The Full Breadth of Mendel's Genetics

Peter J. van Dijk*[,1] and T. H. Noel Ellis
*Keygene N.V., 6708 PW Wageningen, The Netherlands

ABSTRACT Gregor Mendel's "Experiments on Plant Hybrids" (1865/1866), published 150 years ago, is without doubt one of the most brilliant works in biology. Curiously, Mendel's later studies on *Hieracium* (hawkweed) are usually seen as a frustrating failure, because it is assumed that they were intended to confirm the segregation ratios he found in *Pisum*. Had this been his intention, such a confirmation would have failed, since, unknown to Mendel, *Hieracium* species mostly reproduce by means of clonal seeds (apomixis). Here we show that this assumption arises from a misunderstanding that could be explained by a missing page in Mendel's first letter to Carl Nägeli. Mendel's writings clearly indicate his interest in "constant hybrids," hybrids which do not segregate, and which were "essentially different" from "variable hybrids" such as in *Pisum*. After the *Pisum* studies, Mendel worked mainly on *Hieracium* for 7 years where he found constant hybrids and some great surprises. He also continued to explore variable hybrids; both variable and constant hybrids were of interest to Mendel with respect to inheritance and to species evolution. Mendel considered that their similarities and differences might provide deep insights and that their differing behaviors were "individual manifestations of a higher more fundamental law."

KEYWORDS Gregor Mendel; genetics; *Hieracium*; constant hybrids; apomixis

> The publication of Mendel's letters to Carl Nägeli by Correns in 1905 was a service to genetics which seems not to have been fully appreciated by most of those who have since written accounts of Mendel's life and work (Mann Lesley 1927).

"THESE [seedlings] have rooted well, and should flower next year. Whether they will *retain the characteristics of the hybrid, or whether they will show variations*, will be determined by next year's observations" (our emphasis). These lines about the progeny of his first artificial hawkweed (*Hieracium*) hybrid were written by Gregor Mendel on November 6, 1867, in a letter to Carl Nägeli, professor of botany at Munich (Letter III, Stern and Sherwood 1966, p. 73). They indicate that from the beginning of his experiments with *Hieracium*, Mendel expected that constant-hybrid offspring may well occur. Mendel ends the letter with: "I look forward to the coming summer with impatience since the progeny of several fertile hybrids will bloom for the first time. They should be very numerous and I only hope that they repay the yearning [*Sehnsucht*!] with which I await them with much information concerning their life histories." (quoted in Mann Lesley 1927). These are not the words of a frustrated man.

Gregor Mendel's fame is based on his *Pisum* (pea) crossing experiments that were published 150 years ago. His only subsequent publication on plants is a preliminary communication on artificial *Hieracium* hybrids (Mendel 1870). The usual supposition about Mendel's *Hieracium* experiments, which were carried out over 7 years, is that they were intended to verify the results he obtained with his *Pisum* experiments (Nogler 2006; Bicknell *et al.* 2016). Hawkweeds are related to dandelions and, like them, often reproduce by a peculiar and rare breeding system called apomixis. The seeds of apomictic plants are produced clonally and are thus genetically identical to the mother plant. This is achieved by the avoidance of meiosis and the parthenogenetic development of the egg cell. In apomictic hawkweeds, most seeds produced are apomictic, but some may develop after cross-fertilization (for more information on apomixis see Supplemental Material, Section 1, File S1). Hawkweeds are hermaphrodites and produce haploid pollen, so they can act as pollen donors in crosses. Thus the prevalence of apomixis in *Hieracium* would have made it impossible for Mendel to replicate his *Pisum* findings in this genus. Apomixis was unknown in Mendel's time; indeed it

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[1]Corresponding author: Keygene N.V., Agro Business Park 90, 6708 PW Wageningen, The Netherlands. E-mail: peter.van-dijk@keygene.com

was many years after his death that the Danish botanist Carl Hansen Ostenfeld (1904) discovered apomixis in *Hieracium*. The usual interpretation of Mendel's *Hieracium* experiments then is that his work on this genus was a frustrating failure; we suggest this misinterprets Mendel's purpose.

In "Experiments on Plant Hybrids" Mendel (1866) gives an exemplary description of the formation of hybrids and the diversity among their offspring. Most of the work concerns *Pisum*, but he confirmed his findings in the genus *Phaseolus* (common bean). When self-fertilized, F_1 hybrids within these species produce variable progeny. Toward the end of this article, Mendel contrasts his results with the case where "We encounter an *essential difference* in those hybrids that remain constant in their progeny and propagate like pure strains." (Mendel 1866; Stern and Sherwood 1966, p. 41. Mendel used "*reinen Arten*", so "pure species" would be a better translation than "pure strains"). When self-fertilized, F_1 hybrids of these other species breed true: their progeny do not vary. Mendel designated these two distinct classes as variable hybrids (Stern and Sherwood 1966, p. 42) and constant hybrids (Stern and Sherwood 1966, p. 41), respectively[1].

Historians of science (*e.g.*, Olby 1979, 1985, 1997; Callender 1988; Müller-Wille and Orel 2007) have argued that Mendel's main motivation for the *Hieracium* (and *Pisum*) experiments was his interest in hybridization and speciation rather than the inheritance of traits, and they proposed that Mendel stands in the tradition of earlier plant hybridizers like Joseph Gottlieb Kölreuter (1733–1806) and Carl Friedrich Gärtner (1772–1850). Recently this "Mendel as a nongeneticist" view has received considerable attention in popular science books (*e.g.*, Endersby 2007; Numbers and Kampourakis 2015) and education journals (*e.g.*, Peterson and Kampourakis 2015). Although we agree with these historians of science that Mendel selected *Hieracium* to study constant hybrids, we do not think that speciation by hybridization was his only or main motivation. Mendel was also interested in reproductive cells and segregation *vs.* nonsegregation in the successive generations of progeny from a hybrid (*i.e.*, inheritance). Mendel had multiple reasons for selecting *Hieracium* as an object for experimental crossing and the importance of these reasons may have shifted over the years of his study. The opportunity to come into contact with Carl Nägeli, the person most likely to value his *Pisum* findings, would have been additionally attractive.

In addition to his articles, there is a series of 10 letters that record part of his communication with Nägeli. Mendel's notebooks were destroyed after his death, so we must rely on these few documents to form an understanding of his scientific thoughts and motives. From these documents we know that after *Pisum* and *Phaseolus*, Mendel investigated many other species from the genera *Aquilegia*, *Antirrhinum*, *Calceolaria*, *Campanula*, *Cheiranthus*, *Cirsium*, *Dianthus*, *Geum*, *Hieracium*, *Ipomoea*, *Linaria*, *Lychnis*, *Matthiola*, *Mirabilis*, *Tropaeoleum*, *Verbascum*, *Zea*, and more were planned (Letter II). By far, the largest number of these experiments was conducted in *Hieracium* (Cetl 1971). In this article, we argue that a (mis)reading of Mendel's first letter to Nägeli has led to the incorrect idea that Mendel's *Hieracium* experiments were intended to verify his *Pisum* findings.

Correspondence Between Mendel and Nägeli

Carl Nägeli

Carl Nägeli was one of the most important botanists of the 19th century (Junker 2011). His research interests were on natural hybrids, an area where he was recognized as the leading researcher; and *Hieracium*, where again he was the leading authority. Nägeli was the person who could best see the relevance of Mendel's pea results and Mendel also wanted his advice as a *Hieracium* expert (Section 2, File S1).

Mendel's letters to Nägeli

Carl Correns (1900), one of the three "rediscoverers" of Mendel's work, clearly acknowledged Mendel's contribution. Correns was a student of Nägeli's and (after Nägeli's death) was married to his niece. From Mendel's *Hieracium* note and from conversations with Nägeli in the past, Correns knew that Mendel and Nägeli had collaborated closely, so he asked the Nägeli family whether they had any letters from Mendel. Correns published the 10 letters that were discovered (Correns 1905), labeling them with the Roman numerals I to X (Table S1). In 1925, Correns wrote in a letter to Herbert Fuller Roberts that these "first came to light through an accident in 1904" (Roberts 1929, p. 338). Fragments of some of Nägeli's letters to Mendel were found in the monastery in Brno (German: Brünn) and were published by Iltis (1924). The records of their correspondence are thus incomplete. Correns also published some of the keyword summaries that Nägeli had made of his letters to Mendel. The only in-depth analysis of this scientific correspondence we are aware of is Hoppe (1971), in which she discusses it especially in relation to Nägeli's work, but not in relation to Mendel's *Hieracium* results.

Mendel's Hieracium work has been misunderstood as a frustrating failure to replicate his Pisum work

The traditional interpretation of Mendel's motivation for studying *Hieracium* is expressed by Hartl and Orel (1992): Mendel's "studies of *Hieracium* and other species were undertaken to verify, with other plants, the result obtained with *Pisum*," and "the experiments with *Hieracium*, as recounted in the letters to Nägeli, were one long chronicle of failure and frustration." In 2006 the journal *GENETICS* marked the 140-year jubilee of Mendel's *Pisum* article. Crow and Dove (in Nogler 2006) commented negatively about Mendel's *Hieracium* work: "Here, on this anniversary, instead of extolling his success, we present a scholarly account [Nogler 2006]

[1] By "constant hybrids," Mendel means true-breeding *Aa* hybrids. In modern genetic terms these are heterozygotes that remain heterozygotes in subsequent generations. This must be clearly distinguished from true-breeding new trait combinations in variable hybrids (*e.g.*, *AAbb*, *aaBB*).

of Mendel's frustrating attempts to repeat his findings in another species, which, unbeknownst to him, reproduced apomictically." Nogler (2006) starts with: "Mendel hoped that the highly polymorphic genus *Hieracium* would be particularly promising for verifying the laws of inheritance that he had discovered while working on *Pisum*." According to Mawer (2006, p. 167), Mendel's *Hieracium* article is "of no more than curiosity value." Modern articles on the genetics of apomixis often refer to Mendel's frustrating experiences with *Hieracium e.g.*, Koltunow *et al.* (2011): "Apomixis in hawkweed: Mendel's experimental nemesis." At the Mendel Museum at the Monastery in Brno, Mendel's *Pisum* experiments, meteorological studies, and beekeeping activities can be seen, but not his *Hieracium* work, perhaps due to their associated negativity.

It has been argued that Nägeli was instrumental in Mendel's selection of *Hieracium* (as discussed in Nogler 2006), but from Letter I it is clear that Mendel had already made crosses in *Hieracium*, *Geum*, and *Cirsium* in the summer of 1866, so the parental species must have been collected at least one season earlier. Mendel had thus embarked on his *Hieracium* experiments by 1865 at the latest. Therefore Nägeli cannot have pushed Mendel to work on *Hieracium* as is sometimes suggested (Iltis 1924; Mayr 1982); his choice of *Hieracium* predates his communication with Nägeli and Nägeli's expertise with *Hieracium* was a likely motivation for Mendel initiating this correspondence.

Contradiction in Mendel's first letter to Nägeli

Mendel's first letter to Nägeli, written on New Year's Eve 1866, was a covering letter for the reprint of his *Pisum* article. In the letter (Letter I) Mendel clarified his *Pisum* studies, mentioned his future research plans, and asked if he could rely on Nägeli for the determination of difficult *Hieracium* and *Cirsium* (thistle) species, on which Nägeli was an expert. To understand why it is widely believed that Mendel chose *Hieracium* to test the *Pisum* findings, paragraphs four and five are crucial, so these are copied below with the paragraph numbers added in parentheses:

> (4) In order to determine the agreement, if any, with *Pisum*, a study of those forms which occur in the first generation[2] should be sufficient. If, for two differentiating characters, the same ratios and developmental series which exist in *Pisum* can be found, the whole matter would be decided. Isolation during the flowering period should not present many difficulties in most cases, since we are dealing only with few plants; those plants whose flowers are being fertilized and a few hybrids which have been selected for seed production. Those hybrids which are collected in the wild can be used as secondary evidence only, as long as their origin is not unequivocally known.
>
> (5) *Hieracium*, *Cirsium*, and *Geum* I have selected for further experiments. In the first two, manipulation in artificial pollination is very difficult and unreliable because of the small size and peculiar structure of the flowers . . . (Stern and Sherwood 1966, p. 57–58).

From this it has been concluded that Mendel chose the genera *Hieracium*, *Cirsium*, and *Geum* to test the *Pisum* findings. William Bateson (1909, p. 246) wrote: "This genus [*Hieracium*] being one of the most strikingly polymorphic, he chose it after his discovery regarding the inheritance of peas, as the subject of *further* [our emphasis] research. We may surmise that he expected to find in it illustrations of the new principles." Bateson's use of the word "further" suggests that he came to this conclusion based on the two paragraphs mentioned above[3]. This interpretation has become the common belief of geneticists. For example, Iltis (1924, translation of Iltis 1966) wrote: "For Mendel the behavior of the hawkweeds remained an enigma, and his experiments upon these composites shattered the hopes he had entertained of finding confirmation of the principles of inheritance worked out by him in the case of *Pisum*, and thus establishing these principles as universally valid general laws. . . . He had certainly been lucky in his original choice of *Pisum* as the object of his experiments. But fate played him an ill turn when he went on to hybridize the hawkweeds; and when, with peasant doggedness, urged on by Nägeli, he persevered so long in his researches upon this unsuitable genus." (pp. 174–175). Ernst Mayr (1982, p. 723) stated: "Instead, he [Nägeli] encouraged Mendel to test his theory of inheritance in the hawkweeds (*Hieracium*), a genus in which, as we now know, parthenogenesis [apomixis] is common, leading to results that are incompatible with Mendel's theory. In short, as one historian has put it, 'Mendel's connection with Nägeli was totally disastrous.' "

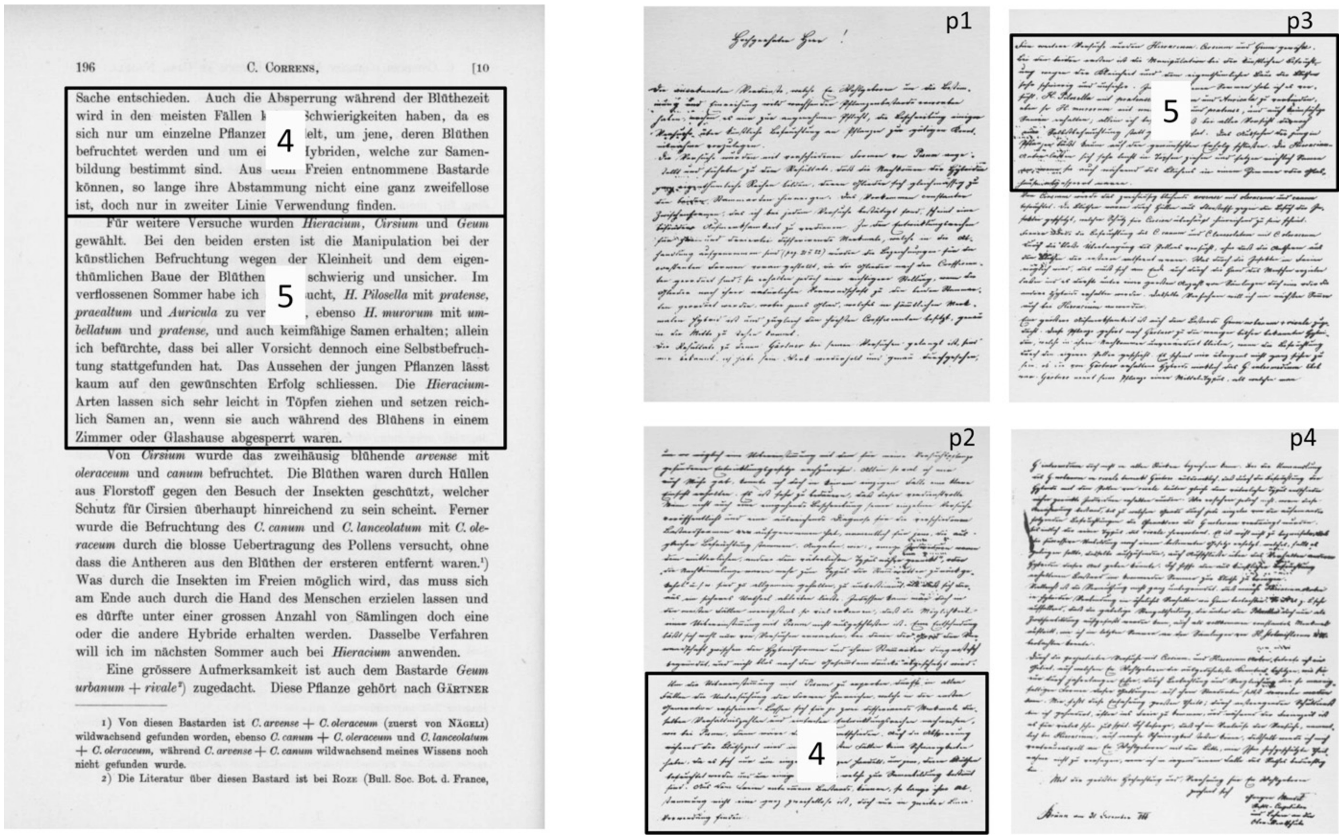
Was it ill fate, as Iltis suggested? One of the very few who has interpreted this differently is the historian L.A. Callender (1988), who wrote: "Mendel, on the other hand, and before he was certain that he had obtained a single *Hieracium* hybrid surmised exactly the opposite [of Bateson's proposal that Mendel expected to verify his *Pisum* results]" and cites a later paragraph from Letter I: "The plant *Geum urbanum* + *rivale* deserves special attention. This plant, according to Gärtner (1849), belongs to the few so far[4] known hybrids which produce nonvariable progeny as long as they remain self-pollinated." And subsequently: "The surmise that some species of *Hieracium*, if hybridized, would behave in a fashion similar to *Geum*, is perhaps not without foundation. It is, for instance, very striking that the bifurcation of the stem, which must be considered an intermediate[5] trait

[2] For Mendel, the "first generation" referred to the first generation derived from the hybrid—today this would be called the F_2.

[3] The wording "further experiments" ("*weitere Versuche*") is somewhat awkward or ambiguous in this context. Since Mendel gave a detailed protocol as to how the *Pisum* findings could be tested in the previous paragraph, his having written "*Hieracium*, *Cirsium*, and *Geum* I have selected for further experiments" rather than ". . . such experiments" might suggest he was referring to a different kind of experiment.

[4] "*bisher*" (meaning "so far") was not translated by Piternick and Piternick (1950). However, it indicates that Mendel expected that more constant hybrids would be found, which is logical if he already suspected *Hieracium* hybrids to be constant.

[5] Piternick and Piternick (1950) use "transitional," but we think "intermediate" is a better translation of "*Zwischenbildung*."



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Sache entschieden. Auch die Absperrung während der Blüthezeit wird in den meisten Fällen [illegible] Schwierigkeiten haben, da es sich nur um einzelne Pflanzen [illegible] elt, um jene, deren Blüthen befruchtet werden und um ei[illegible] Hybriden, welche zur Samenbildung bestimmt sind. Aus [illegible] Freien entnommene Bastarde können, so lange ihre Abstammung nicht eine ganz zweifellose ist, doch nur in zweiter Linie Verwendung finden.

Für weitere Versuche wurden *Hieracium*, *Cirsium* und *Geum* gewählt. Bei den beiden ersten ist die Manipulation bei der künstlichen Befruchtung wegen der Kleinheit und dem eigenthümlichen Baue der Blüthen [illegible] schwierig und unsicher. Im verflossenen Sommer habe ich [illegible] ucht, *H. Pilosella* mit *pratense*, *praealtum* und *Auricula* zu ver[illegible], ebenso *H. murorum* mit *umbellatum* und *pratense*, und auch keimfähige Samen erhalten; allein ich befürchte, dass bei aller Vorsicht dennoch eine Selbstbefruchtung stattgefunden hat. Das Aussehen der jungen Pflanzen lässt kaum auf den gewünschten Erfolg schliessen. Die *Hieracium*-Arten lassen sich sehr leicht in Töpfen ziehen und setzen reichlich Samen an, wenn sie auch während des Blühens in einem Zimmer oder Glashause abgesperrt waren.

Von *Cirsium* wurde das zweihäusig blühende *arvense* mit *oleraceum* und *canum* befruchtet. Die Blüthen waren durch Hüllen aus Florstoff gegen den Besuch der Insekten geschützt, welcher Schutz für Cirsien überhaupt hinreichend zu sein scheint. Ferner wurde die Befruchtung des *C. canum* und *C. lanceolatum* mit *C. oleraceum* durch die blosse Uebertragung des Pollens versucht, ohne dass die Antheren aus den Blüthen der ersteren entfernt waren.[1]) Was durch die Insekten im Freien möglich wird, das muss sich am Ende auch durch die Hand des Menschen erzielen lassen und es dürfte unter einer grossen Anzahl von Sämlingen doch eine oder die andere Hybride erhalten werden. Dasselbe Verfahren will ich im nächsten Sommer auch bei *Hieracium* anwenden.

Eine grössere Aufmerksamkeit ist auch dem Bastarde *Geum urbanum* + *rivale*[2]) zugedacht. Diese Pflanze gehört nach GÄRTNER

1) Von diesen Bastarden ist *C. arvense* + *C. oleraceum* (zuerst von NÄGELI) wildwachsend gefunden worden, ebenso *C. canum* + *C. oleraceum* und *C. lanceolatum* + *C. oleraceum*, während *C. arvense* + *C. canum* wildwachsend meines Wissens noch nicht gefunden wurde.

2) Die Literatur über diesen Bastard ist bei ROZE (Bull. Soc. Bot. d. France,

Figure 1 Letter I (December 31, 1866). A comparison between Correns' publication (left) and Mendel's original handwriting. In Correns' publication, paragraph four and five are on the same page, but in Mendel's original letter, paragraph four is at the end of page two and paragraph five is at the top of page three. The handwriting shows that an entire page could be missing. In Correns' publication a missing page would not be noticed, unless the flow of the content was illogical. Courtesy of the Mendelianum Archives of the Moravian Museum.

among the *Piloselloids*, may appear as a perfectly constant character, as I was able to observe last summer on seedlings of *H. stoloniflorum* W. K.[6]"

This suggests that Mendel expected that *Hieracium* species could be constant hybrids (see also Orel 1998). Why would Mendel select a genus in which he expected to find constant hybrids, to validate the segregation of variable hybrids? This would be irrational. The eminent Mendel-expert Franz Weiling (1970) expressed it very carefully: "From Mendel's first letter to Nägeli one gets the impression that he, with his crosses in *Hieracium*, *Cirsium* as well as *Geum*-species, wanted to test the generalities which he had found in *Pisum*" [*"Aus dem 1. Brief Mendels an Nägeli* (*31. Dezember 1866*) *gewinnt man den Eindruck*, *daß er mit seinen Kreuzungen bei Hieracium*-, *Cirsium*-, *sowie Geum-Arten die bei Pisum gewonnenen Gesetzmäßigkeiten prüfen wollte*." (p. 99)]. The wording "one gets the impression" suggests Weiling was aware of the contradiction in the letter. As far as we know, this major contradiction has never been discussed. Here we suggest that the present paragraphs four and five in Mendel's first letter were originally not linked, but were separated by one or more lost pages. The two paragraphs are not logically connected and we propose that Mendel did not select these species to test the *Pisum* findings.

Could a missing page explain the contradictions in Mendel's first letter?

Because of the contradiction in Letter I, we wondered whether a part of the letter could be missing. Witte (1971), who had photocopies of all the handwritings, compared the original text with the transcript of Correns and found only a few small typographical errors. Therefore an error in the transcription can be ruled out.

We have examined a facsimile of Letter I (December 31, 1866) published by Jelinek (1965) because, despite our efforts, the original could not be traced. In Figure 1 it can be seen that paragraph four ends at the bottom of page two and paragraph five begins at the top of page three. Since the page break does not result in a broken sentence, a missing sheet would go unnoticed, especially in a transcript, where the relationships between paragraphs and pages are different from the original handwriting. In the facsimile, parts of the words written on page two can be seen mirror-wise on page one and vice versa; the same for pages three and four (Figure S4). This means that the sheets of paper are written

[6] Nägeli (1845) mentioned a forked stem as a characteristic of *Pilosella* hybrids.

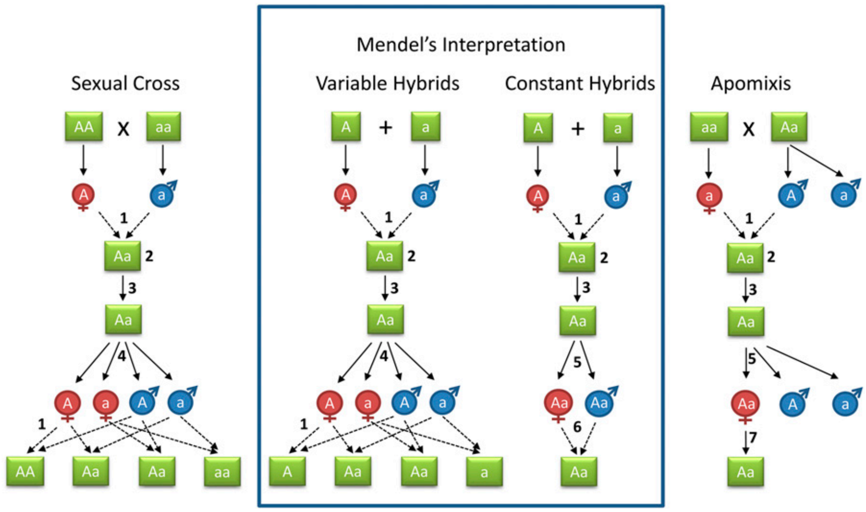

Figure 2 Mendel's 1865/1866 views of inheritance in constant and variable hybrids. Mendel's interpretation (boxed) of the behavior of determining elements is compared to our current understanding. "Sexual Cross" refers to the specific case of a cross between two homozygotes followed by self-fertilization, and should be compared to "Variable Hybrids" which is classically described in his 1866 article. Mendel's interpretation of "Constant Hybrids" should be compared to "Apomixis." Note that Aa has a different meaning in our current understanding from that in Mendel's scheme; Mendel did not know about meiosis and the distinction between diploid and haploid. Numbers indicate: (1) The union of germinal cells from the female and male (egg and pollen). (2) The primordial cell (zygote): differences between antagonistic elements are mediated (in the mediating cell). (3) Vegetative period, the balance/mediation established in the primordial cell continues. (4) In variable hybrids, at the formation of the reproductive cells (gametes) the antagonistic elements are separated and represent "all constant forms which result from the combination of the characters united in fertilization." The "arrangement between the conflicting elements is only temporary," that is, no germinal cells carry the union of conflicting factors. (5) In constant hybrids, at the formation of the reproductive cells (gametes) the antagonistic elements are not separated. The essential difference in the development of constant hybrids is that the union of the factors is permanent. (6) In constant hybrids, the union of germinal cells of identical constitution is proposed (*i.e.*, no parthenogenesis). (7) For comparison, the genetic transmission of apomixis is shown: the unreduced egg cell develops into an embryo by parthenogenesis. Note that in the case of apomixis a breeding system is inherited, which will fix the segregating genetic background of both parents; producing many different apomictic lineages. For simplicity only diploids are shown, but apomixis is often associated with polyploidy. Because apomicts have a simplex dominant genotype (*Aaaa*) this convenience is used. The type of apomixis shown here is typical for the subgenus *Archieracium*, which Mendel also used in crosses.

on both sides and that one or more sheets could be missing (*i.e.*, two or an even number of pages). We examined copies of Mendel's handwritten pages to see whether there were any structural clues that would enable us to discount the possibility that one or more pages is missing. From a statistical consideration of the location of page and paragraph breaks in Mendel's letters, we concluded that paragraphs usually end in the middle of pages, so the location of a paragraph end at the bottom of a page is consistent with this being deliberate. The paragraph need not have ended there: alignment of the text using the ink marks that can be seen through the paper from one side to the other shows that there was adequate room to continue writing on this piece of paper (Section 3, File S1 and Figure S4). If paragraph five begins at the top of the page, as it does according to Correns' transcript, then a missing page is required to end with a paragraph break. The analysis which leads us to conclude that this is not improbable is set out in Section 3, File S1.

Mendel's Research Interests Were Broad

Mendel's hypothesis about the germ cells of constant vs. variable hybrids

In the concluding remarks of the *Pisum* article, Mendel stressed the importance of the "essential difference" between variable and constant hybrids; between hybrids like those of pea, which produced variable offspring; and hybrids that produced constant offspring. He also mentioned that "For the history of the evolution of plants this circumstance is of special importance, since constant hybrids acquire the status of *new species*" (Mendel's emphasis, Stern and Sherwood 1966, p. 41). By "new species" Mendel meant being true breeding and having morphological distinctness. Clearly speciation was one of the interests that Mendel had in constant hybrids.

Mendel was interested in the mechanisms of inheritance and the composition of reproductive cells. So far, this aspect of Mendel's work has not received much attention. According to the report of Mendel's second lecture on March 8, 1865 in the Brünn newspaper *Neuigkeiten*, "he spoke about cell formation, fertilization and seed production in general and in the case of hybrids in particular . . ." (Olby 1985). In his *Pisum* article, Mendel developed a hypothesis about the segregation of antagonistic elements among reproductive cells and their reassortment among progeny, based on the different types of progenies of variable and constant hybrids (Figure 2). This was >20 years before meiosis was discovered and understood by the contributions of van Beneden, Hertwig, Weismann, and others (Mayr 1982).

Mendel (1866) proposed that in variable hybrids that were derived from parents that differed, both the antagonistic elements were temporarily accommodated during the vegetative stage, and separated during the formation of the reproductive cells (egg cells and pollen). In contrast, in constant hybrids, Mendel proposed a permanent mediation. "This attempt to relate the important difference in the development of hybrids as to permanent or temporary association of differing cell elements can, of course, be of value only as a hypothesis which, for lack of well-substantiated data, still leaves some latitude." (Stern and Sherwood 1966, p. 43).

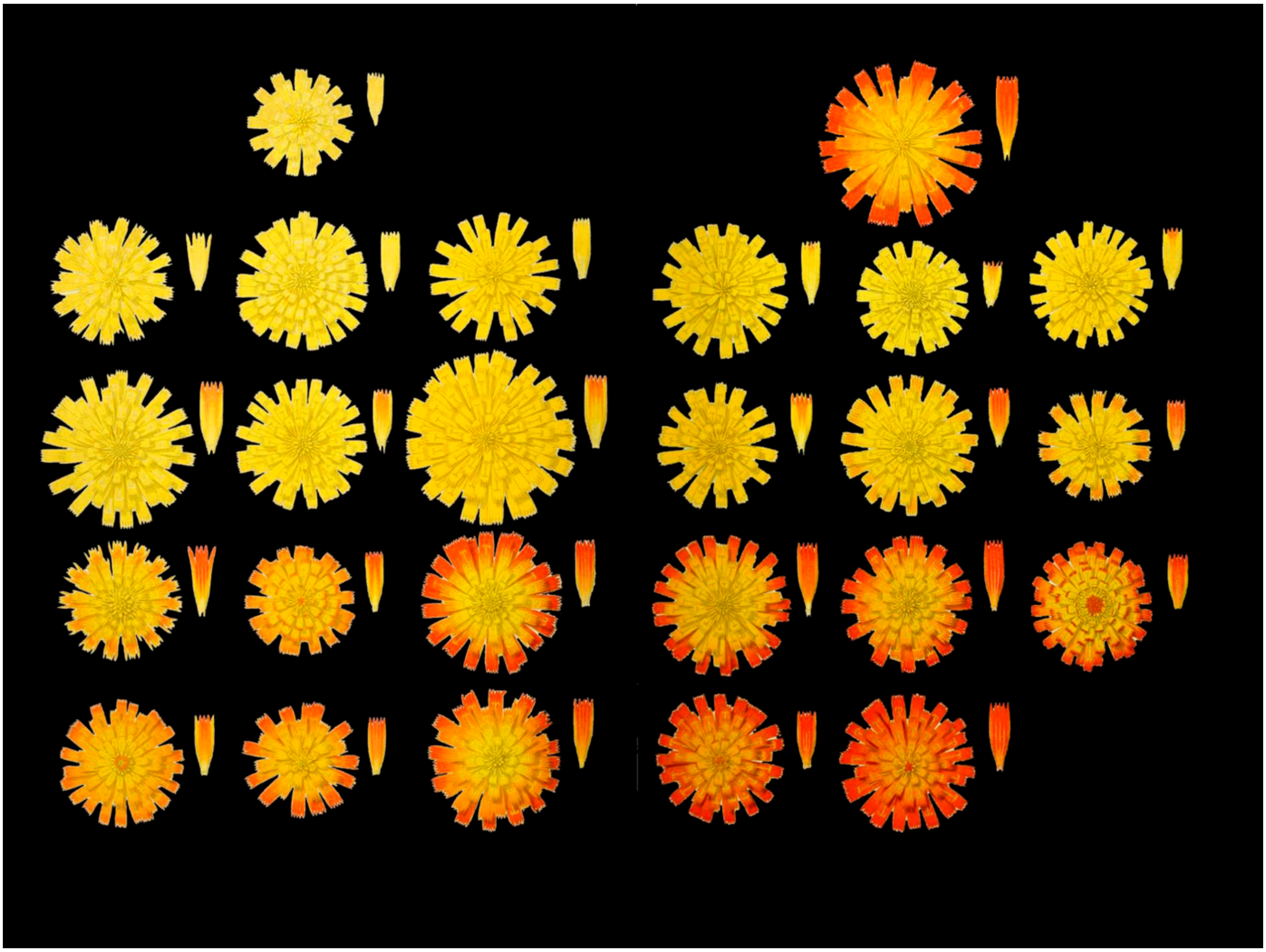

Figure 3 Variation in inflorescence color and size in *Hieracium* hybrids. Ostenfeld (1910) illustrated 23 *H. auricula* × *aurantiacum* hybrids that he obtained. Mendel obtained 84 flowering hybrids from the same cross. The parental species are shown at the top; *H. auricula* left, with a yellow small inflorescence; and *H. aurantiacum* right, with a larger orange inflorescence. Next to the inflorescence a single floret is shown. The original image is from the Biodiversity Heritage Library. Digitized by the Mertz Library, New York Botanical Garden (http://www.biodiversitylibrary.org).

Constant hybrids, such as *Hieracium*, could provide such well-substantiated data; so, after having studied the variable *Pisum* hybrids, it was logical that Mendel would have gone on to study constant hybrids, as presaged by his comments in the *Pisum* article. Moreover, Mendel may not have been satisfied with Gärtner as an "eminent observer" as he wrote in the *Pisum* article, since in Letter I (Stern and Sherwood 1966, p. 57) to Nägeli he criticized Gärtner's observations with respect to variable hybrids ("it is very regrettable that this worthy man did not publish a detailed description of his individual experiments"). Taken together, these considerations would have provided the impetus for Mendel to investigate constant hybrids himself.

Mendel's interest in Hieracium, Cirsium, and Geum

As he neared the completion of his *Pisum* experiments, Mendel had started looking for species for new crossing experiments. In 1864 he had made crosses between *Verbascum* and *Campanula* species and some of his artificial hybrids were shown at the June 14, 1865 meeting of the Natural Science Society (Naturforschender Verein) of Brünn. The *Verbascum* hybrids, however, were completely sterile (Letter III, Stern and Sherwood 1966, p. 77). The timing shows that Mendel's interest in variable hybrids continued while he was also studying constant hybrids.

Why did Mendel select *Hieracium*, *Geum*, and *Cirsium*? Mendel mentioned in Letter I that the artificial hybrid Gärtner had made between *Geum urbanum* and *Geum rivale* was one of the few hybrids known so far that produced constant progeny plants. Both parental species showed discrete alternative states of traits, which had been a methodological requirement for Mendel's study of variable hybrids. Moreover, the taxon *G. intermedium* was found in nature, which could be the constant hybrid between *G. urbanum* and *G. rivale*. The last page of Mendel's personal copy of Gärtner's (1849) *Versuche und Beobachtungen über die Bastarderzeugung im Pflanzenreich* (Experiments and Observations on Hybridization in the Plant Kingdom) contains many notes on *Geum*, and two interesting designations of multigene genotypes of *G. intermedium*: *ABcDEe* and *ABcdEe* (Olby 1985). In these, the heterozygote *Ee* would be constant and would not segregate.

Mendel was an active member of the Natural Science Society where he gave the two 1865 lectures about his *Pisum* experiments. In 1869, he was elected as vice president of the society and in June of that year he gave a lecture about his *Hieracium* hybridization experiments. Both *Hieracium* and *Cirsium* were genera in which intermediate and transitional forms between species were common (Nägeli 1866). Nägeli speculated that these might be constant hybrids or products of transmutation. Natural hybrids of *Hieracium* and *Cirsium* had already been discussed at several meetings of the society (see Section 4, File S1; Weiling 1969; Orel 1996). In general, the society was more interested in interspecific hybridization ("*Bastarde*"), than in intraspecific hybridization ("*Hybriden*"). Although Mendel saw only a graduated difference between varieties and species, he used "*Hybriden*" in the title of his *Pisum* article and "*Bastarde*" in the title of his *Hieracium* article; showing that he was well aware of the difference. His interest in species *vs.* varieties may have been influenced by the publication of Darwin's (1859) *Origin of Species* [Mendel had a copy of the second edition of the German translation of the *Origin of Species* (1863), see Fairbanks and Rytting 2001]. Mendel's selection of *Hieracium*, *Geum*, and *Cirsium* for study is therefore something to be expected in the intellectual atmosphere of Brünn at that time.

Hieracium

Two phases of Mendel's Hieracium research

Mendel's letters to Nägeli give a unique insight into his character, showing the evolution of his views, his openness and honesty, and his admission that some of his earlier expectations were incorrect. In some places the letters are witty and self-deprecating. Also striking, and contrary to what is often claimed, the correspondence between Mendel and Nägeli is friendly: Nägeli was not arrogant or controlling toward Mendel (Schwartz, 2008, and see salutations and signings Table S1). Although Mendel wrote about experiments with other species, in these letters the *Hieracium* experiments were by far the most important. *Geum* and *Cirsium* did not produce constant hybrids and soon Mendel concentrated on *Hieracium*. Mendel's letters and his provisional *Hieracium* communication makes it possible to reconstruct his *Hieracium* crossing experiments (see Table S2 for a timeline, and Table S3 in relation to Mendel's interspecific crosses). A large part of the correspondence is about the identification of *Hieracium* species and the exchanges of plant material, which, although they were important at the time, obscure the purpose of the investigation.

Based on the content of the correspondence, two research phases can be distinguished (see Section 5, File S1); in the first phase Mendel, with great effort, managed to produce some hybrids which indeed propagated constantly. The

Figure 4 Mendel's first constant *Hieracium* hybrid (*H. praealtum* × *H. flagellare*). Mendel observed no variation in three generations of this artificial hybrid. From herbarium "*Hieracia Naegeliana*" (Peter 1885). Courtesy of the Museum of Grenoble (*H. inops* n. hybr., GRM. Arv.-Touv. MHNGr. 191437180).

preliminary communication on *Hieracium* hybrids of June 9, 1869 can be seen to conclude this phase. In the second phase, Mendel tried to find a solution to the fact that, contrary to his expectation, he found multiple types of constant hybrid. Nogler (2006) gives a good biological description and analysis of Mendel's *Hieracium* experiments, although it is chronologically incorrect. He wrote that Mendel was first surprised by the many different F_1 hybrids and then by the fact that these hybrids were true breeding. This chronology reinforced the image of a frustrated Mendel. In reality, Mendel initially obtained very few hybrids. It must have been an exciting vindication that the first hybrid was true breeding, fulfilling his *Sehnsucht*. Only later, to his surprise, he found that there were many different but constant F_1 hybrids. In total, Mendel obtained hybrids in 21 interspecific combinations. Table S3 lists the most important interspecific hybrids and the variability of their offspring.

Mendel's most successful cross was that between *H. auricula* × *aurantiacum* from which he obtained 84 fertile

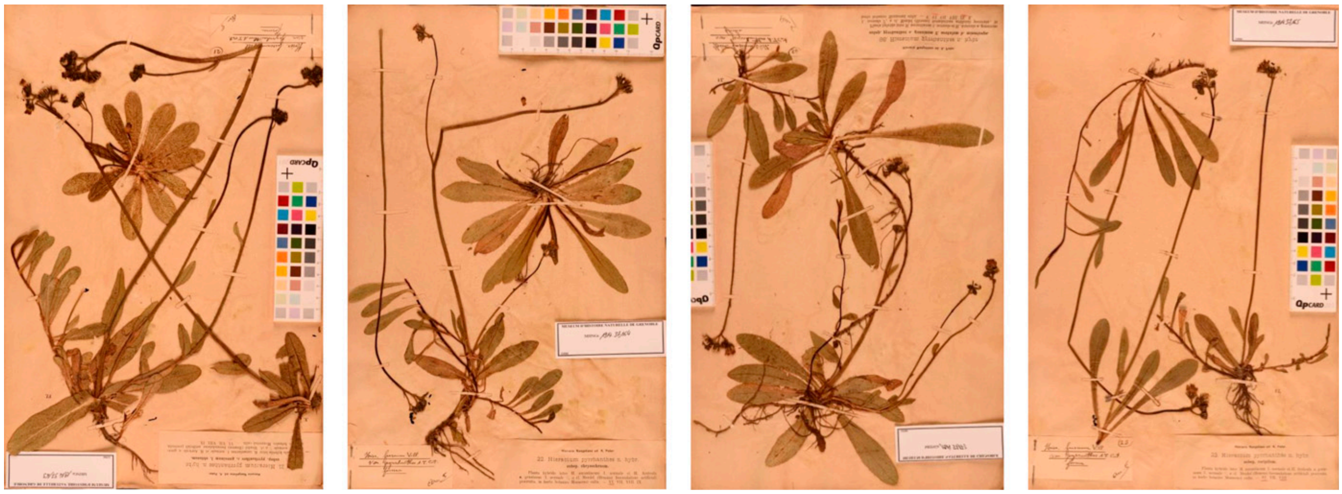

Figure 5 Four of Mendel's *H. auricula* × *aurantiacum* hybrids from the herbarium *Hieracia Naegeliana* (Peter 1885). All hybrids were fully fertile. In 1869, 1870, and 1873 Mendel sent material to Nägeli in Munich where they were cultivated in the common garden. Eight of these are described in Peter (1884). Courtesy of Museum de Grenoble (*H. pyrrhanthes* n. hybr. GRM. Arv.-Touv. MHNGr. 191437163, 191437164, 191437165, and 191437173).

hybrids (40 years later Ostenfeld repeated this cross, Figure 3). Remarkably, some of Mendel's hybrids still exist as dried specimens in the Herbarium of the Museum of Grenoble (Mendel's first constant hybrid, Figure 4; several *H. auricula* × *aurantiacum* hybrids, Figure 5). The hybrids that Mendel sent to Nägeli were grown in the experimental garden of the University of Munich. Nägeli's student and later colleague, Albert Peter, edited a collection of exsiccatae "*Hieracia Naegeliana*" (1885), consisting of 300 herbarium sheets of *Hieracium* subgenus *Pilosella* plants, which included 16 of Mendel's hybrids and 12 parental forms. Weiling (1969) located the "*Hieracia Naegeliana*" in 23 other herbaria in 11 countries throughout Europe, although these are often incomplete.

In the first phase of Mendel's *Hieracium* experiments, he demonstrated the constancy of the hybrid in subsequent generations. He could have hoped to use this, for example, to study dominance relationships among determinants for the differentiating characters. However, the observation of more than one type of constant hybrid was unexpected because the parents were also true breeding and only one F_1 hybrid type was anticipated. The second phase of the *Hieracium* experiments was therefore to determine what caused the multiplicity of F_1 types. Mendel knew from his *Pisum* methodology that he should collect very many F_1 hybrids to "determine the number of different forms in which the hybrid progeny appear . . . and ascertain their numerical interrelationships" (Stern and Sherwood 1966, p. 2). He was well aware of the amount of work this would require and in trying to improve the efficiency of the microscopic *Hieracium* crosses he nearly ruined his eyesight permanently. In his final letter to Nägeli, reflecting his realization that he did not have sufficient time to complete the necessary experiments, he wrote: "I am really unhappy about having to neglect my plants and my bees so completely. Since I have a little spare time at present, and since I do not know whether I shall have any next spring, I am sending you today some material from my last experiments in 1870 and 1871." (Letter X, Stern and Sherwood 1966, p. 97). All he could do was pass on his experimental material to someone who may have the opportunity to continue the work. If he was frustrated, it was not because his experiments had failed, but because they showed what needed to be done next and his duties as abbot prevented him from continuing this work.

Concluding Remarks

In this article we have argued that Mendel's *Hieracium* experiments, and the reasons underlying them, have been misunderstood for more than a century. We propose that this misunderstanding rests on the obscurity of the originals of his written letters and that a missing page (or pages) in his first letter to Nägeli would explain the common misreading of that letter. There is no proof that a page is missing; this could become a certainty only if it were found, which seems highly unlikely. Notwithstanding, the traditional view of Mendel's *Hieracium* experiments is not the only one possible. The interpretation we set out here is consistent with the whole of Mendel's known writings and does not involve the contradiction necessary for the traditional view. We therefore consider our interpretation the more likely. A missing page is not a necessary requirement for our interpretation, but its suggested location would help to explain the prolonged misinterpretation.

Although Mendel's letters to Nägeli mainly concern the *Hieracium* crosses, as would be expected because of their collaboration, the letters also contain important information about his variable hybrids and this has been neglected, perhaps because of the negative view of his *Hieracium* work. In July 1870 (Letter VIII), Mendel wrote: "Of the experiments of previous years, those dealing with *Matthiola annua* and *glabra*, *Zea*, and *Mirabilis* were concluded last year. Their hybrids behave exactly like those of *Pisum*. Darwin's statements concerning hybrids of the genera mentioned in *The Variation of Animals and Plants Under Domestication*, based on reports of others, need to be corrected in many respects." (Stern and Sherwood 1966, p. 93). This clearly shows that Mendel had found additional support for his understanding of inheritance in variable hybrids. In the same letter and in the next (Letter IX, September 27, 1870), Mendel also described repeated experiments to test whether a single pollen grain is sufficient to fertilize a single egg cell and an experiment with two pollen grains, each from a different flower color genotype, to investigate if an egg cell could be fertilized by two pollen grains simultaneously. These experiments are a rigorous test of the basic principles of his theory of inheritance in *Pisum*. Contrary to the historians' view, there can be no doubt that Mendel was above all a geneticist.

"My time is yet to come" are the famous prophetic words attributed to Mendel by his friend Gustav von Niessl. It is not widely known that Mendel said these words in the garden among his *Hieracium* and *Cirsium* plants. ("*aber ich hörte im Garten*, *an den Beeten seiner Hieracien und Cirsien von ihm die prophetischen Worte: 'Meine Zeit wird noch kommen*,' " Von Niessl 1905, p. 8). A more appropriate location is hard to imagine. Mendel's interest in hybrids (both inter- and intraspecific) was broadly based and encompassed the mechanism of their formation, inheritance in general, as well as the consequences of hybridization for evolution. He clearly recognized two contrasting types of hybrid (constant and variable) and he chose to study both. In one of his last letters to Nägeli, he commented: "Evidently we are here dealing only with individual phenomena, which are the manifestation of a higher, more fundamental, law" (Stern and Sherwood 1966, p. 90). With hindsight we see this to be entirely correct. Mendel's observations in *Hieracium* demonstrated the pollen transmission of apomixis that can now be understood in terms of the Mendelian genetics of the process of inheritance itself.

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Communicating editor: A. S. Wilkins

Fig. S1

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An Anton Kerner!

Hochgeehrter Herr!

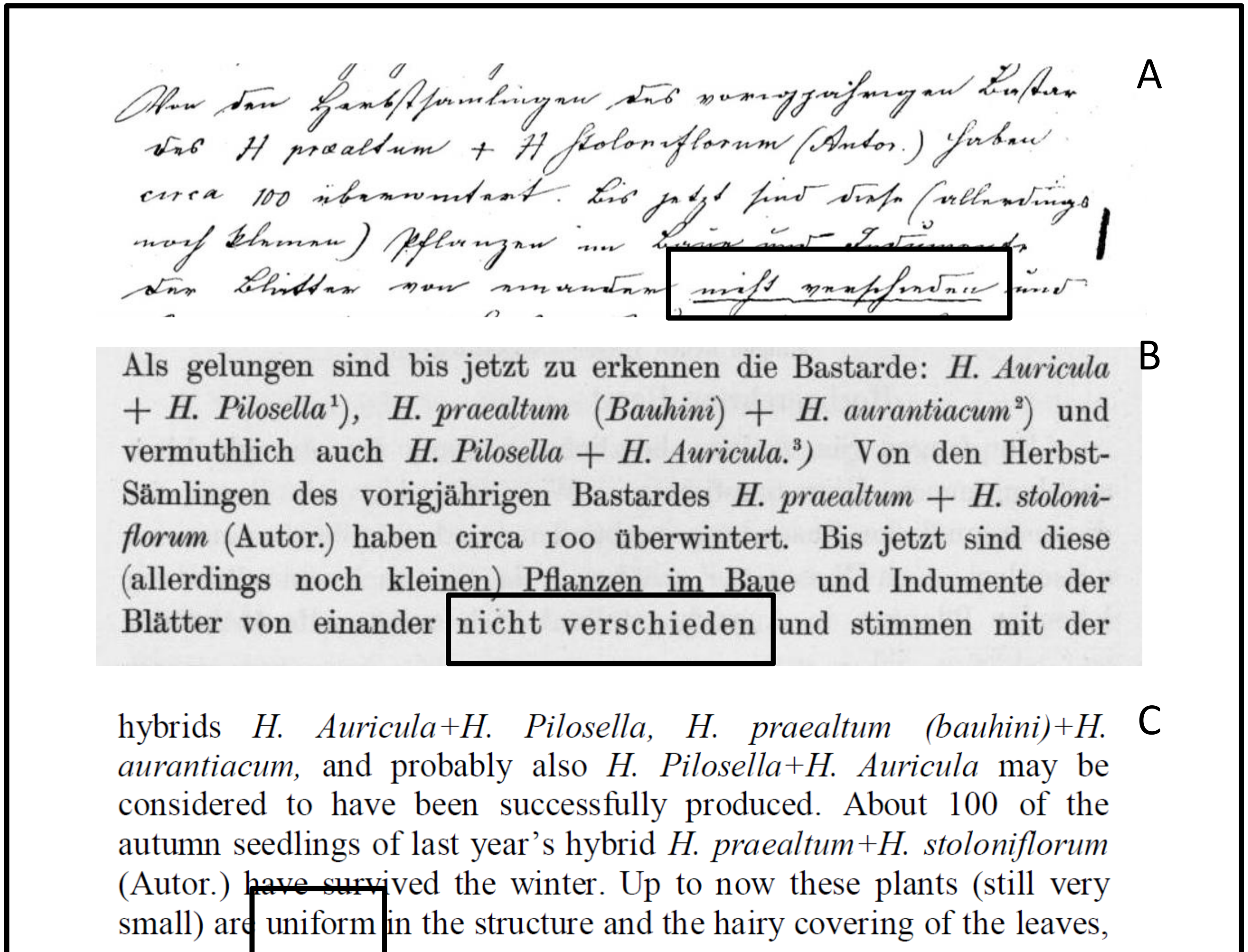
Die anerkannten Verdienste, welche Ew. Wohlgeboren um die Bestimmung und Einreihung wild wachsender Pflanzenbastarte erworben haben, machen es mir zur angenehmen Pflicht, die Beschreibung einiger Versuche über künstliche Befruchtung an Pflanzen zur gütigen Kenntnissnahme vorzulegen.

Mit dem Ausdrucke der grössten Hochachtung für Ew. Wohlgeboren zeichnet sich

Gregor Mendel
Stifts-Capitular
und Lehrer an der
Oberrealschule

Brünn am 1. Jänner 867

Fig. S2



Als gelungen sind bis jetzt zu erkennen die Bastarde: *H. Auricula* + *H. Pilosella*[1]), *H. praealtum* (*Bauhini*) + *H. aurantiacum*[2]) und vermuthlich auch *H. Pilosella* + *H. Auricula.*[3]) Von den Herbst-Sämlingen des vorigjährigen Bastardes *H. praealtum* + *H. stoloniflorum* (Autor.) haben circa 100 überwintert. Bis jetzt sind diese (allerdings noch kleinen) Pflanzen im Baue und Indumente der Blätter von einander nicht verschieden und stimmen mit der

hybrids *H. Auricula+H. Pilosella, H. praealtum (bauhini)+H. aurantiacum,* and probably also *H. Pilosella+H. Auricula* may be considered to have been successfully produced. About 100 of the autumn seedlings of last year's hybrid *H. praealtum+H. stoloniflorum* (Autor.) have survived the winter. Up to now these plants (still very small) are uniform in the structure and the hairy covering of the leaves,

Fig. S3

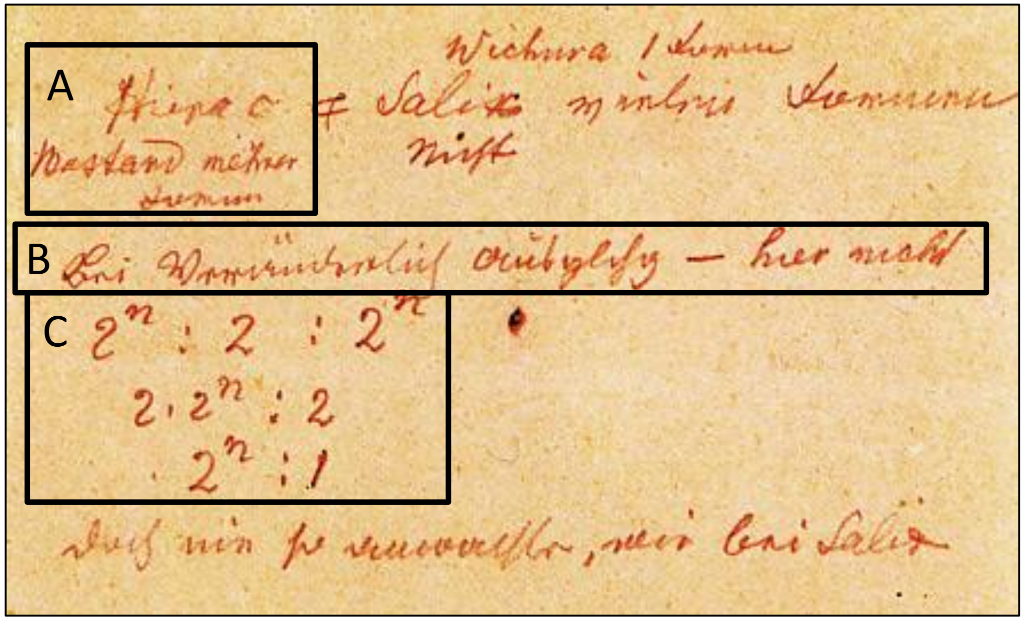
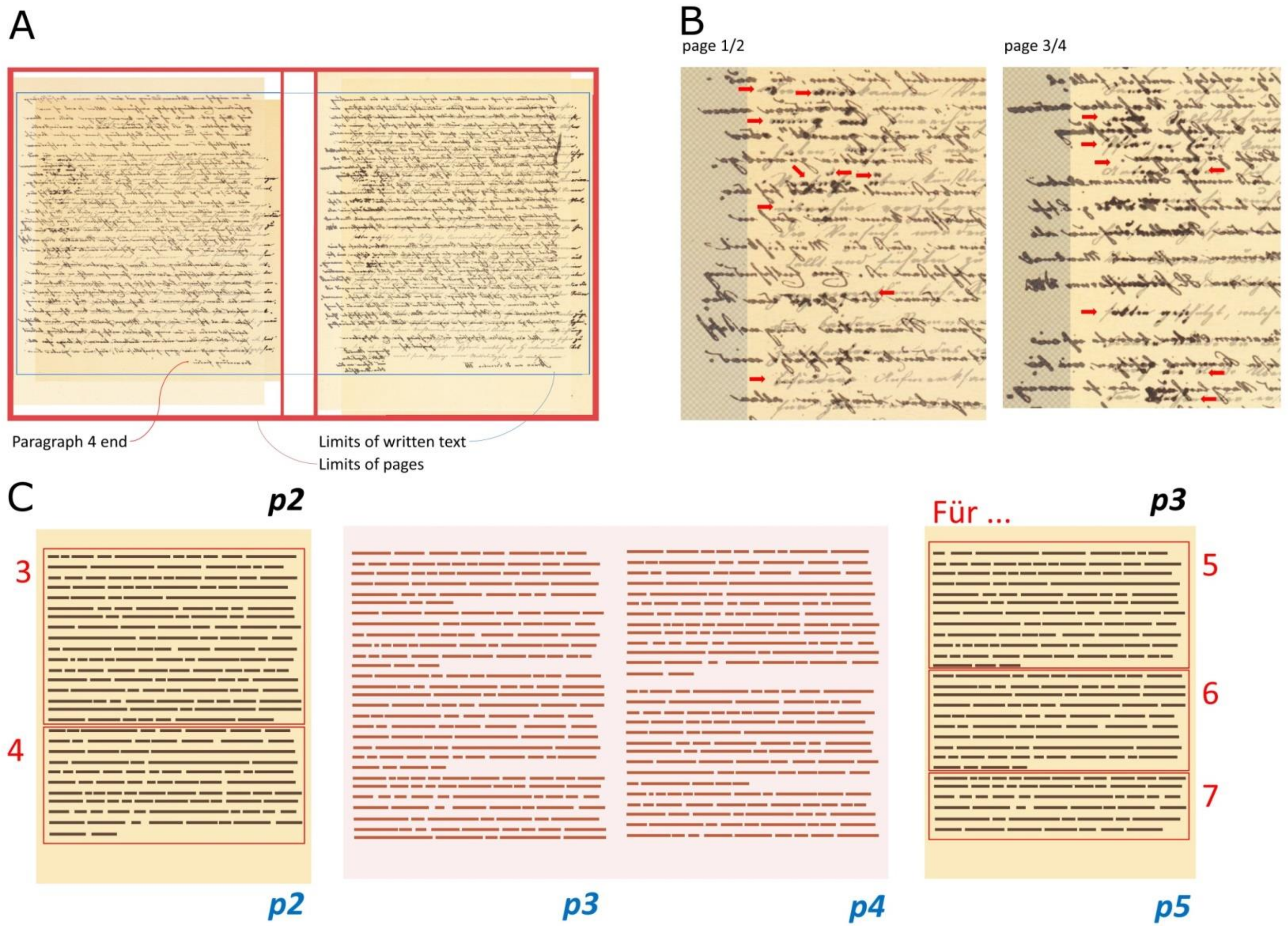


Fig. S4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Supplemental Table ST1. Timeline of the Mendel - Nägeli correspondence

Year	Date	Letter	Mendel's salutation	Mendel's signing	Nägeli' s salutation	Nägeli's signing
1866	December 31st	I	Highly Esteemed Sir	I subscribe myself		
1867	February 24th				Most honored colleague	With esteemed consideration, yours sincerely
	April 18th	II	Highly Esteemed Sir	Your devoted		
	November 6th	III	Highly Esteemed Sir	Sincere admirer		
1868	April 28th				unknown	unknown
	February 9th	IV	Highly Esteemed Sir	With greatest respects for your honor		
	May 4th	V	Highly Esteemed Sir	Your devoted		
	May 11th				Esteemed Sir and friend	With esteemed consideration, your
	June 12th	VI	Highly Esteemed friend	Your devoted friend		
	September*				unknown	unknown
		M1**	unknown	unknown		
1869	April 15th	VII	Highly Esteemed Sir and friend	Your always respectfully		
	April 18th				unknown	unknown
		M2***	unknown	unknown		
1870	April 27th				unknown	With highest esteem and admiration, your most devoted friendship
	July 3rd	VIII	Highly Esteemed friend	Your devoted friend		
	September 27th	IX	Highly Esteemed Sir and friend	Your very devoted		
1871	May 30th				unknown	unknown
1873	spring	M3****	unknown	unknown		
	November 18th	X	Highly Esteemed Sir and friend	Yours very respectfully		
1874	June 23rd				unknown	unknown
1875	date unknown				unknown	unknown

Sources:

Correns (1905)

Hoppe (1971)

Stern and Sherwood (1966)

*) In September 1868, Nägeli sent *Hieracium* plants from the Brenner Pass to Mendel

**) A missing letter from Mendel is inferred from the contents of letter VII

***) A letter from Mendel may be missing because there is no letter from him between two successive letters from Nägeli; there is an unusually long time span between letters VII and VIII (almost 15 months)

****) From letter X, Nägeli concluded that Mendel had sent a letter in the spring of 1873 which he never received.

Supplemental Table ST2. Time line of the key events related to Mendel's *Hieracium* research.

Sources: Correns (1905), Kříženeck□ (1965), Stubbe (1965), Stern and Sherwood (1966) and Orel (1996).

Year	Key events
1840	Nägeli's PhD thesis on *Cirsium*
1841	
1842	
1843	
1844	
1845	Nägeli's paper on the systematics and taxonomy of *Hieracium* , section *Pilosella*
1846	
1847	
1848	
1849	Gärtner's *Versuche und Beobachtungen über die Bastarderzeugung im Pflanzenreich* published
1850	
1851	November 5th, Mendel starts to study at the University of Vienna
1852	Mendel studies at the University of Vienna
1853	July 21st, Mendel returns to Brünn
1854	
1855	
1856	Beginning of the *Pisum* experiments
1857	
1858	
1859	Darwin's *Origin of Species* published December, Natural Science Society of Brünn founded at a meeting attended by Mendel
1860	
1861	
1862	
1863	End of the *Pisum* experiments
1864	*Hieracium* plants collected December, von Niessl discusses wild *Hieracium* hybrids at the Natural Science Society
1865	February 8th, Mendel's first *Pisum* lecture at the Natural Science Society of Brünn March 8th, Mendel's second *Pisum* lecture at the Natural Science Society of Brünn True breeding *Hieracium* lines established
1866	Mendel's *Pisum* paper is published First *Hieracium* crosses December 31st, Letter and *Pisum* article reprint to Nägeli (I)
1867	January 1st, Letter and *Pisum* article reprint to Anton Kerner von Marilaun February 24th, reply letter from Nägeli to Mendel March 5th, reply letter from Kerner to Mendel (lost) April 18th, letter II November 6th, letter III
1868	February 9th, Letter IV

	March 31st, Mendel elected as abbot April 28th, letter from Nägeli May 4th, Letter V May 11th, letter from Nägeli June 12th, Letter VI September, Letter from Nägeli Missing letter Darwin's *The Variation of Animals and Plants under Domestication* published *Idem*, German translation published *Idem*, August 21st review in the newspaper *Neuigheiten* under the header "Plant Breeding"
1869	April 15th, Letter VII April 18th, Letter from Nägeli May - June many *Hieracium* crosses June 9th Reading of Mendel's *Hieracium* paper from July onwards serious eyesight problems Experiment with fertilization by single pollen grain in *Mirabilis* Reports codominance of flower colour in *Mirabilis*
1870	Mendel becomes a member of the Association of Moravian Beekeepers April 27th, letter from Nägeli Publication of Mendel's *Hieracium* paper July 3rd, Letter VIII Summer: 84 hybrids of *H.auricula x H.aurantiacum* in flower Experiment with fertilization by single pollen grain repeated Experiment with simultaneous pollination with two pollen grains from white and yellow flowered *Mirabilis* underway September 27th, Letter IX
1871	May 30th, Letter from Nägeli last *Hieracium* crosses
1872	
1873	Spring, Missing letter (not received byNägeli) November 18th, letter X
1874	June 23rd, Letter from Nägeli
1875	date unknown, letter from Nägeli Nägeli awarded with the Bavarian Order of Merit and becomes: von Nägeli Mid 1870's: Notizblatt 1: segregation in variable hybrids Mid 1870's: Notizblatt 2: multiple constant hybrids in *Hieracium*
1876	Kerner publishes a paper about putative parthenogenesis in *Antennaria alpina*
1877	
1878	
1879	
1880	
1881	Focke publishes *Die Pflanzen-Mischlinge*, mentioning Mendel's research 15 times
1882	
1883	Discovery of meiosis by Van Beneden

1884	January 6th Mendel dies of kidney failure Mendel’s letters and notebooks burned Nägeli publishes his Mechanisch-physiologische Theorie der Abstammungslehre
1885	
1886	
1887	
1888	
1889	
1890	Weissmann concludes that meiosis consists of an equatorial and a reductional division
1891	Nägeli dies
1892	Correns marries Nägeli's niece
1893	
1894	
1895	
1896	
1897	
1898	
1899	
1900	de Vries, Correns & Tschermak's rediscovery of Mendel's work
1901	Correns asks the Nägeli family if letters from Mendel to Nägeli still exist
1902	
1903	Sutton formulates chromosome theory of heredity
1904	Ostenfeld suggests that Mendel's *Hieracium* results can be explained by apomixis
1905	Mendel's letters to Nägeli found 'due to an accident' (!), and published
1906	
1907	
1908	
1909	Bateson suggests that Mendel hoped to confirm his *Pisum* findings in *Hieracium*
1910	

Supplemental Table ST3. Mendel's most important *Hieracium* crosses

The variability / uniformity of the F1 and later generations, based on Correns (1905). Note that the distinct types of hybrid in the first generation had uniform offspring so they are not 'variable hybrids', but distinct lineages of 'constant hybrids'.

female	male	1866	1867	1868	1869	1870	1871
H. praealtum	*H. stoloniflorum (= H. lagellare)*	crossed	1 hybrid	G1 uniform	G2 uniform	G3 uniform	
H. praealtum (?)	*H. aurantiacum*		crossed	2 hybrids, distinct types	G1 uniform	G2 uniform	
H. praealtum (Bauhini?)	*H. aurantiacum*		crossed	2 hybrids, distinct types	G1 uniform	G2 uniform	
H. auricula	*H. pilosella*		crossed	1 hybrid	G1 uniform		
H. echoides	*H. aurantiacum*		crossed	1 hybrid	G1 uniform		
H. auricula	*H. pratense*		crossed	3 hybrids, distinct types			
H. auricula	*H. aurantiacum*		crossed	2 hybrids, distinct types			
H. pilosella	*H. auricula*		crossed	1 hybrid			
H.cymigerum	*H. pilosella (Brünn)*				crossed	29 hybrids, distinct types	
H. auricula	*H. aurantiacum*				crossed	98 hybrids, distinct types	G1 uniform
H. auricula	*H. pilosella vulgare (München)*					crossed	84 hybrids, distinct types*

H. auricula	*H. pilosella vulgare (Brünn)*					crossed	25 hybrids, distinct types*
H. auricula	*H. pilosella niveum (München)*					crossed	35 hybrids, uniform / distinct**

* Mendel’s letter X is ambiguous about F1 variation, but Peter (1884) distinguishes two hybrid forms.

** Mendel’s letter X and Peter (1884) are ambiguous about F1 variation.

Supplemental Files

Supplemental file S1 Apomixis in *Hieracium*

Hawkweeds (genus *Hieracium*) belong to the family Compositae (or Asteraceae), named after the flower head, which is an inflorescence composed of many small flowers (florets) on a basis (capitulum). In 1904 Carl Hansen Ostenfeld discovered apomixis in the genus *Hieracium* and in most of the *Hieracium* species that Mendel had used in his crosses (Ostenfeld 1904). Apomixis is reproduction through clonal seeds as a consequence of two developmental processes: 1. Avoidance of meiosis (apomeiosis) and 2. Parthenogenesis (the development of the egg cell into an embryo without fertilization). Ostenfeld was the first to suggest that the enigmatic results of Mendel's *Hieracium* crossing experiments might be related to the occurrence of apomixis in this genus (Nogler 2006). Apomixis is rare and estimated to be the mode of reproduction in about 1 in 1,000 angiosperm species (Mogie 1992).

The genus *Hieracium* is divided into three subgenera of which the two largest, *Pilosella* and *Archieracium* (now *Hieracium sensu stricto*), have an original Eurasian distribution and were both studied by Mendel. It is now known that in both *Pilosella* and *Archieracium*, diploids are sexual and polyploids are sexual or apomictic. The mechanism of apomeiosis in the subgenera is different: apospory in *Pilosella* and diplospory in *Archieracium* (for details see Hand *et al.* 2015). As a consequence, *Pilosella* species are facultative apomicts, with a small percentage of residual sexual reproduction, whereas *Archieracium* species are virtually obligate apomictic. This largely explains why Mendel was much more successful in making interspecific hybrids in *Pilosella* than in *Archieracium*, *viz.* 19 species combinations in *Pilosella* versus only two in *Archieracium* (Correns 1905).

Species of the *Pilosella* subgenus differ in their degree of apomixis; some are completely sexual, *e.g. H. auricula*, some are partially apomictic, *e.g*. *H. praealtum,* and some are fully apomictic, e.g. *H. aurantiacum*. Initially Mendel used a partially apomictic seed (female) parent, which explained why only one or a few hybrids were produced in a background of apomicts. When two hybrids from the same cross differed, Mendel initially attributed this to contamination with outcross pollen (see Letter VIII). Later, Mendel used fully sexual *H. auricula* as seed parent in conjunction with a male we now know to be apomictic, which explains why he obtained many more hybrids, in which variation was much more obvious and could no longer be explained by contamination; in Letter VIII Mendel records this change in his opinion.

Not knowing of the existence of apomixis, Mendel assumed that *Hieracium* species were true breeding due to self-fertilization. To prevent presumed selfing he had to emasculate the tiny florets in the inflorescence. Since Mendel found maternal offspring even after emasculation, he assumed that emasculation had been unsuccessful and concluded that selfing had occurred before emasculation (at least two days before the florets opened). The immature florets were very sensitive to mechanical damage so the success rate of crossing was low. Mendel complained about exhaustion of his eyes due to the intense light needed for these manipulations and he suffered from a serious eye ailment for six months (Letter VIII). In retrospect all this effort was not necessary, since apomictic offspring do not result from selfing and sexual *Hieracia* are self-incompatible (due to a sporophytic self incompatibility system; Gadella 1987). Ironically, in his first letter, Nägeli advised Mendel to use pollen-sterile plants. Mendel was aware of the fact that such pollen sterile plants occurred in *Hieracium*; in the *Hieracium* paper he writes: "It not rarely happens that in fully fertile species in the wild state the formation of the pollen fails, and in many anthers not a single good grain is developed" (Mendel 1869). Had Mendel followed Nägeli's advice and made crosses onto pollen-sterile plants, an unexpectedly large number of maternal descendants would have led inevitably to the conclusion of parthenogenetic reproduction.

Why did Mendel and Nägeli not consider that parthenogenesis was operating in *Hieracium*? The occurrence of parthenogenesis in seed plants had been passionately discussed a decade before Mendel's *Hieracium* publication; in which Nägeli had taken a prominent part and had stressed that parthenogenetic offspring would be highly uniform (Fürnrohr, 1856). One of the reprints that Nägeli sent to Mendel even mentioned the word "parthenogenesis". Moreover, parthenogenesis was known to occur in bees, and being an ardent bee keeper Mendel must have known this. However, in the second half of the 1850's after thorough evaluation, many cases of supposed parthenogenesis were shown to be caused by pollen contamination and therefore rejected. In 1869, when Mendel gave his lecture, the occurrence of parthenogenesis was widely accepted only in the Australian dioecious species *Coelebogyne ilicifolia* (*Alchornia ilicifolia*). At Kew Gardens three female specimens of this plant produced exclusively female offspring (Smith 1839/1841). Parthenogenesis in a dioecious stonewort *Chara crinita* was also widely accepted and in 1876 Kerner reported on a supposed case of parthenogenesis in dioecious *Antennaria alpina*. All these dioecious cases (separate male and female plants) were supported by reproduction in geographic regions where no male individuals were found, which raised questions about their mode of reproduction. Parthenogenesis in a hermaphroditic pollen producing seed plant like *Hieracium* was not obvious. Nogler (2006) noticed that Correns, De Vries and Bateson did not foresee parthenogenesis in *Hieracium* either and the same can be said about Sutton (1903). It was only in 1904, when Ostenfeld showed that seed development still occurred after removal of both anthers and styles, that parthenogenesis became obvious.

Christoff (1942) repeated Mendel's *H. auricula* x *aurantiacum* crosses and concluded that high levels of heterozygosity were masked by apomictic reproduction. Heterozygosity becomes apparent when the apomict is used as a pollen donor in crosses with sexual plants, resulting in segregation of traits like inflorescence color, but also segregation for the apomictic mode of reproduction. Therefore some (but not all) of the F1 hybrids reproduce by apomixis and become "constant hybrids", as Mendel had found. Christoff also concluded that apomixis was controlled by a dominant gene. In other *Hieracium* species, separate loci for apomeiosis, parthenogenesis and autonomous endosperm development have been identified (Catanach *et al.* 2006; Koltunow *et al.* 2011; Ogawa *et al.* 2013). Genetic studies on the control of apomixis in other genera have shown that apomixis is generally controlled by one or a few dominant apomixis loci that are transmitted through pollen in a Mendelian way (Ozias-Akins and Van Dijk 2007).

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Supplemental file S2 Carl Nägeli and Mendel's letters

Carl Nägeli, the person who could best see the relevance of Mendel’s pea and hawkweed results

Carl Nägeli[1] became professor in botany in Zürich in 1850 and later in Munich in 1857. His PhD thesis (Nägeli, 1841) concerned the systematics of the genus *Cirsium*. Subsequently he published a paper on the species and natural hybrids of *Hieracium*, subgenus *Pilosella* (Nägeli, 1845). At a meeting of the Royal Bavarian Academy of Science on December 15th 1865 he presented a paper reviewing the literature on artificial hybridization in plants ‘The formation of bastards [interspecific hybrids] in the plant kingdom’ (Nägeli, 1865) where he tried to deduce generalities, or rules, out of the many non-structured experiments conducted mostly by Gärtner. Until the appearance of *Die Pflanzen-Mischlinge*, Focke’s book on plant hybridization (Focke 1881)[2], Nägeli’s review remained the most important publication in this field. He published six more papers on the evolution and systematics of plant species, of which three were specifically about the genus *Hieracium* (Nägeli 1866 a,b,c,d,e).

Although Nägeli’s review was presented more than six months after Mendel’s two *Pisum* lectures, the timing was such that it was published too soon to include reference to Mendel's work. All of Nägeli's 1866 (and earlier) papers were available to Mendel in summer of that year and it is likely that he read them before he sent his first letter to Nägeli (Weiling 1969).

Even before the publication of Darwin’s 'Origin of Species' in 1859, Nägeli had accepted that species were not constant but could evolve (Junker 2011). The genus *Hieracium* seemed to be particularly suitable for empirical studies on the process of speciation. This highly polymorphic genus consisted of many different forms with clear species (“*Hauptarten*”) connected by a continuum of intermediate forms

[1] Often also referred to as Carl von Nägeli, however “von” was inserted when he was awarded with the Bavarian Order of Merit in 1875, after the correspondence with Mendel had ended.
[2] Correns, De Vries and Tschermak became aware of Mendel’s work in 1900 through Focke’s book

("*Mittel-* or *Zwischenformen"*). Nägeli, "in the spirit of the Darwinian teaching, defended the view that these forms are to be regarded as [arising] from the transmutation of lost or still existing species" (Mendel 1870, Stern and Sherwood, 1966, p. 51). In other words, in *Hieracium*, the 'missing links' between the species were still present. In contrast to other *Hieracium* experts, Nägeli did not deny hybridization, especially in the early steps of speciation. After his early studies of the subgenus *Pilosella* (between 1841 and 1846), Nägeli returned to studying this subgenus in 1864 when, with the publication of Darwin's work, speciation became topical.

Nägeli was an expert in the identification of natural *Hieracium* hybrids. He collected *Hieracium* seeds and plants from many different taxa and localities and grew these in the common garden at Munich. By 1884 he had cultivated almost 4500 *Hieracium* accessions (Nägeli 1884). Although Nägeli did not carry out artificial hybridizations himself, spontaneous hybrids between different accessions were found in the common garden (Peter 1884).

A collaboration in the field of *Hieracium* would give Mendel the opportunity to bring his *Pisum* work to the attention of Nägeli, who was the best qualified person in the world to appreciate and therefore promote his work. Interestingly, in addition to Mendel's covering letter for the *Pisum* reprint which he sent to Nägeli, the covering letter for the reprint which he sent to Anton Kerner von Marilaun has survived. The latter was written on New Year's day 1867, one day after the former. Kerner was Professor in Botany in Innsbruck and had studied with Mendel in Vienna. Although a lesser authority than Nägeli, Kerner was a distinguished professor who was well known for his research on natural hybrids. Whereas Mendel wrote a long letter to Nägeli of at least 4 pages, his letter to Kerner is only half a page, identical to the first and last formal paragraphs of the letter addressed to Nägeli (Supplemental figure SF1).

Mendel did not consider it worthwhile to explain his *Pisum* work and his future plans to Kerner. Kerner's reprint of Mendel's paper was found later, uncut.

Translations of Mendel's letters to Nägeli

In 1950, at the Golden Jubilee of the rediscovery of Mendel's work, the American Genetics Society published a full English translation of Mendel's letters to Nägeli, together with the 1900 publications of de Vries, Correns and Tschermak. This translation was done by Piternick and Piternick (1950) and was also used in the Mendel Source book of Stern and Sherwood (1966); it can be found at the Electronic Scholarly Publishing website: (http://www.esp.org/foundations/genetics/classical/browse/). In places, the Piternick and Piternick (1950) German to English translation of Mendel's letters tends to be rather negatively biased compared to other translations, but since the Piternick and Piternick translation is the most extensive, we use this translation in our 'Perspective', unless otherwise indicated.

Missing letters from Mendel to Nägeli

We know that at least two of Mendel's letters to Nägeli are lost. In the most obvious case it is clear that Nägeli did not receive Mendel's letter written in the spring of 1873 (Letter M3 of Supplemental Table ST1). In his last letter (X) Mendel wrote that despite his best intentions he could not keep the promises he had made in spring. From this Nägeli deduced that Mendel had sent a letter in spring which he had not received, which he recorded in his notes (Correns 1905).

Secondly, in his letter of April 15th 1869 (Letter VII) Mendel commented on the hybrid samples that he had sent to Nägeli for identification. He remarked, of *Cirsium* hybrid: nr. 15, "I already reported on the interesting progeny of hybrid No 15 in *my last letter*" [our emphasis] (Letter VII, Stern and Sherwood

1966, p. 84). However, in the earlier letters IV, V and VI there is no mention of *Cirsium* (Letter M1, Supplemental Table ST1). In September 1868 Nägeli had sent *Hieracium* plants from the Brenner Pass to Mendel. Whereas in previous letters Mendel thanked Nägeli for material within one month, Mendel's letter VII is dated seven months later and does not contain a word of thanks for the material received. Letter M1 would be appropriate for these thanks as well as discussing the *Cirsium* hybrid No 15. We conclude that a letter by Mendel, written between September 1868 and April 1869, must also be lost.

There may be a third missing letter (Letter M2, Supplemental Table ST1) from Mendel. Between two successive letters from Nägeli (April 18th 1869 and April 27th 1870) no letter from Mendel exists. Correns (1905) wondered if a letter from Mendel in that period was lost. Although Mendel suffered from eye sight problems in June 1869, as he explained in his letter of July 1870 (Letter VIII), he would have had time to write in answer to Nägeli in April or May 1869. On June 9th 1869 Mendel gave his *Hieracium* lecture to the Natural Science Society, mentioning Nägeli twice. A lost letter in that period would also explain why Mendel does not mention his *Hieracium* lecture in any of the surviving letters.

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Supplemental File S3: Phases of Mendel's *Hieracium* experiments

In Letter I Mendel wrote about Gärtner's crosses: "In most cases it can at least be recognized that the possibility of an agreement with *Pisum* is not excluded", indicating that Mendel thought the *Pisum* type of inheritance (variable hybrids) was most common. Concerning constant hybrids he wrote: "This plant [the *Geum* hybrid], according to Gärtner, belongs to the few known hybrids so far, which produce nonvariable progeny as long as they remain self-pollinated", indicating that he thought this type of inheritance was rare. Mendel was right, we now estimate 1 in 1,000 angiosperm species to be apomictic (Supplemental File 1).

Phase 1 – Single Hieracium hybrids with constant progeny (summer 1865 - spring 1869)

As with his *Pisum* experiments, Mendel planned and prepared his *Hieracium* experiments very well. At the beginning of the first phase Mendel collected his parental species, checked whether they were true breeding and developed the crossing methods. Mendel selected parent species from locations where no other parent species occurred and he bred offspring to convince himself that they were true breeding. In the summer of 1866, he tried to make the first crosses. Clearly this project had been conceptualised much earlier (Supplemental Table ST2 gives a chronology of the key events for Mendel's *Hieracium* studies).

Mendel ended his first letter to Nägeli, written on New Year's Eve 1866, by asking for Nägeli's help with the taxonomy of *Hieracium* species: "I am afraid that in the course of my experiments, especially with *Hieracium*, I shall encounter many difficulties, and therefore I am turning confidently to your honor with the request that you not deny me your esteemed interest when I need your advice."(Letter I, Stern and Sherwood 1966, p. 59).

Nägeli replied two months later, on February 24th 1867. This was the start of an exchange of at least 10 letters over a period of 7 years. Nägeli's first letter (the last 4 pages have survived) and Mendel's second letter are interesting because of their discussion of the *Pisum* experiments. Unfortunately Nägeli believed in blending inheritance, as is clear from his draft letter "The constant forms [not hybrids!] have to be tested further (A, a, AB, Ab, aB, ab). I expect that sooner or later (by inbreeding) they will vary again. For example 'A' contains half 'a' of which it cannot get rid of by inbreeding" (Hoppe 1971). Mendel's *Pisum* findings however are outside the scope of this paper. Nägeli advised Mendel to continue his attempts to fertilize *Hieracium*: "It would seem to me especially valuable if you were able to effect hybrid fertilisations in *Hieracium*, for this will soon be the genus about whose intermediate forms we shall have the most precise knowledge" (Iltis 1966, p. 192).

Mendel and Nägeli discussed which *Hieracium* species were most interesting to try to hybridize. Mendel regularly sent seeds and living plants to Nägeli for identification. Between 1867 and 1884 Nägeli cultivated 12 of Mendel's *Hieracium* hybrid combinations in the experimental garden at the University of Munich. In return, Nägeli sent *Hieracium* seed and plants which Mendel could not obtain by himself.

Regarding breeding techniques in *Hieracium*, Mendel wrote in the first letter that "manipulation of artificial pollination is very difficult and unreliable because of the small size and peculiar structure of the florets" (Letter I, Stern and Sherwood 1966, p. 58). Despite "precautions" against self-pollination all *Hieracium* hybridizations from the summer of 1866 failed, only "selfed" offspring was produced (Mendel assumed selfing, but now we know that apomixis was the cause). In *Cirsium*, he also tried mass pollination, without removing the anthers, in the hope of obtaining a few hybrids, since only a few hybrids were required to test the hypothesis of constancy and Mendel expected only one type of constant hybrid per species combination. Mendel was planning to apply the same procedure (mass pollination) next summer (1867) to *Hieracium*. In the summer of 1867 Mendel experimented further with methods for producing artificial hybrids in *Hieracium*. A floret bud emasculation method, using a magnifying lens and a sharp needle produced the first *Hieracium* hybrid and this was the method Mendel used for all his later crosses. On the proposed mass-pollination Mendel did not write any more.

The most important result according to Mendel's third letter (Letter III, November 1867) was an artificial *Hieracium* hybrid between *H. praealtum* and *H. stoloniflorum*. This was obtained by emasculation of florets in bud. Only four seeds developed, one of which was without doubt a hybrid on the basis of morphology. The other three were identical to the maternal plant and Mendel suspected that selfing had occurred before the flower was open. The hybrid was a "healthy, luxuriant plant" that produced 624 seeds in isolation, from which 156 offspring were obtained (Letter III, Stern and Sherwood 1966, p. 72, 73). As is clear from the opening paragraph of the main text, Mendel was very eager to find out whether the plants would be uniform and identical to the mother hybrid plant. We used the Mann Lesley (1927) translation because the Piternick and Piternick (1950) translation of this important passage is very poor and negative (clearly Mann Lesley's "yearning" is a better translation of Mendel's "*Sehnsucht*" than Piternick and Piternick's "anticipation").

In February 1868 (letter IV), Mendel summarized and described his plans: "After having in the past two years collected some experience in the artificial fertilization of *Hieracia*, I intend to perform some systematic experiments with this genus, experiments which will be limited to crosses between the main types." (Letter IV, Stern and Sherwood 1966, p. 78).

On May 4th 1868 Mendel wrote to Nägeli (letter V, see Supplemental Figure SF2), according to the Wilks (1906) translation: "Of the autumn seedlings of the hybrid *H. praealtum* x *H. stoloniferum* which was raised last year, about 100 have overwintered. Thus far these plants (still of course small) in both the structure and the hairiness of the leaves are indistinguishable [Mendel's underlining] from each other and agree with the hybrid mother-plant. I look forward to their further development with some eagerness." This passage is translated by Piternick and Piternick (1950) as follows: "Up to now these plants (still very small) are uniform [no underlining] in the structure and the hairy covering of the leaves and resemble the seed plant. I am awaiting their further development with some suspense" (Letter V, Stern and Sherwood 1966, p. 79/80). This translation fails to recognise importance Mendel gave to the word indistinguishable, and they use negative emotion in "suspense" instead of positive emotion in "eagerness".

The first hybrid progeny flowered in June 1868 (Letter VI): "The first generation of last year's hybrid *H. praealtum* + *H. flagellare* (= *stoloniferum*), consisting of 112 plants, is flowering. As far as I am able to judge, all plants are alike in the essential characteristics, and they differ from the hybrid seed plant, which is now flowering, only to the extent of having weaker, shorter, and less branched stems. This is not remarkable in view of the greater age and strength of the seed plant (Letter VI, Stern and Sherwood 1966, p. 81*)."* In June 1868 therefore Mendel knew that he had succeeded in creating a constant hybrid from his *Hieracium* cross. This is a major success, and by no measure a failure.

In June 1868 Mendel (Letter VI) wrote that he had obtained five other hybrids from different *Pilosella* species combinations. He referred to one hybrid combination (*H. praealtum* x *H. aurantiacum*) where there were two individuals, one he recognised as a hybrid intermediate between the parental species and another that vegetatively resembled *H. praealtum* which Mendel described as 'aberrant'; of the flowers he commented "the flowers are definitely of hybrid color!" (Letter VI, Stern and Sherwood 1966, p. 81). The exclamation mark indicates his surprise, and the beginning of the realisation that there were multiple hybrid types. When more hybrids started to flower, it was clear that in each case where he obtained two or more hybrid plants from a hybrid combination, these always were different from each other (Mendel 1869/1870). Two years later (letter VIII July 1870) Mendel reflected on this revelation: "In *Pisum* and other plant genera I had observed only uniform hybrids and therefore expected the same in *Hieracium*. I must admit to you, honored friend, how greatly I was deceived in this respect. Two specimens of the hybrid *H. auricula* + *H. aurantiacum* first flowered two years ago [1868]. In one of them, the paternity of *H. aurantiacum* was evident at first sight; not so in the other one. Since, at the time I was of the opinion that there could be only one hybrid type produced by any two parental species, and since the plant had different leaves and a totally different yellow flower color, it was considered to be an accidental contamination, and was put aside. Thus, in last year's shipment I enclosed only the specimen which closely resembled *H. aurantiacum* in flower color. But when three specimens, each of the same hybrid produced from the fertilization in 1868, and also the hybrid *H. auricula* + *H. pratense* (var.) later flowered, as three different variants, the correct circumstances could no longer escape recognition" (Letter VIII, Stern and Sherwood 1966, pp. 88 and 89). Thus by autumn 1868 Mendel knew that the *Hieracium* crosses generated a multiplicity of different constant hybrids (Supplemental Table ST3) and knew that he had to find a way of reconciling this with the fact that the parents were true-breeding. This was an unanticipated problem that required further study.

In his lecture of June 9th 1869 (On *Hieracium*-Bastards Obtained by Artificial Hybridization, published 1870) Mendel mentioned that so far he had only [!] obtained hybrids in six species combinations and only one to three hybrids per combination. Although the experiments had only just begun, he still decided to present them because he was convinced that the proposed additional experiments would take a number of years and he was not certain that he could finish them.

Mendel argued that hybrids of *Hieracium* were interesting because this genus was the most polymorphic known with a series of intermediate forms linking the main species. There was much debate about the origin of these intermediate forms. Some experts, including Elias Fries, honorary member of the Natural Science Society, completely denied the existence of hybrids, whereas others considered all intermediate forms to be hybrids. As mentioned above, Nägeli assumed that intermediate forms were transmutations, although he did not completely exclude hybridization.

The first result Mendel mentions is the "striking phenomenon that the forms hitherto obtained by similar fertilization [similar crosses] are not identical…..The conviction is then forced on us that we have here only single terms in an unknown series which may be formed by the direct action of the pollen of one species on the egg-cells of another." If these hybrids were terms of an unknown series, more hybrids would be needed to clarify the series, and experiments would be needed just as Mendel had performed in analysing the F2 for *Pisum*. Further on he wrote: "As yet the offspring produced by self-fertilisation of the hybrids have not varied, but agree in their characters both with each other and with the hybrid plant from which they were derived………If finally we compare the described result, still very uncertain, with those obtained by crosses made between forms of *Pisum*, which I had the honour of communicating in the year 1865, we find a very real distinction. In *Pisum* the hybrids, obtained from the immediate crossing of two forms, have in all cases the same type, but their posterity, on the contrary, are variable and follow a definite law in their variations. In *Hieracium* according to the present experiments the exactly opposite phenomenon seems to be exhibited.

Mendel therefore knew that the behavior of the *Hieracium* hybrids was likely to be of general interest and that they were different in their behavior from *Pisum*, but the methodology for trying to understand the rules that governed this would likely be similar, and amounted to identifying the relevant (mathematical) series and how it was formed.

Mendel investigated the constancy of these first hybrids in successive generations. He wrote to Nägeli in June 1870: "The second generation of the hybrids *H. praealtum* (?) + *H. aurantiacum* and *H. praealtum* (Bauhini?) + *H*. *aurantiacum* has flowered, as has the third generation of *H. praealtum* + *H. flagellare*. Again the hybrids do not vary in these generations. On this occasion I cannot resist remarking how striking it is that the hybrids of *Hieracium* show a behavior exactly opposite to those of *Pisum*. Evidently we are here dealing only with individual phenomena, which are the manifestation of a higher, more fundamental, law." (Stern and Sherwood 1966, p. 90). Although the two types of hybrid differed, Mendel considered that they were likely to be able to be understood in a common framework. This framework was established to some degree, but there remained the problem of understanding the diversity of constant hybrids generated from a single cross.

The *Cirsium* hybrids behaved very differently from the *Hieracium* hybrids. In April 1869 Mendel wrote: "*Cirsium* would be an excellent experimental plant for the study of variable hybrids, if it required less space."(Letter VII, Stern and Sherwood, 1966, p. 84). In *Geum* Mendel produced F1 hybrids, but no information exists about the variation in their progenies. In contrast to *Hieracium*, apomixis has not been found in *Cirsium* and *Geum*.

Phase 2 – different constant hybrids from true breeding parents!

On July 3rd 1870 Mendel wrote to Nägeli: "As a matter of fact, variants appeared in all those cases in which several hybrid specimens were obtained. I must admit to having been greatly surprised to observe that there could result diverse, even greatly different forms, from the influence of the pollen of one species upon the ovules of another species, especially since I had convinced myself, by growing the plants under observation, that the parental types, by self-fertilization, produce only constant progeny."

(Letter VIII, Stern and Sherwood, 1966, pp. 88, 89). In the lecture of June 1869 Mendel had mentioned that the different forms were only single terms in an unknown series. To dissect this series into its terms, as he had done in *Pisum*, Mendel needed much larger numbers of hybrids. We think that this was the reason why Mendel needed to increase the efficiency of his crosses and started to use a mirror with a convex lens, since “diffuse daylight was not adequate for my work on the small *Hieracium* flowers” (Letter VIII, Stern and Sherwood 1966, p. 86). In this long letter of July 3rd 1870 we read that after Mendel made a large number of crosses in May and June 1869 he had serious problems with his eyesight, caused by the very intense light. Although he stopped immediately, it was well into the winter before he was able to read longer texts and perform *Hieracium* crosses without concentrated light. Before the eye problems began, Mendel had fertilized more than 100 emasculated flower heads of *Hieracium auricula* with pollen from *H. praealtum, H. cymosum* and *H. aurantiacum*. The hybridisation procedure was optimized by placing the emasculated plants for 2-3 days in a damp atmosphere in the greenhouse after cross pollination (Mendel 1869).

Mendel emasculated 10-12 florets per flower head (Letter III), which implies that he must have emasculated at least 1000-1200 florets in *H. auricula* alone. Half of the flower heads aborted. All progeny plants were of hybrid origin. Mendel called *H. auricula* a “completely reliable experimental plant” (Letter VIII, Stern and Sherwood 1966, p. 87). We now know that *H. auricula* is a sexual species and self-incompatible (Gadella 1987), so in hindsight Mendel’s emasculations were unnecessary. In contrast to *H. auricula*, Mendel was unsuccessful in obtaining hybrids when *H. aurantiacum*, *H. pilosella* or *H. cymosum* were mother plants, despite “numerous attempts” (Letter VIII, Stern and Sherwood 1966, p. 87). Nowadays we know that these species are highly apomictic. Overall Mendel must have carried out thousands of emasculations and pollinations in May and June 1869, an incredible and painstaking task. Ostenfeld (1906) commented: “This method, however, is so difficult and gives so small results, as the delicate flowers are often destroyed in the operation, that a patience and dexterity like Mendel's are required in order to employ it."

The most successful cross was *H. auricula* x *H. aurantiacum*. In summer 1870 84 hybrids flowered: "Variation among them was considerable. Each hybrid characteristic appears in a certain number of variants which represent different transitional stages between one ancestral character and the other. It seems that the variants of the different characteristics may occur in all possible combinations. This seems probable because in the available hybrid plants the assortment of variants of the characters is exceedingly diverse, so as hardly to be the same in any two instances. If this assumption is correct, many hundreds of possible hybrid types should result because of the large number of characters which differentiate *H. auricula* from *H. aurantiacum*. The observed number of hybrid types is too small in the case of parental species as distant as these to determine the true facts. " (Letter IX, Stern and Sherwood 1966, p. 94)). The only trait by which Mendel sorted these plants was female (self)-fertility (fully fertile, partial fertile and fully sterile), in which he found roughly a 1:2:1 ratio (Letter IX, Stern and Sherwood 1966, p. 94). If we assume self-fertility is due to apomixis and take full and partial fertility together as apomixis, then this could be interpreted as a 3:1 ratio of apomixis : non-apomixis. Modern studies have shown that apomixis in the subgenus *Pilosella* is controlled by three dominant loci: one for parthenogenesis (*LoP*) and two closely linked loci, one for unreduced egg cells (*LoA*) and another for autonomous endosperm development (*AutE*) (Catanach *et al.* 2006, Koltunow *et al.* 2011, Ogawa et al. 2013). Mendel's findings do not easily fit the modern genetic model because of an excess of apomicts in his crosses. However, if his plants were not grown in isolation, but in garden beds, (partial) fertility could also include cross-fertilization, which may explain the excess of self-fertile plants (presumed to be apomicts above).

In Mendel's last letter to Nägeli, dated November 1873, he wrote: "The *Hieracia* have withered again without my having been able to give them more than a few hurried visits. I am really unhappy about having to neglect my plants and bees so completely (Letter X, Stern and Sherwood 1966, p. 97)." The last crossing experiments were conducted in 1871 and no new experiments were carried out in 1872.

In June 1869 Mendel had ended his preliminary *Hieracium* communication with the following sentences: "Already in describing the *Pisum* experiments it was remarked that there are also hybrids whose posterity do not vary, and that, for example, according to Wichura the hybrids of *Salix* reproduce themselves like pure species. In *Hieracium* we may take it we have a similar case. Whether from this circumstance we may venture to draw the conclusion that the polymorphism of the genera *Salix* and *Hieracium* is connected with the special condition of their hybrids is still an open question, which may well be raised but not as yet answered." In his last letter to Nägeli from November 1873, Mendel was ready to answer this question. He assumed that species that easily hybridize (e.g. *H. auricula*) are poor at self-fertilizing due to a poor pollen quality caused by environmental factors. Consequently these species would go extinct, "while one or another of the more happily organized bastard-progeny, better adapted to the prevailing telluric and cosmic conditions, might take up the struggle for existence successfully and continue it for a long time, until finally the same fate overtook it" (Letter X, Stern and Sherwood 1966, p. 102). Mendel disagreed with Darwin about genetics, but not with respect to evolution: he was a Darwinian.

Unfortunately Mendel's misgivings as expressed in the preliminary communication came true; he did not manage to bring the *Hieracium* experiments to a conclusion. Nägeli sent two letters, one in 1874 and another in 1875, however these went unanswered. Mendel died on January 6th 1884. Half a year after his death the new abbot, not knowing what to do with Mendel's notes, and after discussions with

Mendel's nephew who had visited the monastery, had his notes burned (Kříženeck 1965). Only two sheets of Mendel's notes survived (*Notitzblatt* 1 and 2), and both have been dated after 1874 (Orel 1996). The first note is about segregation ratios of seed coat color in *Pisum* (Heimans 1969) species and in the second it is written that *Hieracium* produces several hybrids in contrast to a single hybrid in Wichura's *Salix* (see Supplemental Figure SF3). Neither form a cumulative series of combinations after selfing as variable hybrids do (Heimans 1969, Allen 2016). Interestingly in this second note, Mendel wrote '*Bei Veränderliche Ausgleichung*' (in a varying compromise) *i.e.*, the temporary equilibrium that Mendel hypothesized in variable hybrids and indicating that he was still thinking in terms of gamete formation. This supports our view that variable and constant hybrids were both parts of Mendel's integrated research activities on the rules of heredity.

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Supplemental file S4: The physical structure of Mendel's letters

Supplemental Figure SF4 Page Structure of Letter I

The place where a paragraph ends can be identified by a line of text that does not run to the right margin but a paragraph start must be inferred in the following line, because Mendel did not indent new paragraphs. This inference is easily achieved for paragraphs on the same sheet of paper, because of their physical connectedness. However, this connection is missing when a paragraph ends at the bottom of an even numbered page and a new paragraph starts (or appears to start) on the next sheet, at the top of an odd numbered page. For example, paragraph 5 of Letter I appears to begin at the top of page 3, but this appearance is entirely dependent on the paragraph end at the bottom of page 2. Page 3 begins with a capital letter in "Für", so this starts a new sentence, but not necessarily a new paragraph.

We counted the number of paragraphs (excluding final paragraphs) that coincided with a page break. Six out of 63 paragraphs ended at a page break (35 pages; 4 complete letters, 2 letter fragments, 3 paragraphs at the bottom of an odd numbered page and 3 at the bottom of an even numbered page: 6/63 = 0.095). Since both sides are written on, the probability of a break at an even page is half of this value, thus the probability that this paragraph configuration occurs by pure chance is estimated as 0.5 x 0.095= 0.048. Because paragraphs rarely end at the bottom of an even numbered page, this paragraph structure likely represents an intentional change of subject. Furthermore, when we ask if it is likely that page three of Letter I begins with a new paragraph, the answer is 'no' (p=0.048).

If there is a missing sheet (2 pages), then the first missing page must begin with a new paragraph, as is assumed for the current page 3. If the current page 3 does in fact begin with a new paragraph, then the second (missing) page has to have a paragraph end at the bottom. We can estimate p, the frequency of pages with a paragraph end at the bottom, from those we can observe, thus p = 6/35 = 0.17 and estimate the standard deviation as √(pq/N) = 0.064. Thus the chance that the second page of the proposed missing sheet has a paragraph end at the bottom is p, so roughly 10% to 25% of pages selected at random from Mendel's letters would have the properties required for this proposed missing page. Therefore we cannot rule out the original existence of a page that is now lost.

Since the pages are not numbered, obvious reasons for missing pages would be if the sheet were lost, or inserted in another letter in the wrong place. Mendel's handwritings have not been published completely and we have no information about where the originals are now located. We checked Correns' publications for strange junctions/twists, but could not find any that were clear. On the other hand, given the history of Mendel's letters (Supplemental file 1), it would not be surprising if some were incomplete. As a matter of fact, we know that the correspondence is incomplete (see Supplemental file 1).

The two missing pages (one sheet) could also explain why Mendel did not write about the other species with which he already had started crossing experiments in 1865 and 1866 (*Linaria*, *Calceolaria*, *Zea mays*, *Ipomoea*, *Cheiranthus*, *Antirrhinum* and *Tropaeoleum*), and which were much more suitable for testing his *Pisum* findings than *Hieracium*, *Cirsium* and *Geum*. In his second letter to Nägeli, Mendel reported on the progress of the crossing experiments in these species as though the subject was already known to Nägeli. Only the last four pages of Nägeli's answer to Mendel's first letter have survived, but an important passage is: "your plan to include other plants in your experiments is excellent and I am convinced that with many different forms you will obtain essentially different results" (with respect to the inherited characters[3]) "It would seem to me especially valuable if you were able to effect hybrid fertilizations in *Hieracium* for this will soon be the genus about whose intermediate forms we shall have the most precise knowledge" (transcription: Hoppe 1971, our translation). “Many different forms” suggests more than three (i.e. *Hieracium*, *Cirsium* and *Geum*) and is likely the list of species with which Mendel had started crossing experiments to test the *Pisum* findings; it is surprising these are not mentioned in the existing versions of either letter.

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Stubbe, H., 1965 Kurze Geschichte der Genetik bis zur Wiederentdeckung der Vererbungsregeln von Gregor Mendel. Gustav Fischer, Jena.

[3] Olby (1997) finds it odd that Mendel did not use the terms 'heredity', 'hereditary transmission', and 'laws of heredity' in his *Pisum* paper, if he was interested in the transmission of traits. Therefore Olby argues that Mendel was more a hybridist in the tradition of Gärtner and Kölreuter than a geneticist. However, in his letter to Mendel, Nägeli clearly saw the *Pisum* study as a work on inherited characters. Also in his “*Die Bastardbildung im Pflanzenreiche*” – “On the formation of bastards in the plant kingdom” (1865), the review on hybridization which he sent to Mendel as a reprint Nägeli wrote that hybridization provided insight “in the way parental traits were transferred to their progeny” (See also Orel and Hartl 1994). For Nägeli and Mendel heredity and the transmission of traits was a part of hybridization studies.

Supplemental file S5: Natural hybrids of *Hieracium* and *Cirsium* discussed at meetings of the Naturforschender Verein in Brünn

At the Natural Science Society of Brünn there was a special interest in the genera *Hieracium* and *Cirsium*. Two of the honourable members of the Natural Science Society were distinguished *Hieracium* taxonomists: Elias Fries, botany professor in Uppsala, Sweden and August Neilreich, a well-known florist from Vienna. In 1863, in his acceptance letter upon becoming an honourable member of the Natural Science Society, Fries asked the members to collect *Hieracium* specimens (up to 50 individuals per species). In return he offered to send back duplicates for the herbarium of the Natural Science Society. Neilreich cited Mendel's *Hieracium* study in a publication of his own on *Hieracium* in 1871.

At the monthly society meeting in December 1864, Gustav von Niessl von Mayendorf, the secretary, reported an intermediate form of *H. auricula* and *H. pilosella* which was, in all its traits, intermediate between the two species. Three years later von Niessl reported on different forms of the hybrid *Cirsium palustre* x *rivulare* that were found in the wild, some more resembling one and some the other of the parental species (von Niessl 1867). In 1866 Mendel had cultivated one of these *Cirsium* hybrids which was highly fertile and produced offspring in the same year. Adolph Olborny (a member of the board), also a specialist in *Hieracium,* took care of the *Hieracium* section of the society's herbarium.

Hieracium was a notoriously difficult genus for taxonomists. Besides morphologically distinct forms ("*Haubtformen*"), *Hieracium* was characterized by many intermediate forms ("*Mittelformen*") which formed a continuum between the *Haubtformen*. The question was whether these intermediate forms were hybrids, site modifications (environmentally conditioned variants), or transient forms in the process of speciation (as Nägeli believed). Fries categorically denied the existence of hybrids in *Hieracium*.

Darwin mentioned *Hieracium* in “On the Origin of Species” (Darwin 1859) as an example of a highly polymorphic (or protean) genus*;* "There is one point connected with individual differences, which seems to me extremely perplexing: I refer to those genera which have sometimes been called "protean" or "polymorphic", in which the species present an inordinate amount of variation and hardly two naturalists can agree which forms to rank as species and which as varieties. We may instance *Kubus*, *Kosa*, and *Hieracium* amongst plants, several genera of insects, and several genera of Brachiopod shells.” (p. 46). When Prof. Makowsky (vice president of the society) gave a lecture about Darwinism, in January 1865, a month before Mendel’s first *Pisum* lecture, he cited this passage. The selection of *Hieracium* and *Cirsium* hybrids for study is therefore something fully in keeping with the intellectual atmosphere of Brünn in Mendel's time.

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Supplemental Tables

Supplemental Table ST1. Timeline of the Mendel - Nägeli correspondence

Year	Date	Letter	Mendel's salutation	Mendel's signing	Nägeli' s salutation	Nägeli's signing
1866	December 31st	I	Highly Esteemed Sir	I subscribe myself		
1867	February 24th				Most honored colleague	With esteemed consideration, yours sincerely
	April 18th	II	Highly Esteemed Sir	Your devoted		
	November 6th	III	Highly Esteemed Sir	Sincere admirer		
1868	April 28th				unknown	unknown
	February 9th	IV	Highly Esteemed Sir	With greatest respects for your honor		
	May 4th	V	Highly Esteemed Sir	Your devoted		
	May 11th				Esteemed Sir and friend	With esteemed consideration, your
	June 12th	VI	Highly Esteemed friend	Your devoted friend		
	September*				unknown	unknown
		M1**	unknown	unknown		
1869	April 15th	VII	Highly Esteemed Sir and friend	Your always respectfully		
	April 18th				unknown	unknown
		M2***	unknown	unknown		
1870	April 27th				unknown	With highest esteem and admiration, your most devoted friendship

	July 3rd	VIII	Highly Esteemed friend	Your devoted friend		
	September 27th	IX	Highly Esteemed Sir and friend	Your very devoted		
1871	May 30th				unknown	unknown
1873	spring	M3****	unknown	unknown		
	November 18th	X	Highly Esteemed Sir and friend	Yours very respectfully		
1874	June 23rd				unknown	unknown
1875	date unknown				unknown	unknown

Sources:

Correns (1905)

Hoppe (1971)

Stern and Sherwood (1966)

*) In September 1868, Nägeli sent *Hieracium* plants from the Brenner Pass to Mendel

**) A missing letter from Mendel is inferred from the contents of letter VII

***) A letter from Mendel may be missing because there is no letter from him between two successive letters from Nägeli; there is an unusually long time span between letters VII and VIII (almost 15 months)

****) From letter X, Nägeli concluded that Mendel had sent a letter in the spring of 1873 which he never received.

Supplemental Table ST2. Time line of the key events related to Mendel's *Hieracium* research.

Sources: Correns (1905), Kříženeck� (1965), Stubbe (1965), Stern and Sherwood (1966) and Orel (1996).

Year	Key events
1840	Nägeli's PhD thesis on *Cirsium*
1841	
1842	
1843	
1844	
1845	Nägeli's paper on the systematics and taxonomy of *Hieracium* , section *Pilosella*
1846	
1847	
1848	
1849	Gärtner's *Versuche und Beobachtungen über die Bastarderzeugung im Pflanzenreich* published
1850	
1851	November 5th, Mendel starts to study at the University of Vienna
1852	Mendel studies at the University of Vienna
1853	July 21st, Mendel returns to Brünn
1854	
1855	
1856	Beginning of the *Pisum* experiments
1857	
1858	
1859	Darwin's *Origin of Species* published December, Natural Science Society of Brünn founded at a meeting attended by Mendel
1860	
1861	
1862	
1863	End of the *Pisum* experiments
1864	*Hieracium* plants collected December, von Niessl discusses wild *Hieracium* hybrids at the Natural Science Society
1865	February 8th, Mendel's first *Pisum* lecture at the Natural Science Society of Brünn March 8th, Mendel's second *Pisum* lecture at the Natural Science Society of Brünn True breeding *Hieracium* lines established
1866	Mendel's *Pisum* paper is published First *Hieracium* crosses December 31st, Letter and *Pisum* article reprint to Nägeli (I)
1867	January 1st, Letter and *Pisum* article reprint to Anton Kerner von Marilaun February 24th, reply letter from Nägeli to Mendel March 5th, reply letter from Kerner to Mendel (lost) April 18th, letter II November 6th, letter III
1868	February 9th, Letter IV

	March 31st, Mendel elected as abbot April 28th, letter from Nägeli May 4th, Letter V May 11th, letter from Nägeli June 12th, Letter VI September, Letter from Nägeli Missing letter Darwin’s *The Variation of Animals and Plants under Domestication* published *Idem*, German translation published *Idem*, August 21st review in the newspaper *Neuigheiten* under the header “Plant Breeding”
1869	April 15th, Letter VII April 18th, Letter from Nägeli May - June many *Hieracium* crosses June 9th Reading of Mendel's *Hieracium* paper from July onwards serious eyesight problems Experiment with fertilization by single pollen grain in *Mirabilis* Reports codominance of flower colour in *Mirabilis*
1870	Mendel becomes a member of the Association of Moravian Beekeepers April 27th, letter from Nägeli Publication of Mendel's *Hieracium* paper July 3rd, Letter VIII Summer: 84 hybrids of *H.auricula x H.aurantiacum* in flower Experiment with fertilization by single pollen grain repeated Experiment with simultaneous pollination with two pollen grains from white and yellow flowered *Mirabilis* underway September 27th, Letter IX
1871	May 30th, Letter from Nägeli last *Hieracium* crosses
1872	
1873	Spring, Missing letter (not received byNägeli) November 18th, letter X
1874	June 23rd, Letter from Nägeli
1875	date unknown, letter from Nägeli Nägeli awarded with the Bavarian Order of Merit and becomes: von Nägeli Mid 1870's: Notizblatt 1: segregation in variable hybrids Mid 1870's: Notizblatt 2: multiple constant hybrids in *Hieracium*
1876	Kerner publishes a paper about putative parthenogenesis in *Antennaria alpina*
1877	
1878	
1879	
1880	
1881	Focke publishes *Die Pflanzen-Mischlinge*, mentioning Mendel’s research 15 times
1882	
1883	Discovery of meiosis by Van Beneden

1884	January 6th Mendel dies of kidney failure Mendel’s letters and notebooks burned Nägeli publishes his Mechanisch-physiologische Theorie der Abstammungslehre
1885	
1886	
1887	
1888	
1889	
1890	Weissmann concludes that meiosis consists of an equatorial and a reductional division
1891	Nägeli dies
1892	Correns marries Nägeli's niece
1893	
1894	
1895	
1896	
1897	
1898	
1899	
1900	de Vries, Correns & Tschermak's rediscovery of Mendel's work
1901	Correns asks the Nägeli family if letters from Mendel to Nägeli still exist
1902	
1903	Sutton formulates chromosome theory of heredity
1904	Ostenfeld suggests that Mendel's *Hieracium* results can be explained by apomixis
1905	Mendel's letters to Nägeli found 'due to an accident' (!), and published
1906	
1907	
1908	
1909	Bateson suggests that Mendel hoped to confirm his *Pisum* findings in *Hieracium*
1910	

Supplemental Table ST3. Mendel's most important *Hieracium* crosses

The variability / uniformity of the F1 and later generations, based on Correns (1905). Note that the distinct types of hybrid in the first generation had uniform offspring so they are not 'variable hybrids', but distinct lineages of 'constant hybrids'.

female	male	1866	1867	1868	1869	1870	1871
H. praealtum	*H. stoloniflorum (= H. lagellare)*	crossed	1 hybrid	G1 uniform	G2 uniform	G3 uniform	
H. praealtum (?)	*H. aurantiacum*		crossed	2 hybrids, distinct types	G1 uniform	G2 uniform	
H. praealtum (Bauhini?)	*H. aurantiacum*		crossed	2 hybrids, distinct types	G1 uniform	G2 uniform	
H. auricula	*H. pilosella*		crossed	1 hybrid	G1 uniform		
H. echoides	*H. aurantiacum*		crossed	1 hybrid	G1 uniform		
H. auricula	*H. pratense*		crossed	3 hybrids, distinct types			
H. auricula	*H. aurantiacum*		crossed	2 hybrids, distinct types			
H. pilosella	*H. auricula*		crossed	1 hybrid			
H.cymigerum	*H. pilosella (Brünn)*				crossed	29 hybrids, distinct types	
H. auricula	*H. aurantiacum*				crossed	98 hybrids, distinct types	G1 uniform
H. auricula	*H. pilosella vulgare (München)*					crossed	84 hybrids, distinct types*

H. auricula	*H. pilosella vulgare (Brünn)*					crossed	25 hybrids, distinct types*
H. auricula	*H. pilosella niveum (München)*					crossed	35 hybrids, uniform / distinct**

* Mendel’s letter X is ambiguous about F1 variation, but Peter (1884) distinguishes two hybrid forms.

** Mendel’s letter X and Peter (1884) are ambiguous about F1 variation.