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How did Mendel arrive at his discoveries?

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There are few historical records concerning Gregor Johann Mendel and his work, so theories abound concerning his motivation. These theories range from Fisher's view that Mendel was testing a fully formed previous theory of inheritance to Olby's view that Mendel was not interested in inheritance at all, whereas textbooks often state his motivation was to understand inheritance. In this Perspective, we review current ideas about how Mendel arrived at his discoveries and then discuss an alternative scenario based on recently discovered historical sources that support the suggestion that Mendel's fundamental research on the inheritance of traits emerged from an applied plant breeding program. Mendel recognized the importance of the new cell theory; understanding of the formation of reproductive cells and the process of fertilization explained his segregation ratios. This interest was probably encouraged by his friendship with Johann Nave, whose untimely death preceded Mendel's first 1865 lecture by a few months. This year is the 200th anniversary of Mendel's birth, presenting a timely opportunity to revisit the events in his life that led him to undertake his seminal research. We review existing ideas on how Mendel made his discoveries, before presenting more recent evidence.

The Augustinian friar Gregor (Johann) Mendel (1822–1884) is the founder of the science of genetics. His crossbreeding experiments with peas, reported in two lectures in the spring of 1865 and published in 1866, are so instructive that they are still used to introduce genetics. Textbooks simply state that Mendel conducted his pea crosses to study the rules of inheritance. However, this obscures how little we really know about Mendel. The impetus to study the rules of inheritance was less evident in Mendel's time than it seems today. Uniquely among nineteenth-century scientists, Mendel conducted a coordinated set of quantitative experiments and concluded that inheritance was nonblending. Even after its publication, his work was not understood and remained neglected for 35 years.

The publications by Hugo De Vries, Carl Correns and Erich von Tschermak in 1900 mark the beginning of the broad appreciation of Mendel's work. Mendel had been dead for 16 years, and his notes no longer existed. What remained was the article *Experiments on Plant Hybrids*[1] (*Versuche über Pflanzen-Hybriden*, hereafter the 1866 paper), which was published at the end of 1866 in the *Proceedings of the Natural Science Society* (NSS) of Brünn, the capital of Moravia (now Brno, Czech Republic; Supplementary Note). Searches for other documentation shortly after the rediscovery yielded only a few letters that Mendel had written after 1866 to Carl Nägeli (1817–1891), professor of botany in Munich, together with fragments of Nägeli's responses published by Correns in 1905 (ref. [2]). Until recently, no historical documents were known concerning his pea experiments from the ten years when these were conducted.

Why was Mendel the only one in the nineteenth century to perform a quantitative analysis of traits in crossbreeding experiments? R. A. Fisher[3] was the first to question critically how Mendel had arrived at his discoveries, asking: What did Mendel discover? How did he discover it? What did he think he had discovered? These questions were not seriously addressed until after 1965, and in the absence of historical sources, most answers made assumptions about Mendel's interests. In the past few years, we have found a number of historical sources about Mendel and his immediate environment from digitized newspapers, proceedings and yearbooks, including two short newspaper articles about Mendel's work dating from 1861, while Mendel's experiments were ongoing[4,5]. This Perspective gives a new picture of how Mendel arrived at his discoveries. We review existing ideas on how Mendel made his discoveries before presenting the recent evidence. However, we begin with what Mendel himself writes about it in the 1866 paper.

What does the 1866 paper reveal about Mendel's aims and working method?

Mendel's introduction is brief. It starts by mentioning the recurrence of the same hybrid form when ornamental plants are crossed, that is, that the F_1 from two inbreds is uniform. Mendel gives this observation as his impetus and explains that he aimed to 'follow up the development of the hybrids in their progeny', an expression that recalls Franz Unger, his botany professor in Vienna, who saw the parental plant and its offspring as a unity connected by descent[6] (Unger, 1852). According to Mendel, so far no study had classified all offspring of hybrids and established their numerical relationships. Thus, Mendel set out to study the composition of the F_2 and possibly later generations.

Then follows a whole section on the selection of the experimental plants, where Mendel states: "it cannot be immaterial which plant species were chosen as support for the experiments and in which way these experiments were carried out … On account of their particular flower structure, particular attention was paid to the Leguminosae right from the start. Experiments which were performed on several members of this family led to the result that the genus *Pisum* sufficiently meets the posited requirements"[1,7].

This argument suggests that Mendel deliberately chose *Pisum* as a model species: "A total of 34 more or less distinct pea sorts were procured from several seed shops and subjected to a two-year test… Twenty-two from these were selected for fertilization…"[1,7]. Mendel described the inheritance of seven pairs of differentiating constant characters, writing as though his account should be taken literally, with phrases such as "The next task" and "The results of the previously described experiments led to further experiments." Five traits affected the plant itself; flower color, form of the ripe pods, color of the unripe pods, position of the flowers and the difference in the length of the stem. The remaining two affected the seed: color (yellow versus green) and shape (round versus wrinkled). Mendel

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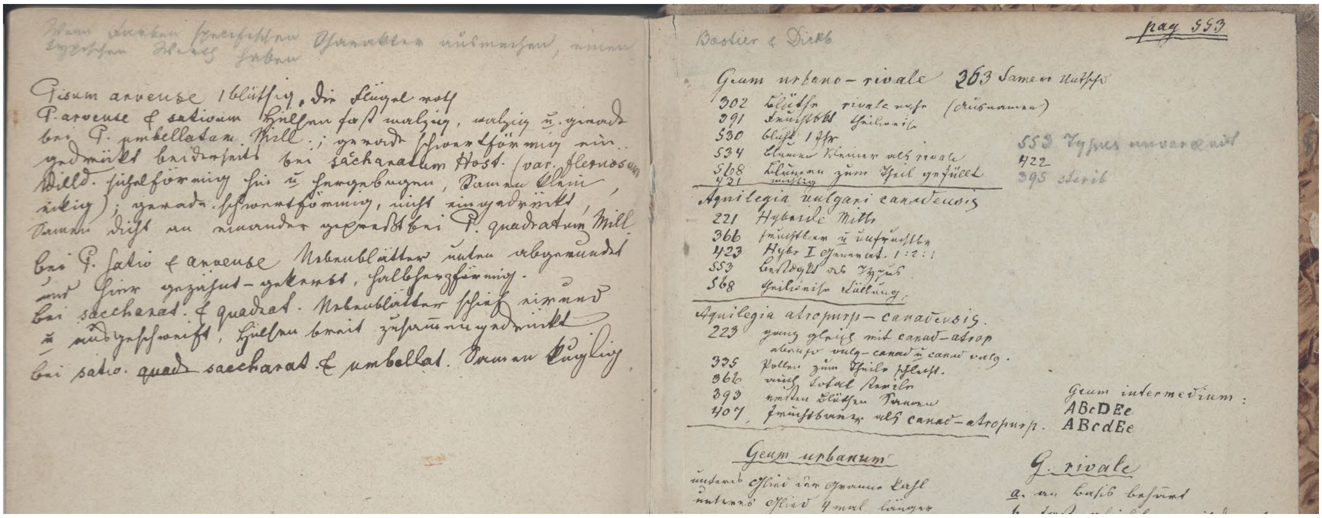
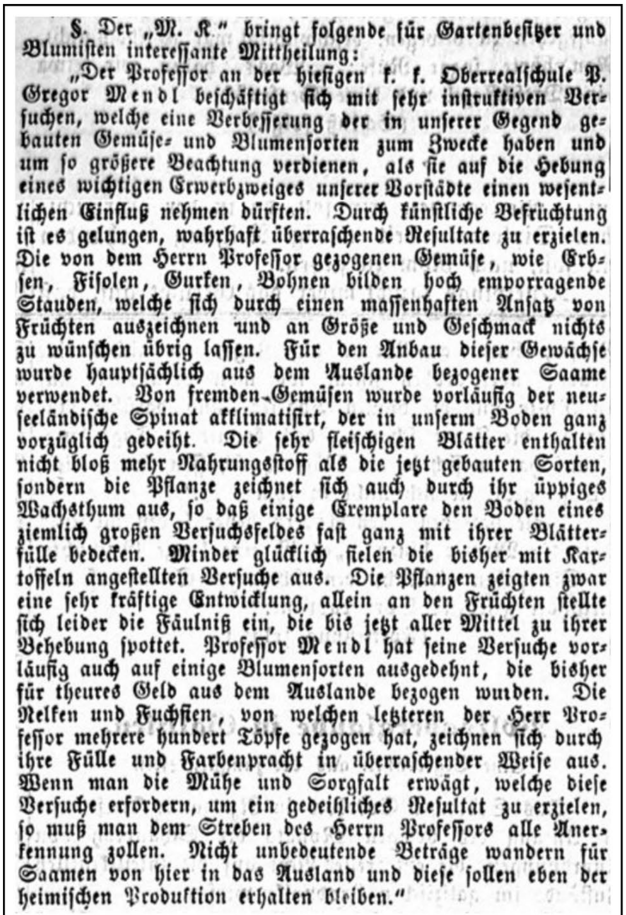


Fig. 1 | Notes on the end page of Mendel's copy of Gärtner's book[10]. On the left page, in ink, Mendel wrote the characteristics of the pea species *Pisum arvense*, *P. sativum*, *P. umbellatum*, *P. saccharatum* and *P. quadratum*. These notes are not from Gärtner's book. On the contrary, the notes in ink on the right-hand page refer to page numbers in Gärtner's book, describing characteristics of hybrids between *Geum* and *Aquilegia* species, crosses that Mendel repeated after 1863. (Courtesy of Mendel Museum of Masaryk University, Augustinian Abbey in Old Brno).



§. Der „M. K." bringt folgende für Gartenbesitzer und Blumisten interessante Mittheilung:

„Der Professor an der hiesigen k. k. Oberrealschule P. Gregor Mendl beschäftigt sich mit sehr instruktiven Versuchen, welche eine Verbesserung der in unserer Gegend gebauten Gemüse- und Blumensorten zum Zwecke haben und um so größere Beachtung verdienen, als sie auf die Hebung eines wichtigen Erwerbzweiges unserer Vorstädte einen wesentlichen Einfluß nehmen dürften. Durch künstliche Befruchtung ist es gelungen, wahrhaft überraschende Resultate zu erzielen. Die von dem Herrn Professor gezogenen Gemüse, wie Erbsen, Fisolen, Gurken, Bohnen bilden hoch emporragende Stauden, welche sich durch einen massenhaften Ansatz von Früchten auszeichnen und an Größe und Geschmack nichts zu wünschen übrig lassen. Für den Anbau dieser Gewächse wurde hauptsächlich aus dem Auslande bezogener Saame verwendet. Von fremden Gemüsen wurde vorläufig der neuseeländische Spinat akklimatisirt, der in unserm Boden ganz vorzüglich gedeiht. Die sehr fleischigen Blätter enthalten nicht bloß mehr Nahrungsstoff als die jetzt gebauten Sorten, sondern die Pflanze zeichnet sich auch durch ihr üppiges Wachsthum aus, so daß einige Exemplare den Boden eines ziemlich großen Versuchsfeldes fast ganz mit ihrer Blätterfülle bedecken. Minder glücklich fielen die bisher mit Kartoffeln angestellten Versuche aus. Die Pflanzen zeigten zwar eine sehr kräftige Entwicklung, allein an den Früchten stellte sich leider die Fäulniß ein, die bis jetzt aller Mittel zu ihrer Behebung spottet. Professor Mendl hat seine Versuche vorläufig auch auf einige Blumensorten ausgedehnt, die bisher für theures Geld aus dem Auslande bezogen wurden. Die Nelken und Fuchsien, von welchen letzteren der Herr Professor mehrere hundert Töpfe gezogen hat, zeichnen sich durch ihre Fülle und Farbenpracht in überraschender Weise aus. Wenn man die Mühe und Sorgfalt erwägt, welche diese Versuche erfordern, um ein gedeihliches Resultat zu erzielen, so muß man dem Streben des Herrn Professors alle Anerkennung zollen. Nicht unbedeutende Beträge wandern für Saamen von hier in das Ausland und diese sollen eben der heimischen Produktion erhalten bleiben."

§. The "M.K." brings the following for garden owners and florists interesting news:

"Father Gregor Mendl, professor at the local k.k. Oberrealschule is concerned with very instructive experiments, which are aimed at improving the vegetable and flower varieties cultivated in our region. They deserve more attention because they should exert a considerable influence on raising a vital economic activity in our suburbs. Through artificial fertilization, astonishing results could be achieved. The vegetables grown by the professor, such as peas, fisols, cucumbers, and beans, are high towering bushes distinguished by a massive production of fruit that leaves nothing to be desired in size and taste. For the cultivation of these plants, mainly seeds from abroad were used. Of the foreign vegetables so far, the New Zealand spinach, which thrives in our soil, was acclimatized. The very fleshy leaves contain more nutritious substances than the now cultivated varieties. The plant is also characterized by luxuriant growth so that some specimens cover their rather large experimental plot almost entirely with their leaves. Until now, the experiments carried out with potatoes were less successful. The plants showed a very vigorous development, but the fruits started to rot, and so far, no remedy has been found. Professor Mendl has temporarily extended his experiments [also] to several species of flowers, which up to now have had to be imported at great expense from abroad. The carnations and fuchsias, of which the professor grew several hundred pots, stand out by their abundance and colorful splendor in a surprising way. Considering the efforts and diligence that these experiments require in order to obtain a successful result, one must give all recognition to the professor's endeavor. The substantial amounts of money that are currently spent on buying seed abroad can better be preserved for domestic production."

Fig. 2 | A short article in *Neuigkeiten* on 26 July 1861, and our English translation. This describes Mendel's ongoing plant breeding and horticultural work. (Courtesy of Digitales Forum Mittel und Osteuropa.)

followed plant traits through four or five generations and seed traits through six generations. This suggests that he started with seed traits, as discussed below. The experiments and results are presented, very clearly, in the 1866 paper.

Gärtner's book as the background to Mendel's work

In his second letter to Nägeli, Mendel wrote that the first trials with the 34 pea varieties took place in the spring of 1854. The plans for the experiments must therefore have been drawn up around

1853, and the two most recent biographies do not explain clearly why these plans were initiated. Orel writes: "Returning from the University of Vienna in 1853, Mendel seems to have recognized a set of phenomena in plant hybridization and plant breeding for which no satisfactory explanation existed"[8]. Klein and Klein phrase it as follows: "Mendel spent the two years in Vienna preparing himself for experiments that he might have been formulating vaguely in his mind"[9].

Mendel scholars generally assume that Mendel was inspired by Carl Friedrich Gärtner's great book on plant hybridization, *Experiments and Observations on Hybridization in the Plant Kingdom*[10], published in 1849, of which Mendel owned a copy. In this book, Gärtner (1772–1850) describes some 10,000 experimental plant crosses, mainly interspecific. Mendel's copy is full of annotations, which testify to his careful study. On the end pages, Mendel made a 12-line note of the characteristics of five different *Pisum* species, of which he used four species names in the 1866 paper (Fig. 1). According to the historian of science R.C. Olby, "These notes are important because they show Mendel at work, hunting for clearly-marked character differences between the various forms of peas. Hence it is reasonable to assume that these notes were written prior to the purchase of the 34 varieties of peas for testing in 1854" (ref. [11]). However, the annotations are not dated, so Mendel could have made the notes when he was preparing his 1865 lectures; the naturalists of the NSS were more interested in natural Linnean species than in pea varieties. It would be interesting to find out from which source these notes were copied. Although Gärtner was clearly an important influence, a role in initiating Mendel's experiment remains speculative.

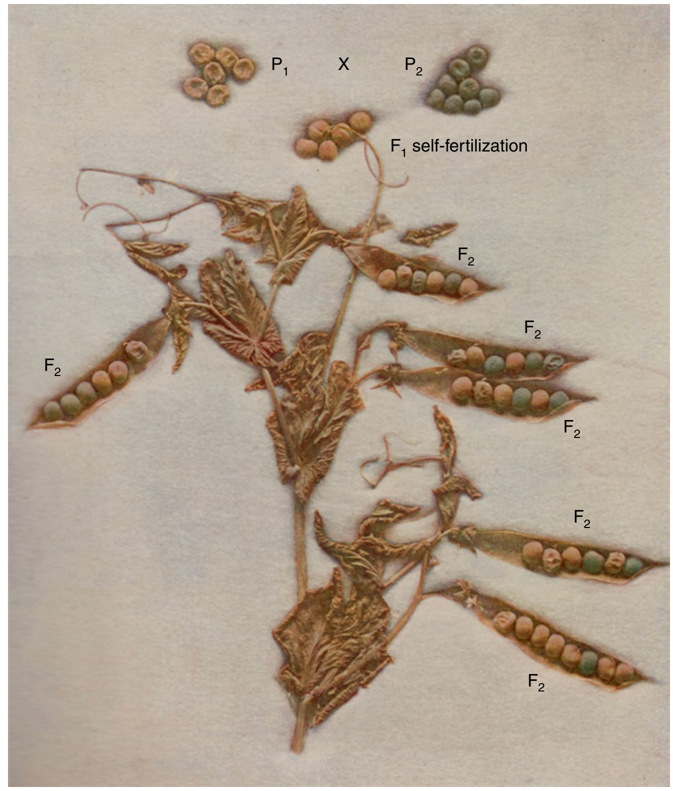


Fig. 3 | Segregation of F_2 seed traits in the pods of a selfed F_1 pea plant. Seed color (yellow versus green) and shape (round versus wrinkled) segregate independently. Mendel may have noticed the 3:1 segregation ratios in both seed traits and their independence in crosses made for plant improvement (modified from ref. [48]).

What did Mendel want to discover, and how did he discover it?

According to Fisher[12], it was clear that a theorist thinking about the inheritance of traits in the nineteenth century would have arrived at a theory of Mendelian transmission. It was not until the approaching centenary of Mendel's lectures in 1965 that Fisher's explicit questions[3] were taken seriously. De Beer[13], Dunn[14] and Mayr[15] agreed with Fisher that Mendel had a comprehensive theory from the start. According to De Beer[13], "this was the sign of genius". Mayr[15] wrote: "The entire planning of [Mendel's] experiments, the explanation of his method, as well as the choice of his material permit no other interpretation than that already early in his work Mendel had a well-formed theory in his mind and that his experiments actually consisted in the testing of his theory".

In 1979, Olby published an article under the provocative title *Mendel no Mendelian?*[16]. He argued that the rediscoverers and later geneticists read more into Mendel's work than Mendel originally intended. According to Olby, there was a historically inappropriate glorification of Mendel among geneticists. He found the lack of the word 'inheritance' (*Erblichkeit*) in the title remarkable and pointed to notation that he thought did not fit in with genetic theory. Olby suggested that Mendel was not primarily interested in the inheritance of traits but in whether new species could arise from F_1 hybrids. This 'species multiplication' question had also occupied previous plant hybridizers, notably Joseph Gottlieb Kölreuter (1733–1806) and Gärtner. Both are often mentioned in the discussion of the 1866 paper, where Mendel shows that his findings can explain many of their results. However, as Gasking[17] had already pointed out, *Pisum* would have been a very poor choice for the study of species multiplication, because that question related to interspecific hybrids between wild plant species and not to intraspecific hybrids between crop varieties.

Olby's paper[16] incited a renewed debate on Fisher's questions between roughly 1980 and 2000. Olby's interpretation gathered much support from other historians of science but was largely ignored by geneticists. The geneticists Orel and Hartl[18] thought that although Mendel did not start from a fully developed theory, his starting point was the hypothesis of nonblending inheritance. During his research, he formulated new hypotheses based on experiments that were then tested with follow-up experiments (hypothetical-deductive method). They proposed a linear progression of nine consecutive hypotheses and experiments, as described in the 1866 paper (Supplementary Fig. 1). By contrast, the geneticists Corcos and Monaghan[19] shared Olby's view, concluding that it was not theory driven but entirely empirical inductive. After 2000, the debate died down, and more recent publications by geneticists[20,21] or historians of science[22,23] do not provide any new answers to Fisher's questions.

Problematically, the above perceptions are based on interpretations of Mendel's text, whereas scientific papers, which are primarily intended to communicate new findings do not necessarily reflect the actual chronology. Although the 1866 paper describes the research in a logical, linear way, in reality a research path is rarely linear and often convoluted. Research plans and goals can be modified as the research is carried out, or reformulated when the paper is written; there was a period of 12 years between the first tentative planning of the pea experiment and the publication.

Contrary to the above conjectures about fundamental scientific questions, Mendel's two earlier short publications[24,25] indicate that he had an applied focus. Both articles relate to pest insects, the first a radish moth[24] and the second a pea weevil (*Bruchus pisorum*)[25]. In the first publication, Mendel warns that the pest could spread from radish to economically more important seed cabbages. In the second, Mendel fears that pea, which he calls "one of the most nutritious crops", is in danger of being lost due to the devastating effects

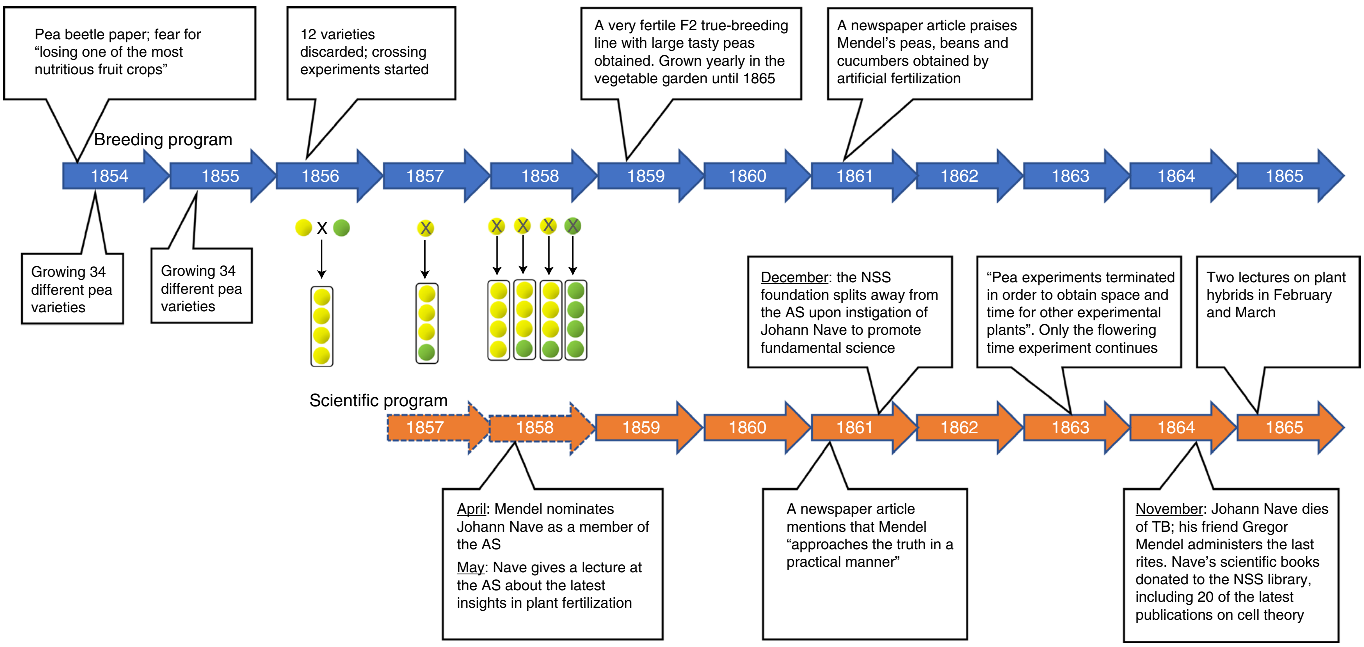


Fig. 4 | The reconstructed timeline of Mendel's applied breeding and scientific breeding programs in peas. The applied breeding program is indicated by blue filled arrows; the scientific breeding program by orange filled arrows, with the notable events indicated. The applied and pure scientific breeding program are connected by observations that Mendel could have made from crosses in the applied breeding program that by chance varied in seed traits — in this case, seed color. The cross between the peas indicates cross breeding; the cross inside the peas indicates self-fertilization. The colors green and yellow are used to illustrate the types of segregation seen. The years 1857 and 1858 in the scientific breeding program are dashed because this programme could have started one year earlier or later. AS, Agricultural Society; NSS, Natural Science Society.

of the pea weevil. So far, these minor publications by Mendel have not received much attention.

Mendel's genetics came from a vegetable breeding program

An article in the Brünn newspaper *Neuigkeiten* in July 1861 praises the peas, beans and cucumbers that Mendel had obtained through artificial crossings for their yield and taste[4] (Fig. 2). In response, the *Brünner Zeitung*, another local newspaper, wrote a few days later that the economic importance of Mendel's work was exaggerated, but that they "honored every endeavor to approach the truth in a practical manner" (Supplementary Note). The phrase 'approaching the truth' refers to solving a scientific problem. In other words, Mendel had both a vegetable breeding and a purely scientific research program. The breeding program is consistent with the applied character of the two brief articles from 1853 and 1854.

By 1846, Mendel had a background in artificial hybridization of fruit trees, having attended courses in pomiculture at the Philosophical Academy in Brünn[8]. In his second letter to Nägeli, Mendel wrote that in 1859 he had "obtained a very fertile descendent with large, tasty seeds" and which was grown annually in the vegetable garden of the monastery for the next six years. Eichling[26], who visited Mendel in 1878, also wrote that Mendel made crosses for breeding purposes.

The monastery had a tradition of animal breeding and horticulture related to the monastery estates. The Abbot, Cyrill (Franz) Napp (1792–1867), played a prominent role in the discussions about sheep breeding in the Agricultural Society in the 1830s. In those days, however, the development of better plant varieties in Central Europe was restricted to ornamentals, but since the early 1850s, there was a growing interest in breeding of vegetable crop varieties. For example, in January 1852, the journal of the Bavarian Horticultural Society published a paper '*Crossing, Especially Garden Vegetables*', which called for breeding locally adapted late-ripening pea varieties, because the peas sold on the market were early varieties bred by the Dutch and the English (Supplementary Note). It is possible that this article could have inspired Mendel's vegetable breeding program, as Napp and Mendel both read the journal. Napp had a subscription, and Mendel made a correcting annotation to an erroneous reference to the journal in Gärtner (1849). Interesting articles in this journal were regularly discussed at meetings of the Agricultural Society.

The monastery possessed fruit tree and grapevine nurseries, with many different accessions to evaluate their performance under the Brünn environmental conditions. Mendel will have understood the importance of diversity for plant breeding, explaining the large number (34) of original pea varieties tested for a two-year period.

This suggests that pure scientific research emerged from plant breeding activities. Beadle[27] (1965) suggested that Mendel might not have needed an a priori theory but could have recognized the 3:1 segregation ratios in the seed traits (color and shape) in his crossing program. These are characteristics of the cotyledons, so they represent one generation later than the plant that bears the seed. This phenomenon had already been described for pea by Andrew Knight in 1799 (ref. [28]). The pea geneticist Wellensiek[29] also wondered whether this phenomenon had not sparked Mendel's experiments.

Seed shape and color can be observed on the mother plant as dried seeds. This needs no planning and can be done in the winter when there is little gardening work. Self-fertilization in peas occurs automatically and is so effective, as Mendel noticed, that special actions to avoid cross-pollination were not needed.

The segregation of seed color and seed shape in the F_2 is very obvious, and we can estimate how likely it is that Mendel would have observed this, by chance alone, among crosses between his 22 selected varieties. Mendel obtained seed mainly from abroad, most likely Germany[30]. Based on the occurrence of variation in seed color and shape in pea varieties on the German market between 1852 and 1855, it appears that about 60% of all possible crosses would vary for at least one seed trait and a quarter for two seed traits simultaneously.

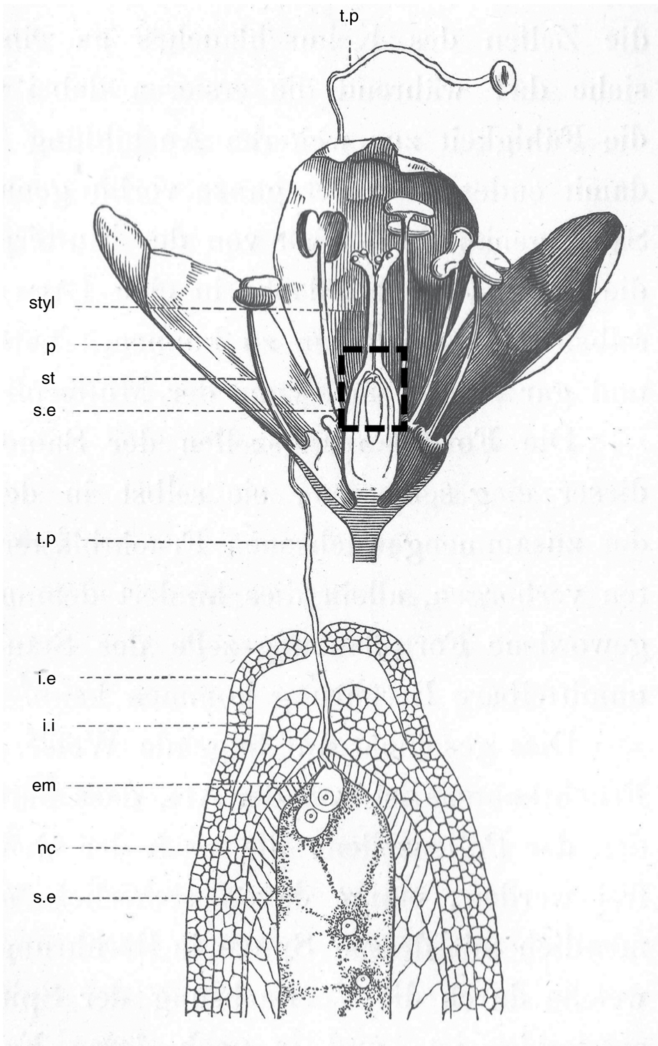


Fig. 5 | Fertilization of the egg cell by the pollen tube, according to Unger[6] in 1852. Unger disagreed with Schleiden's theory that the embryo was formed by the extremity of the pollen tube. By contrast, Unger assumed that the egg cell started to develop into an embryo after the pollen tube made contact with the egg cell and "dynamic transfusion of purified substances" (p108) had occurred. In those days, the differentiation between the two synergids and the egg cell was not known, nor was the fusion of nuclei or the process of double fertilization. Shading is as in the original; in the flower, this indicates its three-dimensional structure, and stippling within the central cell indicates cytoplasmic density. Symbols (originally in German): em, egg cell; i.e, outer integument; i.i, inner integument; nc, nucellusstyl, style; p, perianth; s.e, embryo sac; st, anther (filament); t.p, pollen tube; i.e, outer integument; i.i, inner integument; nc, nucellus.

Figure 3 shows a dried F_1 plant in which the parents (P) differed in seed color and shape. The F_2 seed phenotypes can be scored and counted on the F_1 plant. The ratios of yellow to green and round to wrinkled are 24:13 and 30:7, respectively, which does not deviate significantly from 3:1, but clearly, the seeds of more F_1 plants have to be counted to deduce the 3:1 ratio. However, a single cross between two P plants would have easily generated 30 F_1 seeds, which after self-fertilization could have generated easily 900 F_2 seeds, in which the 3:1 ratio would be evident.

After Knight[28], seed color and shape in peas were investigated by other researchers, such as Seton[31], Goss[32], Gärtner[10], Laxton[33,34] and Giltay[35], but none of these researchers recognized the regular segregation ratios. Mendel had studied plant physiology and physics at the University of Vienna and made meteorological observations in Brünn. He was familiar with experiments, measurements and combinational theory and aware of phenomena like variation and stochasticity. He seems to have had a great interest in numbers and was very meticulous. Orel[8] highlighted this in the context of the meteorological observations made by Mendel and his friend Pavel Olexík; according to Orel "compared to Olexík's somewhat disorganized data, Mendel's are outstanding for their clarity". His "prepared mind"[36] may have enabled him to recognize the recurrent 3:1 segregation patterns in the seed traits of the plant breeding crosses for not only the two characters but also each within a category of the other. By the autumn of 1856, Mendel could have noticed the dominance of the yellow and round seeds in F_1. In the next year, he could have noticed the 3:1 segregation of F_2, and in 1858, he could have noticed the resolving of the 3:1 ratio into the 1:2:1 ratio (Fig. 4). From 1857 or 1858 onward, Mendel may have started with "finding the truth in a practical way". After discovering the remarkable inheritance ratios in the seed traits, Mendel could have, out of curiosity, been interested in whether the plant traits had similar segregation patterns. These plant traits required careful planning and a lot more labor and garden space.

Mendel kept the 22 selected varieties for the entire study period and had sufficient variation to study the five plant and two seed traits[30]. For his large-scale research program, at least 24,000 plants over the entire research period[8], he must have had the consent of Abbot Napp. Although Napp was primarily interested in applied breeding, he understood the importance of pure scientific research. In a discussion about sheep breeding in 1837, Napp had asked, "What is inherited, and how?"[8].

Mendel analyzed the ratios of pairs of differentiating traits, singly and in combination, among the progenies and designed an entirely new notation. Furthermore, Mendel extrapolated expectations to an unlimited number of paired alternative traits and a large number of generations. Finally he sought a biological explanation for the algebraic series, turning to the pollen and egg cells. However the cell theory itself was still incomplete, and his further proposal of differences in the composition of reproductive cells took him into completely uncharted territory.

The explanation of the algebraic series: cell theory

In 1838 Schleiden and Schwann's seminal cell theory had stated that cells are a universal component of organisms[37,38]; however, the theory was marred by the supposition that cells emerge de novo during development. It was not until the 1850s that cells were generally recognized as permanent structures, in the words of Rudolph Virchow, "every cell is derived from a pre-existing cell".

The mechanism of cell division was not considered; it was assumed that the cell and its contents, including the nucleus, split in half when growth had gone beyond a certain limit. It was not until the 1880s that mitosis provided a mechanism for accurate division and accurate distribution of daughter nuclei to daughter cells.

At the outset of Mendel's studies, in 1854, there was also uncertainty about the cellular mechanism of fertilization in flowering plants. Most researchers, including Mendel's botany professor Unger, believed that the embryo developed from the egg cell after contact between the egg cell and the pollen tube (Fig. 5). However, Schleiden's hypothesis that the embryo developed from the end of the pollen tube was still alive[39]. The second theory was definitively abandoned when Radlkofer[40] convinced his professor, Schleiden, that the first theory was correct. Fertilization was now thought to involve the diffusion of a fertilizing fluid from the pollen tube into the egg cell. The fusion of a pollen tube nucleus with the egg cell nucleus was discovered almost 30 years later[41]. When male and female nuclei were seen fusing after fertilization, Weismann drew attention to the need for a reduction division, and meiosis was eventually figured out[38].

Fig. 6 | The board of the Brünn Natural Science Society in 1862. The photograph was taken in the first year since its establishment[43] (Courtesy of the University of Illinois at Urbana-Champaign Archives, image 0009941). This is the only image where Johann Nave is unambiguously identified (number 4, white arrow).

In May 1858, Mendel's friend Johann Nave (1831–1864) gave a lecture '*On the development and reproduction in algae*' for the Scientific Section of the Agricultural Society, the forerunner of the NSS. Nave discussed the latest findings and, in particular, "Pringsheim's brilliant discovery of sexuality" in algae and the fertilization of the egg cell by "probably one" invading male gamete[42]. Nave also emphasized the analogy of the fertilization process between algae, which were easy to study by microscopy, and flowering plants, which are challenging to study because the egg cell is deeply embedded in surrounding tissues. A month earlier, Mendel had nominated Nave as a new member of the Scientific Section[5]. Nave, a civil servant in the Moravian department of finance, had studied botany in Vienna at the same time as Mendel, and the two men likely knew each other well from 1851 onward[43]. Nave was the main instigator of the branching of the NSS from the Agricultural Society at the end of 1861 to promote pure scientific research[8,44] (Fig. 6).

In November 1864, a few months before Mendel held his lectures, Nave died of tuberculosis. He was 33 years of age; according to Iltis[43], his friend Mendel administered the last rites. Nave's scientific books were donated to the NSS[5], and these included multiple papers from leading plant cell biologists, such as Pringsheim, Radlkofer, Braun, Hofmeister, De Bary and Cohn. Nave and Mendel presumably shared their knowledge of cell theory and discussed Mendel's crossbreeding experiments. This could explain the long footnote in the 1866 paper in which Mendel argues that his results reject Schleiden's fertilization theory. Although we know even less about Nave than we do about Mendel, it is clear that Nave stimulated Mendel's interest in the fundamental side of his plant breeding work. Mendel proposed that, for a given character, hybrids make equal numbers of two types of reproductive cell that contain only one type of 'element'. Only one pollen grain randomly fertilizes one egg cell, resulting in the "formation of a cell serving as the foundation of the hybrid" (now zygote), thus combining two elements in a way that defines the character state of the new organism. In self-fertilization and backcrossing experiments, Mendel tested and confirmed the predictions of this proposal.

When his proposal is viewed in the light of what was known of cells in the mid-nineteenth century, Mendel was decades ahead of his time. His prescient deduction of paired elements that break up their association and separate into different daughter cells is what makes him the founder of genetics.

The 1865 lectures and the 1866 paper

In February and March 1865, Mendel presented his work to the NSS. In the 1866 paper, based on these lectures, he logically presented his pea experiments as if they were planned from the beginning to solve the problem of the inheritance of traits. On New Year's Eve 1866, Mendel sent a reprint to Nägeli in Munich, accompanied by a long covering letter[45]. Nägeli was an obvious choice as he had recently written several articles on hybridization, in which he had pointed out that this 'sheds some light on reproduction, more specifically on how traits from the parents are transmitted to the progeny'[46]. This was precisely what Mendel's paper is about. Mendel wrote that he intended to repeat his experiments with other species. Nägeli replied that he thought that was an excellent idea but was convinced that Mendel would also come across other forms of inheritance[4].

In his second letter to Nägeli (April 1867), Mendel wrote that his work was not easy to reconcile with contemporary scientific knowledge and that he had received mixed reactions to his lectures. In this letter, Mendel called himself an empirical worker and described his experiments as 'empirical' (Mendel's emphasis). But further on, he wrote: "If then I extend this combination of simple series to any number of differences between the two parental plants, I have indeed entered the rational domain. This seems permissible, however, because I have proved by previous experiments that the development of any two differentiating characteristics proceeds independently of any other differences". That he probably did not work from a prior theory but proceeded empirically does not preclude his understanding the theoretical (genetic) implications, as evidenced in the Discussion of the 1866 paper and by his many annotations to Darwin's pangenesis theory in his copy of Darwin's *Variation of Animals and Plants under Domestication*[47]. Although Nägeli understood that Mendel's study was about inheritance, as a firm believer in blending inheritance[15], he failed to see the importance of Mendel's discoveries. It took almost 35 years before the significance of Mendel's work was understood.

Conclusion

Most previous reconstructions of Mendel's method of work lacked historical sources from his research period. From the two recently discovered newspaper articles published in 1861, we know that Mendel had both an applied vegetable breeding program and a basic scientific research program. It is plausible that observations of the segregation of seed traits triggered Mendel's prepared mind and led to a purely scientific research program (Fig. 4) during which Mendel discovered the rules of inheritance. This answers Fisher's first two questions: what did Mendel discover, and how did he discover it? However, Mendel went further: he also came up with a cell biological explanation for these rules. Although it was already known that Johann Nave was a close friend of Mendel, a few fragments of historical text now show that they also shared scientific interests. Nave was aware of the latest cell biological insights in plant reproduction, as evidenced by his books and by the lecture he gave on reproduction in algae, and Mendel had nominated him as a new member of the Scientific Section immediately before the lecture. Fisher's third question was: "And what did he think he had discovered?" The cell biological explanation in the 1866 paper answers this question: Mendel discovered the mechanism of how traits were transmitted from parents to offspring. The celebration of the 200th anniversary of Mendel's birth is an appropriate moment to reflect on Fisher's three questions and to understand how the events in Mendel's life, such as his relationships with Napp and Nave, influenced his research path.

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Competing interests

The authors declare no competing interests.

Additional information

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