

Mendel's Search for True-Breeding Hybrids

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It has been widely believed that Gregor Mendel's experiments with peas (genus *Pisum*) were designed to discover the basic principles of heredity (genetics). Those more familiar with the history of genetics know that his work with peas sought to explain the laws governing the origin and development of hybrids (Hartl and Orel 1992, p. 245; Monaghan and Corcos 1985, p. 49–50; Stern and Sherwood 1966, p. 2). His subsequent work with hawkweeds (genus *Hieracium*) was an attempt to find an explanation for the apparent constancy of some hybrid forms in this and other species (Orel 1984, p. 63). One of his main motives was to see if hybrids between different varieties of a species or between different species could breed true, that is, hybrids produce only more hybrids. In those days, the difference between varieties and species was not always well defined. True-breeding hybrids behaved like new species. Mendel published 2 botanical papers in German: his *Pisum* paper was published in 1866, the one on *Hieracium* was published in 1870. Both of these papers and his letters to his friend and advisor Carl Nägeli have been translated into English in Stern and Sherwood (1966). In the text of the present paper, numbers following Mendel's name refer to pages in the Stern and Sherwood book. A complete English translation of

Mendel's 1866 paper by William Bateson can be found on the internet (see Mendelweb). The concept of reproductive isolation as a hallmark of a biological species did not become popular until well into the 20th century (Mayr 1982, p. 270–275). Mendel found that his intervarietal pea hybrids were phenotypically identical but did not breed true when self fertilized, segregating pure breeding parental types (homozygotes) as well as hybrids (heterozygotes) in the now familiar ratio 1:2:1. [N.B. Modern terms are used here and elsewhere in this paper instead of Mendel's terms that have become passé.] Mendel was aware that Charles Darwin's book *On the Origin of Species* was published in 1859 before he began his pea experiments in 1862. Darwin did not really shed much light on how new species originate by natural selection. And the role of hybridization in that process was begging for experimental insights, especially for how an ancestral species can split into 2 or more daughter species that coexist in time (horizontal evolution or speciation, cladogenesis).

After Mendel found some principles of hybrid behavior in his pea varieties, he hoped to find the same regularities operative in other plant species. In his famous 1866 paper (published in *The Proceedings of the Brünn Natural Science Society*), Mendel also reported his interspecific hybridizations with another species of plant (beans, genus *Phaseolus*). He hybridized bean species differing in plant height and shape of ripe pods and found that they followed the same laws of development as *Pisum*. The colors of both flowers and unripe pods, however, seemed to be more complex. Crosses between plants with white flowers and plants with crimson blossoms produced hybrids with flowers less intensely crimson than the parental variety. The progeny of hybrids had flowers varying from crimson to pale violet and white. The unripe seed pods also had various shades of coloring from dark green to yellow. Because white flowered plants seemed to appear in about 1/16 of the progeny of hybrids (F₂), Mendel proposed that there were 2 color

elements (genes) A_1 and A_2 that together in homozygotes contributed to crimson color. Plants with the recessive alleles a_1 and a_2 , when homozygous together, produced white flowers. Other gene combinations contributed to the range of colors between crimson and violet. Mendel seems to be the first to conceive of multiple factors for a single character.

The Hieracium Enigma

Mendel (living in Moravia, part of the Habsburg Monarchy) wrote 10 letters to Carl Nägeli during the years 1866–1873. Nägeli was Professor of Botany at Munich and one of the foremost botanists and hybridizers of his time (Dunn 1965, p. 16). In his first letter, dated 31 December 1866, Mendel (p. 58) told Nägeli that he had selected Hieracium, Cirsium, and Geum for further experiments to see if they could produce "constant hybrids." Thus, the myth that Nägeli was the one who initially caused Mendel to work with Hieracium is untenable. Because Nägeli had extensive knowledge with interspecific hybrids and "was a well-known Hieracium specialist" (Endersby 2007, p. 122), Mendel asked for his advice. Mendel made no reference to Hieracium in his 1866 paper. It is unknown why he chose Hieracium instead of other genera in his research on constant hybrids. Before he began his research with peas, Mendel was aware that the German botanist C. F. Gärtner had reported hybrids that remain constant in their progeny and propagate like pure strains, including the highly fertile hybrids *Aquilegia atropurpurea canadensis*, *Lavatera pseudolbia thuringiaca*, and *Geum urbano rivale* (Mendel, p. 41). "This feature is of particular importance to the evolutionary history of plants because constant hybrids attain the status of *new species*" (Mendel, p. 41). In his first letter to Nägeli, Mendel (p. 58–59) questioned that Gärtner's hybrids from *Geum urbanum* + *rivale* produced "non-variable progeny as long as they remained self-pollinated." In his tenth and last letter to Nägeli, Mendel (p. 98) finally reveals that "The name following the hybridization symbol + refers in all cases to that species from which the pollen was taken." Unfortunately in Mendel's time, the work of other plant breeders mentioned in his papers were not required to be fully cited so that anyone might be able to check the accuracy of statements made or have access to the entire contents of

cited work. So, it is a little known fact that "Gärtner described separately nearly all the phenomena that later occurred in the definitions of the so-called Mendelian Laws of Heredity, with the exception of the most important, those providing the theoretical basis for the numerical segregation ratios" (Orel 1984, p. 39).

Nägeli's letters to Mendel have not been found. Translations of Mendel's letters to Nägeli by Piternick LK and Paternick J (1950) can be found on the internet. In Mendel's letters, he reported on hybridization experiments between species or varieties belonging to 26 different genera. Four of these crosses produced segregating hybrids like those of peas; the remainder did not (Dunn 1965, p. 14). "In 1869 Mendel concluded experiments with *Matthiola* (stock), *Zea* (maize), and *Mirabilis* (four-o'clock), and stated in his eighth letter to Nägeli (3 July 1870) that their hybrids had behaved exactly like *Pisum*" (Orel 1984, p. 62). Mendel made no mention of this in his second and last botanical paper (1869). Titled *On Hieracium-Hybrids Obtained by Artificial Fertilisation*, it was published in the same journal as his 1866 paper. In this paper, Mendel reported on his hybridization experiments with the various "species" in the genus *Hieracium*. He was unable to reproduce the kind of results he had with varietal hybrids of *Pisum*. Mendel wrote: "This genus [*Hieracium*] possesses such an extraordinary profusion of distinct forms that no other genus of plants can compare with it ... Regarding no other genus has so much been written or have so many and such fierce controversies arisen, without as yet coming to a definite conclusion" (Mendel, p. 50–51). Near the end of the paper Mendel confesses: "In *Pisum* the hybrids, obtained from the immediate crossing of two forms, all have 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, p. 55). Unfortunately, it was not known at the time that *Hieracium* species are "frequently parthenogenetic or apogamous, a situation which obviously results in offspring to which the supposed pollinating parent has not made any genetic contribution" (Stern and Sherwood 1966, p. vii). They develop viable seeds without being fertilized. Apomixis is also common in the weedy, invasive dandelions (*Taraxacum*), and the brambles of genus *Rubus* (Endersby 2007, p. 126).

According to Swanson 1957 (p. 520), the cells of *Hieracium* that produce the gametophyte (embryo sac) are of archesporial origin (diplospory), and the meiotic processes are missing or abortive so that a gametophyte of diploid character is preserved. Occasionally, however, some *Hieracium* species reproduce by normal fertilization. But, according to Dunn 1965(p. 14), hybrids between different species of *Hieracium* are "always apogamous, that is, the parent reproduces vegetatively and the offspring are all alike, as though derived from cuttings, and no sexual process, and hence no segregation, can occur." *Pisum* varietal hybrids tend to revert to the parental types over successive generations of self-fertilization, but apogamous *Hieracium* hybrids breed true generation after generation.

Hybrid speciation is more common in plant genera that can reproduce asexually or when self-fertilization is possible. Hawkweeds (*Hieracium*), for example, reproduce mainly asexually but occasionally reproduce sexually. This has produced so many highly variable "species" that no 2 taxonomists agree on how many forms exist (Ridley, p. 418). Even if some of these interspecific hybrids are semifertile, unless they can exploit an ecological niche different from those of the parental species, they usually fail to survive. Thus, true-breeding hybrids by sexual reproduction are uncommon in nature.

Mendel found that interspecific hybrids of *Hieracium* were not easy to obtain, those that did survive were of low fertility even when fertilized by their own pollen (Orel 1984, p. 66); the hybrids were not uniform, and yet their progeny bred true. Eventually Mendel was able to obtain a few true hybrids of *Hieracium* that segregated characters in their progeny but in a series even more complicated than those of *Phaseolus* (Orel 1984, p. 65). Mendel complained that the flowers of *Hieracium* were very small and he needed a magnifying glass and artificial light to accomplish artificial pollination. In his first letter to Nägeli, Mendel confessed that, despite the great pains he took to insure that only foreign pollen was applied to the stigmas of *Hieracium* (e.g., *Hieracium pilosella* with *Hieracium pratense*, *Hieracium praealtum*, and *Hieracium auricula*; and *Hieracium murorum* with *Hieracium umbellatum* and *H. pratense*), he thought that self-fertilization had occurred (Mendel, p. 58). His failing eyesight and appointment in 1868 as abbot of the Augustinian monastery of St Thomas eventually put an end to his plant work. However, in his last

letter to Nägeli, Mendel (p. 102) anticipates the arrival of "shipments from the corresponding members of our society." Mendel died in 1884, unaware of the importance his *Pisum* work would later prove to be.

Some historians claim that Mendel's 1866 paper was published in an obscure journal (Lander and Weinberg 1778). It is now well publicized that the *Verhandlungen* (transactions, deliberations, proceedings) of the Brünn Society was sent to the libraries of 115 or more institutions, including the Royal Society and the Linnean Society in Great Britain (Mayr 1982, p. 723). Mendel also made 40 reprints of his paper, but in his second letter to Nägeli (dated 18 April 1867) Mendel (p. 61) remarks "The paper which was submitted to you is the unchanged reprint of the draft of the lecture mentioned; thus the brevity of the exposition, as is essential for a public lecture." Unfortunately, Nägeli was a firm believer in the theory of pure blending inheritance. To acknowledge Mendel's theories would have meant, for Nägeli, a complete refutation of his own theories (Mayr 1982, p. 723). When Mendel asked Nägeli for his advice, he seems to have encouraged Mendel to go ahead with his plans to see if *Hieracium* hybrids could produce segregating progeny and later exchanged many specimens or seeds with him. In his 1884 book on evolution and inheritance, however, Nägeli made no mention of Mendel (Mayr 1982, p. 723). Perhaps, this was because Mendel (p. 51) never used Nägeli's name but referred to him only once indirectly in his 1869 paper as "a famous *Hieracium* specialist." Mendel (born 1822) coincidentally died in 1884.

The Bizarre *Oenothera* Complex

The evening primrose *Oenothera lamarckiana* is a beautiful, freely branching plant that can grow more than 5-ft tall. It is a self-fertilizing biennial plant, taking 2 years to produce seeds. In 1900, the Dutch botanist Hugo de Vries was one of the botanists who rediscovered Mendel's 1866 paper. He found his first "mutants" in the evening primrose *O. lamarckiana* in 1886 (Dunn 1965, p. 57), 2 years after Mendel's death. These rare variant types (races) differed from the normal form of the species in several characteristics and naturally bred true by self-fertilization from seed through subsequent generations. He collected seeds from plants growing wild in an abandoned potato field near Amsterdam and planted them

in his own garden. Among the numerous resulting plants, he found 3 phenotypically identical plants that had different characteristics than *O. lamarckiana*, but they did not produce fertile pollen. He named these 3 plants *Oenothera lata*. Later this species was found growing in the wild (Endersby 2007, p. 152). "It was the survival of the *Oenothera* mutants alongside the parental forms [in the wild], resisting blending and swamping, that had first excited de Vries" and led him to believe that the mutants were reproductively isolated new species (Endersby 2007, p. 185). de Vries also discovered a robust variant he named *Oenothera gigas* (later shown by others to be a polyploid) that bred true without any sign of reverting to the original type. When other plant breeders hybridized 2 pure-breeding lines (races) of *Oenothera* (*O. lamarckiana* and *Oenothera biennis*), it was found that the hybrids split into three distinct phenotypes, each of which bred true when self-fertilized. This was the exact opposite of Mendel's results with pea hybrids. deVries thought that each of these "mutations" represented a different "elementary species" (Dunn 1965, p. 59). In 1901–1903, he published his theory that new species originate in a single step, in his 3-volume book *Die Mutationstheorie* as a counterpoint to Darwin's theory that gradual changes occur within a single lineage over many generations as an adaptive response to natural selection (Dunn 1965, p. 60). de Vries also thought that all *Oenothera* mutants were produced by a common mechanism. Both these ideas were later disproved by other geneticists.

The origin of de Vries' "mutations" began to be explained in 1917 by Otto Renner, who found that most *Oenotheras* are complex translocation hybrids (structural heterozygotes) that nonetheless breed true from seed when they are normally self-pollinated. The ancestral species from which most *Oenothera* species evolved most likely had 7 pairs of chromosomes ($2n = 14$), as found in *Oenothera hookereri* (Swanson 1957, p. 496). If two nonhomologous chromosomes exchange chromosome tips, the resulting structural heterozygotes will tend to synapse only at their homologous regions and form a circle of 4 chromosomes, the remaining 10 chromosomes will pair as 5 bivalents during meiosis. If another chromosome exchanges end segments with a

ring of 4, a ring of 6 and 4 pairs will form at meiosis. In this way, a series of reciprocal interchanges (translocations) of chromosome tips are thought to have eventually involved all 7 pairs of chromosomes that characterize most of the numerous *Oenothera* races existing today. There are many thousands of ways to arrange 14 chromosome ends in 7 groups of 2. More than 160 different segmental arrangements have already been identified (Herskowitz 1965, p. 235).

In 1918, H. J. Muller was the first to discover a balanced lethal system in the fruit fly *Drosophila* (Gardner and Snustad 1984, p. 441–442). Individuals homozygous for one allele (*aa*) die. At another locus on the same chromosome, individuals homozygous for one allele (*bb*) die. If dihybrids are linked in repulsion phase (*Ab/aB*) and crossovers between the 2 loci is infrequent or entirely absent, intercrossing dihybrids would be expected to produce mainly or only dihybrid progeny; zygotes homozygous for either of the lethal alleles die. Because in this strain of *Drosophila*, both loci lie within an inversion heterozygote, any crossovers occurring in this region are likely to produce genetically unbalanced gametes (duplications or deficiencies) that are dysfunctional. Thus, genes within the inversion heterozygote remain inseparably linked via the balanced lethal system. Muller suggested that "permanent hybrids like *O. lamarckiana* could also be maintained as balanced lethal systems if crossing over between homologues was reduced almost to zero. Exceptions due to rare crossovers would then, because of their rarity, be interpreted as mutations" (Dunn 1965, p. 160). Other geneticists later found that reciprocal translocations (rather than inversions) "suppress" crossing over in the balanced lethal system of *O. lamarckiana*.

The work of many geneticists contributed to our modern knowledge of the *Oenotheras*, including O. Renner, F. Oehlkers, J. Belling, S. Emerson, and A. H. Sturtevant and others. R. E. Cleland and A. F. Blakeslee demonstrated in 1930 that the peculiar patterns of the transmission groups in

various *Oenothera* races result from a system of balanced lethal genes within each of the reciprocal translocation complexes. In *O. lamarckiana*, the balanced lethal system causes the death of zygotes that inherit identical recessive lethal gene complexes GG or VV, only the heterozygous GV zygotes survive.

"In most species of *Oenothera* the seven pairs [of chromosomes] form one large ring [of 14 chromosomes] during meiosis. When the reducing division occurs, alternate members of the ring (which have come from opposite parents) go to one pole and the balance to the other, so that no recombination of genes or chromosomes ordinarily occurs and the whole complex of each parent with its lethals is transmitted as a unit" (Dunn 1965, p. 160). All the genes of *O. lamarckiana* appear, by genetic mapping, to be linked to one another. In nature, self-fertilization insures that only gametes of the parent plant will normally unite to form the zygote. If maternal and paternal chromosomes alternate in the circle of 14 and alternate members of the ring segregate to opposite poles in meiotic anaphase I, as they usually do, the resulting gametes would be identical to those which united to form the plant. Any other segregation pattern would likely produce genetically unbalanced and nonfunctional gametes. The mechanism responsible for predominately alternate segregation of chromosomes to opposite poles is still unknown (Herskowitz 1965, p. 234). The chromosomes of *Oenotheras* that form a ring of 14 during meiosis are about the same size, with median centromeres, a feature that permits mobility of chromosomes on the metaphase plate. Thus, the surviving translocations have been not only reciprocal but also approximately equal in length (Swanson 1957, p. 495). During the evolution of modern *Oenotheras*, the only scenario that makes sense is the initial formation of ring complexes by reciprocal translocations, followed by the incorporation of balanced lethal systems and then by establishment of self-pollination (Swanson 1957, p. 498). Occasionally, outcrossing may occur, bringing together independently evolved complexes from which F1 and F2 plants with rings of various sizes and some paired bivalents arise. Segregation of genes from the paired homologues produces a burst of biotypes on which natural selection can act (Swanson 1957, p. 498). "Nothing like it is found in other species of plants or animals (aside from a few rare,

similarly balanced systems)" (Mayr 1982, p. 744). *Oenothera muricata* is one such species, but its balanced lethal system involves gametic lethals in which pollen containing gene R is sterile, embryo sacs containing gene C are inactive, and only the union of a C male gamete with an R female gamete produces a viable zygote (Swanson 1957, p. 495).

The Evolutionary Role of Interspecific Hybridizations

Closely related species may naturally hybridize occasionally, but as a rule the hybrids are sterile. When partly interfertile with one or both parental species, the hybrids may intercross among themselves or backcross to the parents. Each successive backcross generation becomes more like the recurrent parent. Very shortly, in terms of geological time, the backcrosses become almost indistinguishable from the recurrent parent, and it is difficult to detect that gene flow has occurred without laboratory tests. Some of the genes from species A have now become incorporated into the gene pool of species B. This process is known as "introgressive hybridization" or simply "introgression" (Anderson 1949). In his 1866 paper, Mendel appears to be describing the process of introgressive hybridization in the formation of new species when he says:

When species A was to be transformed into B, the two were combined by fertilization and the resulting hybrids once more fertilized with pollen from B; from among their various descendants those closest to species B were then chosen and repeatedly fertilized by pollen from B, and so on, until finally a form that was like B and remained constant in its progeny was obtained. Thus species A was transformed into the other species, B. Gärtner himself has carried out 30 experiments of this kind with plants from genera *Aquilegia*, *Dianthus*, *Geum*, *Lavatera*, *Lychnis*, *Malva*, *Nicotiana*, and *Oenothera* (Mendel, p. 44).

Unlike gene mutations, introgression is a mechanism for introducing many genes simultaneously into a species. Many of these blocks of genes (linked in chromosomes) were functionally integrated in the species of their origin (adaptive gene complexes), but some of them might prove

harmful, benign, or even beneficial in their new hosts. Some of the backcross products might, in time, become stabilized by selection for favorable recombinants, but the true-breeding introgressive variants that Mendel proposed would today probably be considered varieties or races, not new species.

Viable interspecific hybrids are usually sterile because of chromosome mismatches during meiosis, as occurs in the mule, a hybrid between a female horse and a donkey male. However, if an interspecific hybrid spontaneously doubles its chromosome number (e.g., $2n \rightarrow 4n$), it becomes, in a single step, fertile as well as reproductively isolated from its parental species. It is thus, by definition, a new biological species. For example, union of the diploid ($2n$) gametes of an allotetraploid (amphidiploid) hybrid and the haploid (n) gametes of the diploid parental species produces a triploid ($3n$) zygote that, if it survives, will be sterile because the unbalanced gametes of triploids contain different numbers of chromosomes that result in genic disharmony. This process is much more common in plants than in animals. It has been estimated that about half of all angiosperm species in nature and almost all pteridophytes (ferns) are polyploids, usually allopolyploids. Plant breeders have used the chemical colchicine to induce chromosome doubling in hybrids to produce many new allopolyploid species. "Polyploidy is known in certain of the *Oenotheras*, but where it accompanies structural heterozygosity it has no survival value" (Swanson 1957, p. 498).

Aside from some plant apomicts and allopolyploids, the search for true-breeding interspecific hybrids, beginning with Gärtner and Mendel and ending with the demise of deVries' mutation theory, has proved to be mostly futile.

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