology prior and after Mendel's discovery**. Czech. J. Genet. Plant Breed., **50**: 52–64.

Pea (*Pisum sativum* L.) has been extensively used in early hybridization studies and it was the model organism of choice for Mendel's discovery of the laws of inheritance, making pea part of the foundation of modern genetics. Pea has also been used as model for experimental morphology and physiology. However, subsequent progress in pea genomics has lagged behind many other plant species, largely as a consequence of its genome size and low economic significance. The availability of the genome sequences of five legume species (*Medicago truncatula*, *Lotus japonicus*, *Glycine max*, *Cajanus cajan* and *Cicer aerietinum*) offers opportunities for genome wide comparison. The combination of a candidate gene and synteny approach has allowed the identification of genes underlying agronomically important traits such as virus resistances and plant architecture. Useful genomic resources already exist and include several types of molecular marker sets as well as both transcriptome and proteome datasets. The advent of greater computational power and access to diverse germplasm collections enable the use of association mapping to identify genetic variation related to desirable agronomic traits. Current genomic knowledge and technologies can facilitate the allele mining for novel traits and their incorporation from wild *Pisum* sp. into elite domestic backgrounds. Fast neutron and targeting-induced local lesions in genomes (TILLING) pea mutant populations are available for reverse genetics approaches, BAC libraries for positional gene cloning as well as transgenic and *in vitro* regeneration for proof of function through gene silencing or over-expression. Finally, recently formed International Pea Genome Sequencing Consortium, holds promise to provide the pea genome sequence by 2015, a year of 150 anniversary of Mendel's work.

Keywords: heredity; hybridization; legume; Mendel; pea

Pea as model for hybridization experiments

Pea has been an object of experimental work well before Mendel's genetic discoveries. This might be attributed likely to the appearance and availability of large number of varieties with distinct traits, such as seed, pod and flower colours, seed shape, plant height etc. There were other plants with even higher variation like the cabbage family, but these were either biannual plants or displayed outcrossing pollination and incompatibility.

Experiments in plant hybridization can be traced back to 1694 when Rudolph Jacob Camerer (1665–1721) started systematic crossing of plants considered to be different varieties and species (Camerer 1694). Camerer's work was continued by Joseph Gottlieb Kölreuter (1733–1806) and by Carl Friedrich von Gärtner (1772–1850). However, the first report, of using pea variation to gain insight into the transmission of traits among generations is from Thomas Andrew Knight (1759–1838). Although Knight's interest was in fruit trees improvement, he soon realized that an annual plant with good trait variation is needed to address his questions. He wisely chose pea. Notably, Knight's introductory statement is a curious reminder in point of form of Mendel's own introduction nearly half a century later (Knight 1799; Hellens *et al.* 2010). Knight determined that, in crossing a pea with grey (e.g. pigmented) upon one with white (we say transparent today) seed coat, the resulting hybrid seeds were uniformly grey seeded, as well as having purple-coloured flowers of the male parent. Knight

further discovered that by crossing plants grown from these (heterozygous) grey seeds, with pollen from what he called a "permanent" white variety, plants of two types appeared, one bearing grey and the other white seeds. No numbers were reported, so that a scientific foundation based on ratios was not laid (Roberts 1929). Twenty-five years earlier, Knight undertook experiments with plants to test the theory of "superfetation", e.g. the possibility of two males combining in the fecundation of a female. At the time, the behaviour of the fertilizing cells was absolutely unknown, as was the fact that but one sperm cell was required to fertilize the egg. In that report, Knight first recorded colour-dominance (grey, pigmented colour of testa) and possibly also heterosis in peas. Beside A. Knight, there was John Goss, which in 1820 pollinated flowers of variety *Blue Prussian* with pollen of a dwarf pea known as *Dwarf Spanish*, obtaining, three pods of hybrid seeds. In the following spring, when he opened pods, he was surprised to find that the colour of the seeds (i.e. cotyledons), instead of being a deep blue like those of the female parent, was yellowish-white like that of the male. He observed a case of segregation in the next generation, as well as recording evidence of dominance and segregation, however he did not recognize them as such (Goss 1824). Goss either did not sow the seeds of different plants separately, or did not make counts as Mendel later (Roberts 1929). At the meeting of the Horticultural Society on 20th of August 1822, a communication on the same subject was presented by Alexander Anderson-Seton (1769–1850), secretary of Horticultural Society. He pollinated the flowers of the *Dwarf Imperial*, a green-seeded pea, with the pollen of a tall white-seeded variety. One pod with four peas was produced, all of which were green, possibly demonstrating the dominance of green cotyledon colour over its absence (white). The plants growing from the four peas (F_1 seeds) were intermediate in size between the two parents (Seton 1824). Finally, in 1872, Thomas Laxton (1830–1893) published the results of hybridization experiments, which have several points of interest: first, the fact of dominance in colour and form of the seeds was brought out; secondly, a statement of numerical results was attempted (Laxton 1866, 1872). Notably, Laxton corresponded on these findings with Charles Darwin. In 1849 Gärtner described the result of crossing the yellow-seeded variety *Pariser Wachserbse* which he called *Pisum sativum luteum*, fertilized either with pollen of *P. sativum macrospermum w*hich had greenish-yellow seeds, or with pollen of the green-seeded *P. sativum viride*. In the first case the hybrid seeds were all pure yellow; in the second case twelve seeds were produced in four hybrid pods; and these were all a greenish-yellow colour, although the greenish tinge disappeared from some of them on drying. Another yellow-seeded pea (*P. sativum nanum repens*) fertilized with the pollen of the green-seeded (*P. sativum viride*) gave five hybrid pods with seeds, of which one contained five "dirty green" seeds, a second had five seeds which were "not distinctly yellow, but yellowish green". The others were not yellow like the mother, but "dirty yellow" (Gärtner 1849). It is clear that the "greenness" of *P. sativum viride* did in these cases affect the colour of the seeds, when its pollen was used to fertilize plants of yellow-seeded variety, though it is difficult to judge exactly how great the effect (Roberts 1929).

In general, these 18th- and 19th-century researchers dealt with questions regarding the variation or fixity of natural forms and the physiological process by which either variety or homogeneity was transferred from one generation to the next (Wynn 2007).

Gregor Johann Mendel (1822–1884) chose pea for his work after preliminary experiments with several plant species and an examination of botanical literature on plant hybridization, particularly of Gärtner (1849). For his hybridization experiments, Mendel selected 22 pea varieties that he had confirmed through two years of testing to be true-breeding. He reported data from hybridization experiments on seven traits that differed among the varieties (Orel 1971). He does not discuss the question whether all his variants belong to one "species" or not, but describes the result of crossing any two of them as "hybrid." The fact that Mendel used the same species, *Pisum sativum*, fuelled many of the later criticisms (Orel 1971). However the use of pea was in fact essential in order to dissect inheritance of characters. Mendel paid special attention to seven sets of characters, with regard to each of which it was possible to separate into two categories. Thus the shape of the seeds might be round, with only slight and shallow wrinkles on the surface, or irregular and deeply wrinkled. The cotyledons of the seeds might be yellow or green in colour, and so on. The pairs of characters, recognised in this way for each organ or set of organs studied, are distinguished, according to their power of affecting hybrid offspring, into dominant and recessive, as we call them today. He precisely recorded the numbers of plants with such traits in any studied generation and was thus able, all what was necessary and sufficient to deduce his

three "laws". The high number of studied plants enabled Mendel to see the underlying nature of the ratios between the different sets of experiments that would not have been so discernable with smaller plant numbers. The only qualifications Mendel offers, in applying his general statements to these very varied characters, are (1) that the violet dots on the seed-coat are often more numerous and larger in hybrids than in pure-bred forms, and (2) the observation that the mere fact of hybridisation produces an increase in the size of the vegetative organs, so that hybrid plants are often taller than either of their parents (sign of heterosis), an observation made previously by Knight and Darwin. In Mendel's time, statistics were not so advanced to permit testing of fit between observed and expected data. This was invented later by Fisher (1936), who recalculated Mendel's original data and discussed the suspicious fit (Franklin 2008). Mendel made several series of observations to test the validity of his statement in cases involving more than one pair of differential characters. The number of possible combinations quickly becomes too great to deal with experimentally, and the most complicated case recorded is that of hybrids between female parents of round smooth seeds with yellow cotyledons and grey-brown seed-coats, and male parents with angular green seeds and white seed coats. The original hybrids were 24 in number, and from these 639 hybrids of the second generation were grown and observed (Wendon 1902). It is notable that in the Introductory Remarks section of his text, Mendel suggests his opposition to the variability of species characteristics by citing as his precursors Gärtner, Kölreuter, and Wichura, all of whom believed in the fixity of the species. The work and ideas of Unger, Nägeli, and Darwin, who believed that species changed over time, are not mentioned here or anywhere else in the text (Wynn 2007). In 1893, E. Giltay who does not appear to have known Mendel's work, crossed several yellow-seeded peas with the green-seeded *Reading Giant*, and found that the colour of the cotyledons was always yellow, showing that Mendel's law of dominance was completely valid in this case (Roberts 1929). It is appropriate here to mention, that comparing results obtained by various authors at that time, one must keep in mind, that some trait classification might be subjective. For example pea with "round smooth" seeds does not produce seeds which are exactly alike, and also "greeness" is variable. Moreover, there is frequent inconsistency in descriptions even of the same pea varieties (Bateson 1913). The most striking exception to the law of dominance is that observed by Carl Correns (1864–1933). The "*grüne späte Erfurter Folger-Erbse*" has a nearly colourless seed-coat; the "*Purpurviolettschottige Kneifel-Erbse*" and the "*Pahl-Erbse mit purpurrothen Hülsen*" have the seed-coat uniformly orange, becoming brown with age. In hybrids of the first generation, between either of these coloured varieties and the *Erfurter Folger-Erbse*, the coats of seeds (often in the same pod) were sometimes nearly colourless, sometimes intensely orange-red, but generally orange-red with blackish violet spots. The seeds of extreme colour, those with orange or those with almost colourless seed-coats, gave plants of the second generation which again showed the same extremes of colour in the seed-coats. This is clearly a case in which one of Mendel's characters obeys neither the law of dominance nor the law of segregation. We can speculate if such variation could be due to genetic non homogeneity of parental types.

Charles Darwin (1809–1882) also used pea in his experiments to study plant movement and sensitiveness of the apex of the radicle to contact (Darwin 1880). Pea was among plants he tested for effect of cross- versus self-pollination. Interestingly, in contrast to Knight and Laxton, Darwin observed growth depression of selfed compared to cross-pollinated pea (Darwin 1876). However he worked with only four plants per cross and grew them in pots. Although Darwin conducted monumental work that lead to his theory on evolution, there were some parts missing, especially regarding the mechanism of inheritance. Thus Darwin was unable to answer critics such as those pointing out that if variations were small and needed to be accumulated regularly over vast amounts of time, variations would become swamped and blended away over repeated generations of cross-breeding within the normal population. Importantly, whereas Darwin held that species varied over time, a widely held belief among breeders, Mendel believed that species characteristics remained constant. Moreover Mendel's results and his methods of arguing from the principles of probability and combinatorics were fundamental. Mendel's use of mathematical and quasimathematical formula, operations, and laws added rigor to his biological arguments that appealed to his early 20th century supporters for whom mathematically describable laws were quickly becoming the gold standard for making arguments about evolution, heredity, and variation (Wynn 2007). Interestingly, Mendel's use of mathematics appealed to later audiences, this approach

provoked an adverse reaction in his contemporaries who thought that Mendel was being presumptuous (Fairbanks & Rytting 2001). The reason for his success is in part the result of Mendel being a hybrid himself: part biological scientist, part physical scientist, and part mathematician. As is well known, Mendel's work was largely forgotten or betterto say not understood for about 40 years later, when Carl Correns, Hugo De Vries and Erich Tschermak have re-discovered his work (Orel 1971). Of the three authors, priority in respect of publication of results lies with Hugo De Vries, then Professor of Botany at the University of Amsterdam, in his paper, "Das Spaltungsgesetz der Bastarde", (received for publication in March 14, 1900.) The second in order of publication was that of Carl Correns, then Professor of Botany at the University of Tubingen (Correns 1900, received for publication, April 24, 1900). Third in publication was the paper of Erich von Tschermak, Professor at Vienna University, received for publication June 2, 1900. It was particularly W. Bateson who introduced Mendel's work to the English speaking world (Bateson *et al*. 1913). He experimented with sweet pea and also with garden pea, and together with Edith Rebecca Saunders, and Reginald C. Punnett, reported one of the earliest exceptions to normal Mendelian ratios (Bateson *et al*. 1905). In their work with pea plants, these researchers noticed that not all of their crosses yielded results that reflected the principle of independent assortment-specifically, some phenotypes appeared far more frequently than Mendelian genetics would predict. Based on these findings, the Bateson, Saunders, Punnett trio proposed that certain traits must somehow be coupled with one another, although they weren't sure how this linkage occurred. The answer to this question came just seven years later, when Thomas Hunt Morgan used fruit flies to demonstrate that linked genes must be real physical objects that are located in close proximity on the same chromosome. Erich von Tschermak- Seysenegg (1871–1962), one of the three "re-discovers" of Mendel's work was interested in study of xenia, a genetic phenomenon where under special genetic preconditions, characters on the mother plant offer hybridisation effects caused by the pollen after fertilization. He chose pea, where the occurrence of yellow and green colours of the cotyledons in different types could have led to the twofold detection of the knowledge about inheritance of parental characters (Tschermak 1951). In a number of pea crosses, the F_1-hybrids resemble the difference of cotyledon colours in the seeds and the alternative seed shapes of the parental partners already in the pods of the F_1-plant and are easily to distinguish from each other. He composed his observations into his D.Sc. thesis ("Habilitationsschrift") which he delivered to the University of Vienna authorities in January 1900 (Tschermak 1900; Ruckenbauer 2000). The detection of a citation of Mendel's work in the book of Wilhelm Olbers Focke (Focke 1881) and the study of Mendel's publication, guided Hugo De Vries (1848–1935) to work with peas. In his first report about the segregation of his pea hybrids (De Vries 1900a) he did not mention Mendel's name, but used the expressions "dominant" and "recessive". In the second, more precise paper De Vries (1900b) confirmed Mendel's result but concentrated again on his "Mutation theory", which De Vries published in two volumes (De Vries 1901, 1903). De Vries was convinced that breeding efforts should concentrate in looking for spontaneous variations within population caused by "retrogressive and degressive" mutations. He presumed the effects of "pangenes", which modify the expansion of characters within populations. Speaking of pea and breeding, one should not forget on Philippe De Vilmorin (1872–1917), who contributed most to the introduction of Mendelism in France (Bonneuil 2006). Being from Vilmorin breeding company, he established a "laboratory of botany and genetics" at the company's headquarters and selection station in Verrieres, near Paris in 1910, the first ever explicitly designated "genetics" laboratory in France. He also assembled likely the first collection of pea mutants (De Vilmorin 1910; De Vilmorin & Bateson 1911).

Pea as model in experimental plant morphology

Pea has been used as model for experimental morphology and physiology. Particularly Brno, Czech Republic has played an important role in plant biology once again, when Rudolf Dostál (1885–1973) experimented with pea to study correlations in plant morphology (Dostál 1930, 1941). Dostál started his work as student and assistant of Bohumil Němec (1873–1966) at Prague's University, with his dissertation on correlations of pea cotyledons. The pioneering studies of Dostál with pea seedlings showed that not substances of a nutritive character but those of a growth-regulating character play the decisive role in the regulation of the phenomenon of apical dominance. Later the inhibitory effect of the shoot apex was successfully simulated by an exogenous application of the plant hormone auxin. This de-

veloped into a long history of elegant physiological experiments that established the plant signalling molecule auxin as a key player in organogenesis and vascular tissue formation. This work was continued by Dostál's student, Jiří Šebánek (1926–2013), which started the international fame of Czech school of experimental plant morphology and phytohormonal studies (Šebánek *et al.* 1983, 1991) which continues to this day. It would be a further fifty years, following the advent of molecular biology,that this auxin transport in pea seedlings was directly visualized (Sauer *et al.* 2006; Balla *et al.* 2011). Pea has been extensively used as model in study of shoot branching, a trait not accessible in *Arabidopsis*, due to several features such as long internodes separating axillary buds and the shoot tip, are easy to graft, are amenable to root xylem-sap extraction, and their axillary buds are accessible for hormone applications, growth measurements, and other related analyses. Some of these traits, in addition to the availability of mutants, made working with pea and other legumes attractive to the early plant physiologists, and remain relevant today. Thus pea was one of the plants where new phytohormones, strigolactones have been discovered (Gomez-Roldan *et al.* 2008). From being a long time object of classical genetics, pea has a large number of mutant lines, either of spontaneous or induced. Numerous morphologically well described mutants exist, many of them being used in genetic mapping (De Vilmorin 1913; Blixt 1972). Induced mutagenesis has become widespread for creation of novel genetic variation for selection and genetic studies (Lamm & Miravalle 1951; Lamprecht & Svensson 1963; Blixt 1972) with mutants in physiological, chlorophyll, seed, root, shoot, foliage, inflorescence, flower and pod traits as well as nodulation (Duc & Messager 1989). Unfortunately, until recently cloning genes responsible for pea mutant phenotypes has largely been via candidate gene approach by using the genes characterized in other plant species, usually *Arabidopsis*. With promise of pea genome sequence, novel gene identification would become feasible task.

Mendel's pea genes today

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 (Reid & Ross 2011). 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 (Reid & Ross 2011). With the improved linkage maps available today the seven traits in fact are thought to occupy only five of the seven linkage groups. 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 (Ellis *et al.* 2011; Reid & Ross 2011).

Round versus wrinkled (R versus r) seeds. Wrinkled seeds possess elevated sucrose, fructose, and glucose levels on expense of starch, and this results 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 and a reduced percentage of some storage proteins such as legumin (Ellis *et al.* 2011). 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, the biochemical evidence accumulated to date established that the primary lesion in *r* embryos was in starch biosynthesis. This trait results in the failure of sugars to starch conversion and was the first gene identified by biochemical approach. Today there are known to be several genes in pea that confer a wrinkled (*rugosus*) phenotype and all are lesions in different enzymes involved in starch biosynthesis. However, only the *r* mutant is known to have been available to Mendel (Ellis *et al.* 2011). Thus the first of Mendel's mutants to be characterized corresponded to a mutation in a gene encoding a biosynthetic enzyme and it was associated with an active transposon (Bhattacharyya *et al.* 1990).

Yellow versus green cotyledons (I versus i). Another of Mendel's genes to be sequenced was the gene responsible for cotyledon colour. This gene was given the symbol *I* by White (1917). Ripe wild-type (*II*) seeds are yellow because the chlorophyll is lost as

the seeds mature, whereas (*ii*) seeds remain green. 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 (Ellis *et al.* 2011). It was shown that not only do the cotyledons in pea exhibit a green colour 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). The corresponding gene; homolog of *Stay-Green* (SGR) has been identified based on candidate gene approach using knowledge from rice and *Arabidopsis*. SGR appears to direct chlorophyll to the degradation pathway (Armstead *et al.* 2007; Sato *et al.* 2007). However, they provide no evidence that this was indeed the specific mutation that Mendel had used.

Seed coat and flower colour (A versus a). Mendel noted that coloured seed coats were always associated with coloured (purple) flowers. He also noted that these coloured varieties possessed pigmentation in the leaf axils. On the other hand, a clear or colourless 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. In pea, as in many other plants, the red, purple or blue pigmentation is due to the accumulation of anthocyanin compounds. The mutation in (*a*) gene abolishes anthocyanin pigmentation throughout the plant. 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. 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 (Hellens *et al.* 2010). The mutation in splicing site leads to a frame shift and a premature stop codon. A second *a* allele was found to be present in a number of accessions (Hellens *et al.* 2010), and was concluded that it was probably of African origin. 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 (Reid & Ross 2011).

Tall versus short (Le versus le). Wild pea and most of the older cultivated varieties have tall vines, whereas most of modern varieties have shortened internodes. A similar phenomenon has been exploited during Green Revolution in wheat and rice, and was identified to be associated with gibberellin (GA) pathway. Based on the phenotype the *Le* gene is considered to be the one studied by Mendel (Ellis *et al.* 2011). It was White (1917) who gave the dwarf trait the gene symbol *le*. *LeLe* plants are tall, whereas *lele* plants are dwarf and this difference is due to internode length rather than the number of nodes. A combination of genetic, physiological, and analytical techniques suggested that *Le* might code for a GA_3β-hydroxylase enzyme. The *Le* gene product was implicated in GA biosynthesis in early experiments that showed that stem elongation in dwarf plants was stimulated by application of GA_3. The activity of the *Le* gene product was established because the conversion of GA_{20} to GA_1 (the active form of GA) was much greater for *LeLe* than for *lele* plants, and GA_1 levels were higher in the shoots of *LeLe* versus *lele* plants, whereas GA_{20} amounts were elevated in *lele* plants. Consequently it was hypothesized that *Le* encodes a GA_3-oxidase (GA_3β -hydroxylase). Indeed, GA_3-oxidase activity was shown to be reduced in *lele* plants and subsequent identification of the *Le* gene demonstrated that it encodes a GA_3-oxidase. Le was the second of Mendel's genes to be cloned when in 1997 two groups working independently reported the isolation of this gene (Lester *et al.* 1997; Martin *et al.* 1997).

The remaining three genes underlying traits Mendel's studied await their identification. These are:

Inflated versus constricted pod (P versus p or V versus v). 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. 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 (Ellis *et al.* 2011). Such pods without 'rough skinny membrane' were already described in

Gerard's 1597 Herball, and in general this cell layer is absent in vegetable pea types where the whole pod is eaten. Absence of this cell layer leads to a pod that is constricted around the seeds at maturity. Mendel referred to peas with this pod characteristic as *P. saccharatum* suggesting that he used a 'sugar snap' type. There are two possible genes involved and 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 (Ellis *et al.* 2011). Double-mutant plants totally lack sclerified cells on the inner side of the pod. The deduction has been made, based on Mendel's two and three trait crosses. 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 (Rasmusson 1927). However, he did not work extensively on this gene combination and may not have even made the bi-factorial cross (Blixt 1975). This trait has clearly received less attention than any of the other seven traits of Mendel, making the prediction of putative candidate genes difficult (Reid & Ross 2011).

Axial versus terminal flowers (Fa versus fa or Fas versus fas). 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). Mendel's authoritative translator, William Bateson, was clearly convinced that Mendel had worked with a type known as "Mummy Pea" (Bateson 1909). White (1917) was the first to ascribe a gene symbol, in this case *Fa* for the wild-type form. The *Fa* gene has not yet been cloned although fasciation has been linked with clavata genes in other species (Leyser & 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* (Reid & Ross 2011).

Green versus yellow pod (Gp versus gp). Of Mendel's three genes that have not been sequenced, the colour 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 colour of the pods. Price *et al.* (1988) studied the structural and physical basis of this difference and 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. 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, it may now be possible to identify candidate genes that may control the green/yellow pod colour difference (Reid & Ross 2011).

Pea genetics and genomics

The standard pea karyotype comprises seven chromosomes: five acrocentric and two (4 and 7) metacentric chromosomes. The numbering of pea chromosomes is unconventional. The largest chromosome is conventionally called 5 rather than the usual 1. A set of translocation stocks was generated but there was considerable disagreement about which linkage groups and chromosomes were involved (Lamm 1977; Folkeson 1990). For this reason the chromosome numbers and linkage group numbers are referred to using Arabic and Roman numerals respectively (1 = VI, 2 = I, 3 = V , 4 = IV, 5 = III, 6 = II and VII = 7, Fuchs *et al.* 1998). Several lines with reconstructed karyotypes were used for flow sorting of individual pea chromosomes with over 95% purity suitable for PCR-based physical mapping in pea (Neumann *et al.* 2002). Therefore, the only mean to reliably distinguish between all chromosome types is to label the chromosomes with markers showing chromosome-specific FISH pattern (reviewed in Smýkal *et al.* 2012). There is a long history of genetic mapping studies in pea (reviewed in Smýkal & Konečná 2014). Different types of polymorphisms were successively used: morphological markers, isozymes, RFLP, RAPD, SSR and ESTs through PCR-based techniques or more recently through high-throughput parallel genotyping. The most commonly used is composite genetic map of 1430 cM (Haldane) comprising 239 microsatellite markers (Loridon *et al.* 2005). These markers are quite evenly distributed throughout the seven linkage groups of the map, with 85% of intervals between the adjacent SSR markers being smaller than 10 cM. This map was used to localize numerous QTLs for disease resistance as well as quality and morphology traits (reviewed in Smýkal *et al.* 2012). More recently, functional maps, i.e., composed of genes of known function, were developed (Aubert *et al.* 2006; Bordat *et al.* 2011). The latest consensus map published in pea

provides a comprehensive view. This map includes 214 functional markers, representing genes from diverse functional classes such as development, carbohydrate metabolism, amino acid metabolism, transport and transcriptional regulation. It also includes 180 SSR, 133 RAPD and three morphological markers and is thus intrinsically related to previous maps (Bordat *et al.* 2011). Comparative mapping of pea and lentil, pea and chickpea, pea and *Medicago*, as well as among several legume species has been reported (Kalo *et al.* 2004; Choi *et al.* 2004). All suggested a good conservation of synteny among legume crop species. Recently, new opportunities have arisen from advances in the sequencing of model legumes *M. truncatula* and *L. japonicus*, as well as the crop legume *Glycine max*. Of these, *Medicago truncatula* is taxonomically the closest model species to pea, while the model legume species *Lotus japonicus* belongs to the closely related Robinioid clade and soybean to the more distant Milletioid clade. Translational genomics is also beginning to assist in identifying candidate genes or saturating markers in a zone of interest of pea.

Pea diversity

The classification of *Pisum* L. has changed over time from a genus with five species, to a monotypic genus, to currently accepted genus with two species (reviewed in Smýkal *et al.* 2011, 2013). While most authors agreed with the original suggestion of Linné, who described the genus *Pisum* as distinct from *Lathyrus* (Linnaeus 1753), the recent molecular phylogenetic analysis (Schaefer *et al.* 2012) finds it deeply nested in *Lathyrus*. Despite being small genus, *Pisum* is very diverse and the diversity is structured, showing a range of degrees of relatedness that reflect taxonomic identifiers, eco-geography and breeding gene pools (Jing *et al.* 2010; Ellis 2011; Smýkal *et al.* 2011, 2013). Pea genetic diversity is sampled in germplasm collections, with over 1000 accessions found in national genebanks in at least 25 countries, with many other smaller collections worldwide totalling 98 thousand accessions (Smýkal *et al.* 2013). There are gaps in the collections, particularly of wild and locally adapted materials (landraces), that need to be collected before these genetic resources are lost forever. Numerous studies have been conducted to investigate genetic and trait diversity of *Pisum* germplasm. Several major world pea germplasm collections have been analysed by molecular methods and core collections were formed (Smýkal *et al.* 2011). There are efforts to make either genome-wide introgression lines or at least simple crosses with the intent of broadening the genetic base. The molecularly analysed major world pea collections and formulated core collections might act as toolkits for association mapping, a strategy to gain insight into genes and genomic regions underlying desired traits. Recent advances in genomic technology, the impetus to exploit natural diversity, and development of robust statistical analysis methods make association mapping affordable to pea research programs (reviewed in Smýkal *et al.* 2012).

Pea mutagenesis and transgenesis

Pea has also long tradition of mutant collections for single or combined characters, first established by De Villmorin (1913) and further developed by Blixt (1972). Induced mutagenesis is currently undergoing renaissance to create novel genetic variation for selection, especially as linked to molecular techniques. The genomics tools such as fast neutron and TILLING mutant populations were developed for reverse genetics approaches (Dalmais *et al.* 2008; Wang *et al.* 2008). The TILLING (targeting-induced local lesions in genomes) method combines the induction of a high number of random point mutations with mutagens like ethyl methane sulfonate (EMS) and mutational screening systems to discover induced mutations in sequence DNA targets. Sufficiently large TILLING population made in the French variety Cameor is available for pea and data were developed into on-line database (UTILLdb) that contains phenotypic as well as sequence information on mutant genes (Dalmais *et al.* 2008). Currently it has 4817 lines, of which1840 are with phenotype and 464 identified mutations by sequencing. In addition, fast neutron generated deletion mutant resources (around 3000 lines) are also available for pea. The major pea mutant collections include: John Innes Collection, Norwich, UK (575 accessions); Plovdiv, Bulgaria (122 accessions); a targeted-induced local lesions in genomes (TILLING) population of 4817 lines at INRA, Dijon, France.

Although pea is accessible to genetic transformation, this remains a challenge and precludes the systematic characterization of gene functions (Somers *et al.* 2003). This is both due to the recalcitrance nature of pea to *in vitro* regeneration as well as *Agrobacterium*-mediated transformation. Despite this, co-cultivation process was elaborated and several successful pea transformations were published (reviewed in Atif *et al.* 2013). In addition to *Agrobacterium*-mediated transformation, direct

gene transfer methods such as electroporation of isolated pea protoplasts and biolistic are also available. Despite the fact that pea transformation was reported over 20 years ago its efficiency remains low (in range of 0.1 to 6.5%) (reviewed in Atif *et al.* 2013). The majority of these studies used only selection and reporter marker genes, but some used agronomically useful genes such as bean alpha-amylase inhibitor, tested in field conditions and found effective against pea weevil (Morton *et al.* 2000). Transgenic pea came into focus in relation to plant-made vaccines, to which the protein rich seeds of legumes are very suitable. Virus-induced gene silencing (VIGS) has become an important reverse genetics tool for functional genomics and VIGS vectors based on *Pea early browning virus* (PEBV, genus Tobravirus) are available for the legume species *Pisum sativum* and were successfully used to silence pea genes involved in the symbiosis with nitrogen-fixing *Rhizobium* as well as development (Constantin *et al.* 2004; Gronlund *et al.* 2010).

Pea entering (post) genomic era

For cultivated pea, nuclear genome size estimates have been produced for several accessions using different methods and estimated to be 9.09 pg DNA/2C corresponding to the haploid genome size (1C) of 4.45 Gbp with a large component (75–97%) made up of repetitive sequences (Macas *et al.* 2007; Dolezel & Greilhuber 2010). The pea variety Cameor used to create mutant TILLING population (Dalmais *et al.* 2008), has also been used for BAC library development, essential tool for positional cloning and also for pea genome sequencing (Hellens *et al.* 2010). Another BAC library was developed from PI 269818 accession, which was used to introgress genetic diversity into the cultivated germplasm pool and which could be useful for the isolation of genes underlying disease resistance (such as *Fw,* Fusarium resistance loci) and other economically important traits (Coyne *et al.* 2007). Both BAC libraries will be essential for good quality pea genome sequencing. Despite the large and complex genome, the International Pea Sequencing Project consortium has been formed, with aim to provide pea genome by 2015, a 150 years anniversary of Mendel's discovery (McGee 2013).

The complete pea chloroplast genome is also available, knowledge of which might be used both for evolutionary as well as transgenic applications (Magee *et al.* 2010). Importantly, the pea aphid *Acyrthosiphon pisum* has been selected as the model aphid species and its genome sequenced (The International Aphid Genomics Consortium 2010). As aphids are responsible for serious crop damage and as the vectors for the transmission of viral diseases, this knowledge has enormous potential for evolutionary studies and practical applications. Several transcriptome analyses have been performed using a pea oligo-array (Ps6kOLI1) developed from diverse sources of sequences and particularly seed EST libraries. Notably, the effect of mutations in genes involved in primary metabolism or hormone deficiency on seed transcriptome has been assessed (Weigelt *et al.* 2009). Recently, a set of SNP markers using Illumina Veracode genotyping technology has been applied and used to build consensus map (Deulvot *et al.* 2010; Bordat *et al.* 2011). Within the Crop EST project a further 9377 ESTs with BLAST identified 8238 ESTs were obtained from 2 cDNA libraries of pea (Franssen *et al.* 2011). Recently, a set of 37 455 contig sequences were assembled from 3 084 253 high quality 454 reads (1.2 Gbp) of pea variety Aragorn using the Newbler algorithm. These 37 368 putative transcripts were of average length 1045 bp and represent 25 353 isotigs, and represent 34 846 unigenes, 8817 contigs and 26 029 singletons (http://www.gabcsfl.org). Similarly, INRA Dijon, France has produced pea RNA-Seq Gene Atlas (http://bios.dijon.inra.fr). This web-portal provides the first full-length Unigene set expression atlas for pea. In pea, the proteome of mitochondria (ca. 60 spots identified), and the peribacteroid space and membrane from symbiosomes (ca. 20 pea spots), of mature leaves and stems (190 spots identified), of mature seeds (156 spots identified) were analysed. To extend our knowledge of the pea genome structure, the current studies are focused on pea metabolome (reviewed in Smýkal & Konečná 2014).

The current status of completed and annotated genomes of model legumes includes the 373 Mb genome of *Medicago truncatula*, the 472 Mb genome of *Lotus japonicus*, and of three legume seed crops: the 1,112 Mb genome of *Glycine max*, the 833 Mb genome of pigeonpea (*Cajanus cajan*), the 738 Mb genome of *Cicer aerietinum* and the ongoing genome sequencing efforts in *Phaseolus vulgaris* (550 Mb), *Pisum sativum* (4600 Mb), *Lupinus angustifolius* (924 Mb), *Trifolium praetense* (440 Mb) and *Arachis hypogaea* (2800 Mb), there is strong potential for comparative genomics and its applications, including specific gene/allele mining and deeper diversity studies of legume germplasm collections. There is no doubt that genomic sequence knowledge provides powerful tools and resources to improve agronomic and nutritional traits so important to maintain-

ing and improving their nutritional status. Second, while pea has been an important temperate season legume crop, it is losing competitiveness because it does not have the biotechnology tools currently available to other crops. The sequence will greatly facilitate marker assisted selection, an important genetic alternative that will help improve the economic competitiveness of the crop. And third, as a close relative of soybean, chickpea, cowpea, common bean, peanut, vetches and pigeonpea, its sequence is important for the study of the function of genes within this economically important group of legumes. In relation to current development of sequencing methodology, there is issue if to use whole genome shotgun (WGS) method based on the Sanger technique, or BAC clone approach. New approaches such as the 454 Roche pyrosequencing or Illumina, offer a cost advantage along with increased speed and throughput. Considering the large proportion of repetitive sequences and size of pea genome, it will be important to have sufficient genetic and physical tools for scaffold assembly and merging the scaffolds into pseudo chromosomes. There is a community-wide effort with input and support from many individuals resulting in establishment of an International Consortium for Pea Genome Sequencing (McGee 2013). Moreover, owing to the seminal work of J.G. Mendel, pea has a unique position among any other species. Celebrating 190 anniversary of Mendel birthday (1822) and soon 150 years of inheritance law formulation (1865), the full pea genome sequence would have not only scientific but also great educational and social impact. Knowledge of pea genome architecture will facilitate the identification of a wide range of DNA markers, genes, and pea genotypes that influence important traits such as resistance to biotic and abiotic stress; plant architecture, yield stability and nutritional quality. Newly identified genes and alleles controlling these traits will enable marker-assisted breeding and transgenic strategies for accelerating pea enhancement.

Acknowledgements. Discussion over earlier versions of the manuscript, helpful comments and edits from C. Coyne, USDA-WSU, USA and M. Ambrose, JIC, UK are greatly acknowledged. P.S. work was supported by the Grant Agency of Palacký University in Olomouc, IGA_PrF_2014001 Project.

References

Armstead I., Donnison I., Aubry S., Harper J., Hörtensteiner S. *et al.* (2007): Cross-species identification of Mendel's *l* locus. Science, **315**: 73.

Atif R.M., Patat-Ochatt E.M., Svabova L., Ondrej V., Klenoticova H., Jacas L., Griga M., Ochatt S. (2013): Gene transfer in legumes. In: Luttge *et al.* (eds): Progress in Botany. Springer-Verlag, Berlin, 37–100.

Aubert G., Morin J., Jacquin F., Loridon K., Quillet M.C., Petit A., Rameau C., Lejeune-Henaut I., Huguet T., Burstin J. (2006): Functional mapping in pea, as an aid to the candidate gene selection and for investigating synteny with the model legume *Medicago truncatula*. Theoretical and Applied Genetics, **112**: 1024–1041.

Aubry S., Mani J., Hörtensteiner S. (2008): Stay-green protein, defective in Mendel's green cotyledon mutant, acts independent and upstream of pheophorbide a oxygenase in the chlorophyll catabolic pathway. Plant Molecular Biology, **67**: 243–256.

Balla J., Kalousek P., Reinohl V., Friml J., Procházka S. (2011): Competitive canalization of PIN-dependent auxin flow from axillary buds controls pea bud outgrowth. The Plant Journal, **65**: 571–577.

Bateson W. (1909): Mendel's Principles of Heredity. Cambridge University Press, Cambridge.

Bateson W. (1913): Mendel's Principles of Heredity. Cambridge University Press, Cambridge.

Bateson W., Sauders E.R., Punnett R.C. (1905): Experimental studies in the physiology of heredity. Reports to the Evolution Committee of the Royal Society, **2**: 1–55, 80–99.

Bhattacharyya M., Smith A.M., Ellis T.H.N., Hedley C., Martin C. (1990): The wrinkled-seed character of pea described by Mendel is caused by a transposon-like insertion in a gene encoding starch-branching enzyme. Cell, **60**: 115–122.

Blixt S. (1972): Mutation genetics in *Pisum*. Agricultural and Horticultural Genetics, **30**: 1–293.

Blixt S. (1975): Why didn't Gregor Mendel find linkage? Nature, **256**: 206.

Bonneuil C. (2006): Mendelism, plant breeding and experimental cultures: Agriculture and the development of genetics in France. Journal of the History of Biology, **39**: 281–308.

Bordat A., Savois V., Nicolas M., Salse J., Chauveau A., Bourgeois M., Potier J., Houtin H., Rond C., Murat F., Marget P., Aubert G., Burstin J. (2011): Translational genomics in legumes allowed placing *in silico* 5460 unigenes on the pea functional map and identified candidate genes in *Pisum sativum* L. G3. Genes-Genomes-Genetics, **2**: 93–103.

Camerer R. J. (1694): De sexu plantarum. Encyclopaedia Britannica. Encyclopaedia Britannica Online Academic Edition, available at http://www.britannica.com/EBchecked/topic/90882/Rudolph-Jacob-Camerarius

Choi H.K., Mun J.H., Kim D.J., Zhu H.Y., Baek J.M., Mudge J., Roe B., Ellis N., Doyle J, Kiss G.B., Young

N.D., Cook D.R. (2004): Estimating genome conservation between crop and model legume species. Proceedings of National Academy of Sciences USA, **101**: 15289–15294.

Constantin G.D., Krath B.N., MacFarlane S.A., Nicolaisen M., Johansen I.E., Lund O.S. (2004): Virus-induced gene silencing as a tool for functional genomics in a legume species. Plant Journal, **40**: 622–631.

Correns C. (1900): G. Mendel's Regel über das Verhalten der Nachkommenshaft der Rassenbastarde. Berichte der Deutschen Botanischen Gesellschaft, **8**: 158–168.

Coyne C.J., Mcclendon M.T., Walling J.G., Timmerman-Vaughan G.M., Murray S., Meksem K., Lightfoot D.A., Shultz J.L., Keller K.E., Martin R.R., Inglis D.A., Rajesh P.N., Mcphee K.E., Weeden N.F., Grusak M.A., Li C.M., Storlie E.W. (2007): Construction and characterization of two bacterial artificial chromosome libraries of pea (*Pisum sativum* L.) for the isolation of economically important genes. Genome, **50**: 871–875.

Dalmais M., Schmidt J., Le Signor C., Moussy F., Burstin J., Savois V., Aubert G., Brunaud V., de Oliveira Z., Guichard C., Thompson R., Bendahmane A. (2008): UTILLdb, a *Pisum sativum* in silico forward and reverse genetics tool. Genome Biology, **9**: R43.

Darwin C.R. (1863): Vindication of Gärtner—effect of crossing peas. Journal of Horticulture and Cottage Gardener, **29**: 93.

Darwin C.R. (1876): The Effects of Cross and Self Fertilisation in the Vegetable Kingdom. John Murray, London.

Darwin C.R. (1880): The Power of Movement in Plants. John Murray, London.

De Vilmorin P. (1910): Experiments on Mendelian inheritance (transl. from French). Comptes Rendus de l'Académie des Sciences, Paris **2**: 548–551. (in French)

De Vilmorin P. (1913): Quelle a été jusqu'á présent l'influence des nouvelles méthodes de sélection sur la stabilité des variétés de plantes cultivées. [Reprint from the Actes du Congrés International d'Agriculture de Gand] Masson, Paris, 312–316.

De Vilmorin P., Bateson W. (1911): A case of gametic coupling in *Pisum*. Proceedings of the Royal Society London B. Biological Sciences, **84**: 9–11.

De Vries H. (1900a): Sur la loi de disjunction des hybrides. Comptes Rendus de l'Académie des Sciences. Paris, 249–255.

De Vries H. (1900b): Das Spaltungsgesetz der Bastarde. Berichte der Deutschen Botanischen Gesellschaft, **18**: 83–90.

De Vries H. (1901, 1903): The Mutation Theory. German edition, Bd. 1–2, Veit & Company, Leipzig.

Deulvot C., Charrel H., Marty A., Jacquin F., Donnadieu C., Lejeune-Hénaut I., Burstin, J., Aubert G. (2010): Highly-multiplexed SNP genotyping for genetic mapping and germplasm diversity studies in pea. BMC Genomics, **11**: 468.

Dolezel J., Greilhuber J. (2010): Nuclear genome size. Are we getting closer? Cytometry, **77**: 635–642.

Dostál R. (1930): Experimental Plant Morphology. Praha. Aventinum. (in Czech)

Dostál R. (1941): Wuchsstoffstudien betreffend die Korrelationen zwischen Wurzel und Sproß bei *Pisum sativum*. Práce Moravské Přírodovědné Společnosti, **13**: 1–31.

Duc G., Messager J. (1989): A mutagenesis of pea (*Pisum sativum L.*) and the isolation of mutants for nodulation and nitrogen-fixation. Plant Science, **60**: 207–213.

Ellis T.H.N. (2011): Pisum. In: Kole C. (ed.): Wild Crop Relatives: Genomic and Breeding Resources. Chapter 12, Springer-Verlag, Berlin-Heidelberg, 237–248.

Ellis T.H.N., Hofer J.I., Timmerman-Vaughan G.M., Coyne C.J., Hellens R.P. (2011): Mendel, 150 years on. Trends in Plant Science, **16**: 590–596.

Fairbanks D.J., Rytting B. (2001): Mendelian controversies: a botanical and historical review. American Journal of Botany, **88**: 737–752.

Fisher R.A. (1936): Has Mendel's work been rediscovered? Annals of Science, **1**: 115–137.

Focke W.O. (1881): The Plant Hybrids, a Contribution to the Biology of Plants. Gebrüder Borntraeger Verlag, Berlin. (in German)

Folkeson D. (1990): Assignment of linkage segments to the satellite chromosomes 4 and 7 in *Pisum sativum*. Hereditas, **112**: 257–263.

Franklin A. (2008): The Mendel-Fisher controversy: an overview, Chapter 1. In: Franklin A., Edwards A.W.F., Fairbanks D.J., Hartl D.L., Seidenfeld T. (eds): Ending the Mendel-Fisher Controversy. University of Pittsburgh Press, Pittsburgh, 1–68.

Franssen S.U., Shrestha R.P., Bräutigam A., Bornberg-Bauer E., Weber A.P.M. (2011): Comprehensive transcriptome analysis of the highly complex *Pisum sativum* genome using next generation sequencing. BMC Genomics, **12**: 227.

Fuchs J., Kühne M., Schubert I. (1998): Assignment of linkage groups to pea chromosomes after karyotyping and gene mapping by fluorescent *in situ* hybridization. Chromosoma, **107**: 272.

Gärtner C. F. (1849): Versuche und Beobachtungen über die Bastarderzeugung im Pflanzenreiche. K.F. Herring, Stuttgart.

Gomez-Roldan V., Fermas S., Brewer P.B., Puech-Pagès V., Dun E.A., Pillot J.P., Letisse F., Matusova R., Danoun S., Portais J.C., Bouwmeester H., Bécard G., Beveridge C.A., Rameau C., Rochange S.F. (2008): Strigolactone inhibition of shoot branching. Nature, **455**:189–194.

Goss J. (1824): On the variation in the colour of peas, occasioned by cross-impregnation. Transactions of the Horticultural Society of London, **5**: 234.

Gronlund M., Olsen A., Johansen I.E., Jakobsen I. (2010): Protocol: using virus-induced gene silencing to study the arbuscular mycorrhizal symbiosis in *Pisum sativum.* Plant Methods, **6**: 28.

Hellens R.P., Moreau C., Lin-Wang K., Schwinn K.E., Thomson S.J. , Fiers M.W.E.J., Frew T.J., Murray S.R., Hofer J.M.I., Jacobs J.M.E., Davies K.M., Allan A.C., Bendahmane A., Coyne C.J., Timmerman-Vaughan G.M., Ellis T.H.N. (2010): Identification of Mendel's white flower character. PLOS ONE, **5**: e13230.

Jing R., Vershinin A., Grzebyta J., Shaw P., Smýkal P., Marshall D., Ambrose M.J., Ellis, T.H.N., Flavell A.J. (2010): The genetic diversity and evolution of field pea (*Pisum*) studied by high throughput retrotransposon based insertion polymorphism (RBIP) marker analysis. BMC Evolutionary Biology, **10**: 44.

Kalo P., Seres A., Taylor S.A., Jaka J., Kevei Z.A., Kereszt G.E., Ellis T.H.N., Kiss G.B. (2004): Comparative mapping between *Medicago sativa* and *Pisum sativum*. Molecular and General Genomics, **272**: 235–246.

Knight T.A. (1799): An account of some experiments of the fecundation of vegetables. Philosophical Transactionsof the Royal Society of London, **1**: 195–204.

Lamm R. (1977): Transpositions in *Pisum.* Pisum Newsletter, **9**: 28–29.

Lamm R., Miravalle R.J. (1951): A translocation tester set in *Pisum*. Hereditas, **45**: 417– 440.

Lamprecht H., Svensson V. (1963): Die Mutationsfrequenz der Membrangene von *Pisum* und ihre züchterishe Bedeutung. Agricultural and Horticultural Genetics, **21**: 1–18.

Laxton T. (1866): Observations on the variations effected by crossing in the color and character of the seed of peas. Report of the International Horticultural Exhibition and Botanical Congress, 156.

Laxton T. (1872): Notes on some changes and variations in the offspring of cross-fertilized peas. Journal of the Royal Horticultural Society, **3**:10–14.

Lester D.R., Ross J.J., Davies P.J., Reid J.B. (1997): Mendel's stem length gene (*Le*) encodes gibberellin 3b-Hydroxylase. Plant Cell, **9**: 1435–1443.

Leyser H.M.O., Furner J.I. (1992): Characterisation of three shoot apical meristem mutants of arabidopsis thaliana. Development, **116**: 397–403.

Linnaeus C. (1753): Species Plantarum. Vol. 2, Salvius, Stockholm.

Loridon K., McPhee K.E., Morin J., Dubreuil P., Pilet-Nayel M.L., Aubert G., Rameau C., Baranger A., Coyne C.J., Lejeune-Hénault I., Burstin J. (2005): Microsatellite marker polymorphism and mapping in pea (*Pisum sativum* L.). Theoretical and Applied Genetics, **111**: 1022–1031.

Macas J., Neumann P., Navrátilová A. (2007): Repetitive DNA in the pea (*Pisum sativum* L.) genome: comprehensive characterization using 454 sequencing and comparison to soybean and *Medicago truncatula*. BMC Genomics, **8**: 427.

Magee A.M., Aspinall S., Rice D.W., Cusack B.P., Sémon M., Perry A.S., Stefanović S., Milbourne D., Barth S., Palmer J.D., Gray J.C., Kavanagh T.A., Wolfe K.H. (2010): Localized hypermutation and associated gene losses in legume chloroplast genomes. Genome Research, **20**: 1700–1710.

Martin D.N., Proebsting W.M., Hedden P. (1997): Mendel's dwarfing gene: cDNAs from the Le alleles and function of the expressed proteins. Proceedings of the National Academy of Sciences USA, **94**: 8907–8911.

McGee R. (2013): Mendel's legacy: An international pea sequencing project. Pisum Genetics, **44**: 13–14.

Mendel G. (1866): Versuche über Pflanzen-Hybriden. Verhandlungen des Naturforschenden Vereines in Brünn, IV. Verlage des Vereines, Brünn, 67–112.

Morton R.L., Schroeder H.E., Bateman K., Chrispeels M.J., Armstrong E., Higgins T.J.V. (2000): Bean a-amylase inhibitor 1 in transgenic peas (*Pisum sativum L.*) provides complete protection from pea weevil (*Bruchus pisorum*) under field conditions. Proceedings of National Academy of Sciences USA, **7**: 3820–3825.

Neumann P., Pozárková D., Vrána J., Dolezel J., Macas J. (2002): Chromosome sorting and PCR-based physical mapping in pea (*Pisum sativum* L.). Chromosome Research, **10**: 63–71.

Orel V. (1971): Mendel and the evolution idea. Folia Mendeliana, **6**: 161–172.

Price D.N., Smith C.M., Hedley C.L. (1988): The effect of the *gp* gene on fruit development in *Pisum sativum L.* I. Structural and physical aspects. New Phytologist, **110**: 261–269.

Rasmusson J. (1927): Genetically changed linkage values in *Pisum*. Hereditas, **10**: 1–150.

Reid J.B., Ross J.J. (2011): Mendel's genes: Toward a full molecular characterization. Genetics, **189**: 3–10.

Roberts H.F. (1929): Plant hybridization before Mendel. Princeton, Princeton University Press.

Ruckenbauer P. (2000): E. von Tschermak-Seysenegg and the Austrian contribution to plant breeding. Vorträge für Pflanzenzüchtung, **48**: 31–46.

Sato Y., Morita R., Nishimura M., Yamaguchi H., Kusaba M. (2007): Mendel's green cotyledon gene encodes a positive regulator of the chlorophyll-degrading pathway. Proceedings of National Academy of Sciences USA, **104**: 14169–14174.

Sauer M., Balla J., Luschnig Ch., Wisniewska J., Reinohl V., Friml J., Benková E. (2006): Canalization of auxin flow by Aux/IAA-ARF-dependent feedback regulation of PIN polarity. Genes and Development, **20**: 2902–2911.

Schaefer H., Hechenleitner P., Santos-Guerra A., de Sequeira M.M., Pennington R.T., Kenicer G., Carine M.A. (2012): Systematics, biogeography, and character evolution of the legume tribe *Fabeae* with special focus on the middle-Atlantic island lineages. BMC Evolutionary Biology, **12**: 250.

Šebánek J., Sladký Z., Procházka S. (1983): Experimental Plant Morphology. Academia, Praha. (in Czech)

Šebánek J., Sladký Z., Procházka S. (1991): Experimental Morphogenesis and Integration of Plants. Elsevier, Amsterdam, Oxford, New York, Tokyo.

Seton A. (1824): On the variation in the color of peas from cross-impregnation. Transactions of the Horticultural Society of London, **5**: 236.

Smýkal P., Konečná E. (2014): Advances in pea genomics. In: Gupta S., Nadarajan N., Gupta D. (eds.): Legumes in the Omic Era. Chapter 15. Springer, Dordrecht, 301–337.

Smýkal P., Kenicer G., Flavell A.J., Corander J., Kosterin O., Redden R.J., Ford R., Coyne C.J., Maxted N., Ambrose M.J., Ellis T.H.N. (2011): Phylogeny, phylogeography and genetic diversity of the *Pisum* genus. Plant Genetic Resources, **9**: 4–18.

Smýkal P., Aubert G., Burstin J., Coyne C., Ellis N., Flavell A., Ford R., Hýbl M., Macas J., Neumann P., McPhee K., Redden R., Rubiales D., Weller J., Warkentin T.D. (2012): Pea (*Pisum sativum* L.) in the genomic era. Agronomy, **2**: 74–115.

Smýkal P., Coyne C., Redden R., Maxted N. (2013): Peas. In: Singh M., Upadhyaya H. (eds): Genetic and Genomic Resources of Grain Legume Improvement. Elsevier, Amsterdam, 41–80.

Somers D.A., Samac D.A., Olhoft P.M. (2003): Recent advances in legume transformation. Plant Physiology, **131**: 892–899.

The International Aphid Genomics Consortium (2010): Genome Sequence of the Pea Aphid *Acyrthosiphon pisum*. PLOS Biology, **8**: e1000313.

Tschermak E.V. (1900): Über künstliche Kreuzung bei *Pisum sativum*. Habilitationsschrift Zeitschrift für das landwirtschaftliche Versuchswesen in Österreich, **3**: 465–555.

Tschermak E.V. (1951): The rediscovery of Gregor Mendel's work. Journal of Heredity, **42**: 163–171.

Wang Z., Luo Y., Li X., Wang L., Xu S., Yang J., Weng L., Sato S., Tabata S. Ambrose M., Rameaus C., Feng X., Hu X., Luo D. (2008): Genetic control of floral zygomorphy in pea (*Pisum sativum* L.) Proceedings of National Academy of Sciences USA, **105**: 10414–10419.

Weigelt K., Küster H., Rutten T., Fait A., Fernie A.R., Miersch O., Wasternack C., Emery R.J.N., Desel C., Hosein F., Müller M., Saalbach I., Weber H. (2009): ADP-glucose pyrophosphorylase-deficient pea embryos reveal specific transcriptional and metabolic changes of carbon-nitrogen metabolism and stress responses. Plant Physiology, **149**: 395–411.

Weldon W.F.R. (1902): Mendel's laws of alternative inheritance in peas. Biometrika, **1**: 228–254.

White O. E. (1917): Studies of inheritance in *Pisum*. II. The present state of knowledge of heredity and variation in peas. Proceedings of American Philosophical Society, **56**: 487–588.

Wynn J. (2007): Alone in the Garden: How Gregor Mendel's inattention to audience may have affected the reception of his theory of inheritance in Experiments in Plant Hybridization. Written Communication, **24**: 3–27.

Received for publication January 4, 2014
Accepted after corrections February 24, 2014

Corresponding author:

Ing. Petr Smýkal, Ph.D., Univerzita Palackého v Olomouci, Přírodovědecká fakulta, katedra botaniky, Šlechtitelů 11, 783 71 Olomouc, Česká republika; e-mail: petr.smykal@upol.cz