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No. 50 July 2022

Contents ***Page***

This 50th issue of the ECA Newsletter marks the 25th anniversary of the E.C.A!

E.C.A. on Facebook

As mentioned in the earlier Newsletters, E.C.A. is on Facebook.
Each week you will find announcements of interesting articles, related to cytogenomics or to biology in general, and also pictures and stories from social events related to E.C.A. and its members. Also our E.C.A. conferences will be covered on Social Media.
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You will find a selection of interesting Facebook posts in this Newsletter starting at page 24.
Please contact us (mariano.rocchi@uniba.it) if you wish to share an interesting news item or a pertinent article.

200 years after the birth of Gregor Mendel

Hynčice, July 20, 1822 - Brno, January 6, 1884

Mariano Rocchi

It is 8 May 1900 and William Bateson, a zoologist at St. John's College in Cambridge, is traveling by train to London to give a seminar at the Royal Horticultural Society. He had prepared a speech on the important results obtained by the Dutch botanist Hugo De Vries, whom he knew well, also because he had hosted him at his home the year before on the occasion of the First International Conference on Hybridization and Plant Breeding in London, which they both attended. Bateson later recounted that a few days earlier he had read the German version of De Vries' latest work, which the German Botanical Society had published the previous month. In a note, and only in this note, De Vries reported, *through gritted teeth*, that he had realized that his own fundamental results had been published in 1866 by a Moravian monk, Gregor Mendel. We will see the reason for the "*gritted teeth*" later on. For now, it is just worth noting that in the French version of the work, which had just preceded the German one, the note was not there. Having read the note, Bateson managed to obtain Mendel's article and now, he was reading it on the train. And on the train, says Bateson, the shock led him to change the outline of his presentation. Gregor Mendel took centre stage! Mendelism thus landed triumphantly in Darwin's homeland.

The story of this landing is, most likely, very different from the above legend created by Bateson himself with the aim of tying his name closely to that of Mendel, after realizing that the achievements of this obscure Moravian monk could represent a fundamental stage in the history of biology. But he was not the only one; there were three other people who understood this. In the absence of exact dates of when these "rediscoverers" had Mendel's work in their hands, it is difficult to reconstruct the merits and demerits of each of them, despite the fact that there are various publications on the subject.

Anyway, a few decades after the rediscovery of Mendel's work, Mendelism and Darwin's theory of natural selection finally met and a whole new phase of biology began.

Telling biologists about the importance of Mendel for the development of genetics seems to me to be superfluous, even though some hints of eugenics, born from a first simplistic interpretation of natural selection combined with genetics, would make interesting reading. My drive to write this article arose from my desire to fill a gap. We know, so to speak, of Darwin's life, his death, and his miracles, whereas much less is known about Mendel. While many of us have surely read *The Origin of Species*, which is available in all languages and even in digital format, very few will have read Mendel's original article. And I must say that it was also not so easy to find it online in pdf. Those who are interested can find it here, in English.

The desire to fill this gap led me to look for books that were on psychological, cultural and social aspects of Mendel's life,

as well as details on the rediscovery of his work.

The most interesting source that I found, is *The monk in the garden: the lost and found genius of Gregor Mendel, the father of genetics* by Robin Marantz Henig (Mariner Books, 2001, available also digital format). For Italians: *L'eredità di Mendel* (Hoepli books, by Alfonso Lucifredi, 2018). It is from these books that I have taken many ideas, and it is to these books that I refer the reader for further information.

Early life

Johann (later Gregor as monk) Mendel was the only son, the second of three children. He was born in Heinzendorf (now Hynčice), in the north of Moravia (Austrian Empire), on 20 July 1822, to Anton, a farmer who had redeemed his land, and Rosine.

At the parish primary school, Johann was an excellent pupil and the constant appreciation by his teachers paved the way for him to move on to the next steps, middle school in Leipnik (now Lipník nad Bečvou) and the Imperial Royal Gymnasium in Troppau (now Opava). In 1839 Johann, then 17 years old, while on a summer vacation from the Gymnasium and while his father had health problems, became bedridden for four months. He did not see himself as a farmer, and this weighed heavily on him because, as the only son, he would have had to take over the management of the farm from his father. Was being confined to bed a sign of anxiety? Being in bed with a mysterious illness was to occur on other stressful occasions in later life, such as after his academic defeats. In the autumn, however, he returned to the secondary school which he finished, and subsequently enrolled at the Phylosophical Institute in Olmutz (today Olomouc) (1840-43). Do not be misled by the term "Phylosophical", a legacy of the pre-eminence of philosophy over other sciences in previous centuries. Scientific subjects such as mathematics and physics were also studied that institute. Incidentally, Galileo, in 1610, addressed his Sidereus Nuncius, to "*Philosophis* atque Astronomis" ('to philosophers and astronomers', in that order), and the full title of Newton's Principia (1687) is *Philosophiae Naturalis Principia Mathematica* (Mathematical Principles of Natural Philosophy). Even today the highest academic degree in any subject, PhD, means Doctor of Philosophy.

In order to keep up with his studies, given his relationship with his father, Mendel provided private tutoring to get by, but he was still in financial difficulties. His relationship with his family improved when his elder sister Veronika's husband took control of the farm management. From an economic point of view, the turning point was when his younger sister, Theresia, generously gave him a share of her dowry to help support his studies. Out of gratitude, Mendel was of great help to Theresia's three children who went on to attend the classical studies in Brno and who later became medical doctors.

Friedrich Franz, a priest and his physics teacher in high school, had great esteem for Mendel. In consideration of his financial difficulties, he directed him to the Augustinian convent of St. Thomas in Brno, where he himself had been for 20 years. This was in 1843.

In the convent

The convent was built in 1322 as a cold fortress to protect the Cistercian nuns. The Augustinians, towards the end of the 18th century, had their convent in a luxurious building in the center of Brno, the capital of Moravia at that time. But the Emperor Francis II of Habsburg-Lorraine wanted to make it his main residence in Moravia, and had the friars moved to the former convent of the Cistercians. The convent had been greatly improved with renovation to create larger and more hospitable lodgings.

In general, a convent is not associated with a center of scientific excellence, but the convent of St. Thomas was. The Augustinians had as their motto "*Per scientiam ad sapientiam*" (Through Knowledge to Wisdom). Erasmus of Rotterdam was an Agostinian. Anyway, it was above all for their motto that Franz, his professor, had directed Mendel to the Augustinian convent, with a letter of introduction for the abbot Cyrill Napp, in which he presented Mendel not as the most pious of his students, but as one of the best physics students. Franz knew his friend Napp well.

Brno (at that time Brünn) was a large cultural center of 70,000 inhabitants. There was a polytechnic, various orchestras, the above-mentioned philosophical institute and various scientific societies. These including the Moravian and Silesian Imperial Society for the Improvement of Agriculture, Natural Sciences and Knowledge of the Nation, of which abbot Napp was the president, since 1827. In 1807 the Emperor Franz II, moreover, had commissioned the monastery to provide teachers for mathematics and religion for the Philosophical Institute in Brno. It should not be forgotten that Ernst Mach (known for the speed of sound) and Milan Kundera are from Brno. The convent's library had more than 20,000 volumes and Mendel was to become a regular visitor. Brno was also a center of excellence in cooking, the cuisine of the convent in particular (which certainly influenced Mendel's waistline). The girls flocked there in order to learn and to later find work in the aristocratic palaces of Vienna, where Moravian cuisine was in high regard.

In addition to theology, Mendel, as a novice, had studied archeology, Hebrew and Greek. Abbot Napp, moreover, encouraged him to cultivate meteorology, botany, physics and mathematics. He also granted Mendel free access to the greenhouse. It should be noted that Mendel's interest in horticulture had already begun in elementary school.

In 1843, the year of Mendel's arrival at the convent, his co-brother Matouš Klácel, with whom Mendel had a long friendship, had become a gardener. Klácel was a philosophy teacher, but had made important observations on trees transplanted from the Moravian mountains to Brno, unaware of the changes that were thought to occur due to the change in the environment. Klácel had just lost his teaching position for some writings in favor of Naturphilosophie, in which concepts of evolution were foreshadowed. The publication of one of his writings was never authorized because it was seen as a defense of linguistic minorities that the Austrian Empire did not really appreciate. In 1848, the year of upheavals in Europe, Klácel and Mendel were among the seven signatories of a

petition in favor of the civil rights for priests in Moravia. The petition was ignored.

In 1847 Mendel was ordained as a priest, and for more than a year he carried out pastoral activities, also in contact with poor people, with the sick and dying. The stress, considering his character, was enormous and, for the second time, he was bedridden for a month without a specific illness. It was then that Napp started a series of initiatives that were to be crucial for Mendel's future. Without Napp we would never have known Mendel. Napp decided that Mendel should devote himself to teaching. He defended this decision against the opinion of Schaffgotsch, bishop of Brno, who was higher in rank than Napp in the ecclesiastical hierarchy but was much lower in the scientific one. Mendel had begun breeding experiments with mice, hosted in his rooms. Napp was obviously aware that Mendel was working with mice. However, when the bishop became aware of it, he forbade it; it was not appropriate for a monk. However, every cloud has a silver lining; Mendel left mice and turned to peas. By the way, whenever Mendel happened to talk about crossbreeding in class, there were some mischievous smiles of the students – to which Mendel would reply: don't be stupid, these are natural things!

In the autumn of 1849 Mendel thus started teaching mathematics and Greek in the Gymnasium of the city of Znail (today Znojmo), as a substitute. Mendel was, as always, successful in teaching; so much so that the Gymnasium itself recommended him for the certification exam in natural sciences and physics. The certification exams were held in Vienna. This was in August 1850. Months earlier Mendel had sent the commission a written test on zoology, meteorology and geology. The exams were personalized, meaning that the six examiners would be there just for him. In a letter dated 1 August, Baumgartner, a well-known physicist and chairman of the examination board, had summoned Mendel for mid-August for the oral exam. But then he realized that the teachers' holidays began on 12 August and sent a second letter postponing the oral exam to the autumn. This second letter, however, never reached Mendel, and Mendel unexpectedly appeared at Baumgartner's office. One can imagine that Baumgartner tried to persuade him to postpone the exam, also in order to avoid summoning the examiners, who were ready for the holidays. Contrary to his character, Mendel persisted, and the exam was set for 15 August. Mendel's insistence, despite his shy nature, was probably dictated by the desire to start the new school year as an official teacher. However, the poor written test, the probable bad disposition of the examiners due to the sudden convocation, and Mendel's awkward oral test ended in a rejection. It should be noted that Mendel's colleagues, in contrast, considered Mendel a brilliant teacher. Baumgartner sent a note to abbot Napp, perhaps due to the great good will shown by the candidate, suggesting that the self-taught Mendel should attend academic courses. This suggestion was fully accepted by abbot Napp, further demonstrating his benevolence and considerable esteem for Mendel. Vienna, the capital of the empire, was a scientific environment of the highest order. Mendel had to juggle, first of all, to find accommodation because the abbot, despite the acceptance for the courses, had not been able to find one in a

convent. The bishop objected to the fact that Mendel would have to give up his pastoral duties and also that he would be living in the city of Vienna and not in a convent. But Napp, it should be emphasized, still had the upper hand, and so Mendel left, albeit late with respect to the start of the courses, and spent two years in Vienna following courses not only in natural sciences, but also, perhaps above all, in mathematics and physics, in which he excelled. To confirm his abilities, Christian Andreas Doppler (of the Doppler effect of sound) offered him an additional position as "élève" (students in the role of laboratory assistants). This was a sign of appreciation, given that Mendel had started attending classes late due to the problem of accommodation, and that the 12 available places as élève had all already been assigned. However, Doppler became ill and left teaching early. He was succeeded by Andreas von Ettingshausen, a talented mathematician and physicist, also the author of a book on combinatorial calculus. Juggling with numbers and their combinations was to become fundamental for Mendel. His scientific training, his sense of humour and his ability to laugh at himself are evident when he blamed Newton's law of universal gravitation for the difficulty he had in climbing up the hill to get to the bee hives, given his afore-mentioned waistline.

Equally important for Mendel was the teaching of the botanist Franz Unger on the hybridization experiments that had been conducted by the German botanists Josef Kölreuter and Karl Friedrich von Gärtner. This was the period in which Matthias Jakob Schleiden and Theodor Schwann discovered that all plants and animals are composed of cells, fully landing biology into the field of exact experimental sciences. Unger introduced Mendel to *Pisum sativum*, which English botanists had described as being very suitable for controlled hybridizations, for the shape of the flowers, for anthers that could be easily manipulated, and for the easy identification of some of the characteristics. Carl von Nägeli, a renowned botanist from Munich, was a strong promoter of the thesis that the phenomena of life could be traced back to physical and chemical laws. Unger had great deal of reverence for Nägeli, which he passed on to Mendel; this turned out to be unfortunate for Mendel as we will see later on. Mendel had undoubtedly heard from Unger about metamorphosis and transmutation (a word later replaced by evolution), even if the ideas were far from clear at the time. Later on, some of Mendel's fellow students, such as Johann Nave, a law student, were very interested in Unger's lessons. Nave moved to Brno in 1854 and, even as a lawyer, he continued to cultivate botany and friendship with Mendel. Later they became two of the founding members of the Society for the Study of Natural Sciences of Brno.

Upon returning from Vienna Mendel resumed teaching, this time in the Realschule (Royal School) of Brno itself, though with half the salary of a licensed teacher, and continued to cultivate peas to be sure that he had purebreds in hand.

In 1856, six years after the debacle of the exam for teaching certification, it was time to try again. But the result was worse than the first time. After stumbling on the first question, Mendel walked out. We don't know what happened exactly. However, we have some clues involving Eduard Fenzl, his examiner and one of his botany teachers.

Fenzl was a convinced "preformist", i.e. an advocate of the idea that pollen, or the male gamete in general, was already preformed and that it lacked only an appropriate environment in which to develop. Mendel absolutely did not share this theory. Did he prefer to capitulate rather than surrender? We don't know for sure. What we do know is that Mendel was in bed again. And it took a visit from his father and uncle to persuade him to get out of bed and get on.

We do not know who met Mendel during his last and short stay in Vienna. Was it his botany professor Franz Unger? If so, it is likely that the two returned to talk about the non-fixity of species. Unger had nearly been fired for such ideas. Darwin's *The Origin of Species* would not be published for another three years (1859). It should be noted that the Moravian Catholic Church was very progressive, with excellences in the scientific field (Napp among them) and was very distant from the heavily conservative positions of Pope Pius IX.

After the debacle and the bed, Mendel kept himself very busy with peas and the garden that gave the friars a lot of vegetables, including the cucumbers that Mendel loved. “Prepare the cucumbers, I am coming”, he wrote to his relatives announcing a visit.

An important and positive event of this period is the fact that Napp built a much larger structure than the existing greenhouse at the time, in which Mendel was able to start cultivating on a larger scale. After having followed the lessons of von Ettingshausen, Mendel was very clear about the concept that small numbers correspond to very large fluctuations, which are difficult to interpret. Did this demanding work give Mendel a sense of revenge / redemption / refuge following his academic debacle? One cannot exclude it.

It was again Napp who, in 1855, built a second, smaller greenhouse which was heated in winter, for citrus fruits. Mendel made it his favorite refuge, equipping it with a writing table, six cane chairs and a chess table; chess was his passion. We can imagine him playing chess with his youngest nephew during the time he was at the Gymnasium in Brno, holding in his mouth one of the twenty cigars he smoked every day as a slimming cure prescribed by his doctor. Later, when he became an abbot, he usually entertained his guests in this greenhouse.

By 1856 all of Mendel’s problems seemed to have been overcome. The purebreds were ready for hybridization and teaching was very rewarding. During this period he took on the official post of a meteorologist, with three daily observations to be recorded and with reports to be sent monthly to Vienna. His fame as a meteorologist was Mendel's greatest scientific recognition during his life.

Hybridization experiments

It was 1856 when the first hybridization experiments started: smooth x wrinkled peas. The sowing of the two purebreds had possibly begun on St. Gregory's day (12 March); according to a Moravian saying "Whoever does not cultivate his land within Gregory's day is a lazy man". In May Mendel worked hard on hybridizations. The two varieties were in alternate rows. Armed with tweezers, he would run along the first row of peas castrating the flowers of the male part, the anthers, before they mature, and covering

each flower with a cap. For the second variety, in the second row, he waited for the anthers to be ripe, for the pollination of the first row, which he did with a camel hair brush, carefully putting the cap back on the flower to avoid intruding pollen. To get an idea of the amount of work and the patience required, see Youtube documentary.

Plant hybridization was a widespread experimental practice, but the interpretations were often opposite to those that Mendel later obtained. Josef Kölreuter, a German botanist, had crossed two species of tobacco, *Nicotiana rustica* and *Nicotiana paniculata*. After two years, *N. rustica* had transformed into *N. paniculata*. Kölreuter was amazed and puzzled, given that his philosophical / religious background was very similar to that of Linnaeus: "*Species tot numeramus quot a principle creavit infinitum Ens*" (There are as many species as the Infinite Being produced diverse forms in the beginning). It was therefore with relief that he found that the plant had become eventually practically sterile. Charles Naudin, a Frenchman described the hybridization experiments he had carried out in his 1862 essay (which Darwin had read) for the Parisian Academy of Sciences. But he too had the above mentioned Linnaean prejudices. According to him, the return to the parental form of his primroses occurred because "Nature is eager to dissolve hybrid forms ... which art or chance has violently brought together ". If Naudin had read Galileo's Il Saggiatore he would have learned that "The book of nature is written in the language of mathematics." In all the observations of Naudin and his contemporaries (including Darwin!), mathematics was conspicuously absent. In Mendel's mind, however, mathematics was very much present, especially in his most recent expressions of combinatorial calculus, which he had learned from von Ettingshausen in Vienna.

Even if these prejudices were not present in other cultural contexts, the search for new varieties was almost always subordinate to an economic / practical return, as specified by the call (sic!) of the Dutch Academy of Sciences to which Karl Friedrich von Gärtner (nomen omen) had answered. Call for applied research, we would say today.

Gärtner had published his work but his influence on Mendel was limited because, as Mendel complained, the "Materials and Methods" of his papers were very very lacking. In any case Mendel did not have the aforementioned prejudices. In a certain sense it is surprising if we consider that Mendel was a monk, but it is really no surprise when we consider the cultural context of abbot Napp's convent. It should, of course, be noted that no one before Mendel had made such an accurate experimental plan for the sole purpose of scientific curiosity. A full-blown basic research program, with two years devoted to preliminary data (purity of varieties). Mendel's basic research would be one of the most impactful in the history of science, but that did not happen immediately. Today, a similar example of our dangerous times is Katalin Kariko's research on RNA, the importance of which was not immediately recognized but which was later reconsidered to produce vaccines against COVID-19.

Let's go back to Mendel's peas. Mendel chose seven varieties for his experiments.

- seed shape, round or wrinkled
- seed color, green or yellow
- pod inflated or constricted (to sur round the peas like a tight dress)
- unripe pod color, green or yellow
- position of the flowers, only at the apex or distributed on the stem
- flower color, purple or white
- stem length, tall or dwarf

We now know that 5 of these 7 loci are on different chromosomes, and the two on the same chromosome are at opposite ends. So, there was no linkage to complicate the interpretation of the results. One might say that it was really luck! But that was not the case or if it was, it was only partially so, because initially Mendel had considered 34 traits. The two years for the "preliminary data" served not only to check purebreds, but also to choose those traits that would not give problems in interpretation.

There is one more point: the temporal sequence of the experiments and the underlying plan are deduced from the two presentations in 1865 to the Society for the Study of Natural Sciences of Brno, which later merged into the publication of 1866, and from some letters addressed to German botanists. Many of his notes were burned, most likely, by Abbot Anselm Rambousek, his successor (see below). The order of the experiments reported in the publication may have been adjusted, a posteriori, to give logical clarity to the presentation.

The annual rhythms of his experiments which began in 1856 were: preparation of the soil and sowing in March, pollination in the spring and data collection in summer or autumn depending on whether the feature to be examined was flowers, pods or peas. For the last two cases Mendel carried the sacks of the pods, whose origin had been well marked, to the greenhouse. Then sitting on one of the reed chairs, he patiently opened all the pods and placed the peas in other bags, labeled according to the characteristic and the row from which they came. This was done from 1856 to 1863. Just in one year, 1856, he analyzed about 7,000 peas, derived from crossings for the characteristics of the shape. He then moved on to color and finally to height. Then there were two experiments of dihybrids (yellow and round peas, i.e., double dominant and green and wrinkled i.e., double recessive). The complexity increased in the case of trihybrids, with three dominant and three recessive traits. Dihybrids and trihybrids were to answer the question of whether segregation was independent (so, not much was left to luck). The backcross experiments probably came last, as crucial evidence on the theories gleaned from earlier experiments. Here one should note Mendel's meticulousness. For each experiment he could rely on a precise reconstruction of the experimental path of the seeds in question.

Mendel is estimated to have analyzed a total of 10,000 plants, 40,000 flowers and 300,000 peas; counting, counting, counting… It was a mindset learned in Vienna (von Ettingshausen) that Mendel applied in his meteorology tasks and also in other things. Just to give me an idea of these figures, I bought one kg of peas and I counted ~800 of them.

Details of experimental results

From the F_1 hybrids of the round x wrinkled peas, Mendel could not conclude much as they were all round. We then come to F_2, the result of the self-pollination of 250

F_1 plants. The counts were 5,474 round and 1850 wrinkled peas (25%). The most important point here is: the wrinkled line had not disappeared, and Mendel set the result as a 3:1 ratio. He then moved on to color, which obviously cost him more work being linked to direct observation on the plant, but again, he obtained identical results. The ratio was 3:1 and the recessive trait had reappeared. Mendel certainly began to speculate, but in the meantime the concepts of "dominant" and "recessive" were very clear to him. He used the notation "*A*" capital letter for dominant and lowercase "*a*" for recessive. No one else had used these terms and this notation, which tells us that Mendel held the right key for all subsequent interpretations, including the concept that only one form, "*A*" or "*a*", passed from parent to offspring. Naturally Mendel did not have the concepts of diploidy, haploidy and meiosis, which were still to come with the chromosomal theory of heredity.

Let us go back to the 3:1 ratio. We do not know when exactly the whole mechanism of inheritance was clear to him, but between 1858 and 1860 Mendel knew with certainty that the self-pollination of F_1 (we would say heterozygous) produced 3 types of plants: homozygous *AA*, heterozygous *Aa*, homozygous *aa*. So, looking at the phenotype, as we call it, can be deceiving. Yellow peas could be *AA* or *Aa*. With subsequent experiments Mendel was able to demonstrate which were *AA* and which *Aa*. The 3:1 ratio thus became 1:2:1.

All these experiments clearly demonstrated the independence of traits, but what concept did Mendel have of a "trait" or "characteristic"? It was certainly far from today's concept of gene. But how far? It should be noted that Mendel used the German word *Merkmal* (trait, in the phenoltypic sense) 150 times, while *Elemente* (element), was used only 10 times and only in the plural. The reference to the German terms is important because the English translation of the early 1900s translates *Merkmal* as “unit” or “factor” or “determinant”, which appear as a discreet tug of a jacket, or rather of a cassock, to Mendel. The concepts of chromosome, mitosis and, above all, of meiosis and DNA were still unknown.

These years were happy years for Mendel. His teaching of science subjects at the Realschule in Brno was much appreciated, he enjoyed the Moravian cuisine (they say his waistline attested to this) and he found full satisfaction in his engaging work on peas. To invite one of his visitors to see his pea crops he would ask "Would you like to see my children?"

Mendel traveled very little in his first 40 years, but 1862 marks a major exception. On 24 July Mendel, as a member of a delegation from the Realschule of Brno, left for the grandiose London International Exhibition, mainly focused on science and organized per nation. This confirms the scientific level of the Realschule and of Brno in general, as well as the regard in which Mendel was held. The contribution of the Realschule to the exhibition was on crystallography. Another important reason was that Brno was planning to set up a museum of technology and the exhibition could certainly have given some ideas. A photo shows the large delegation in front of the Grand Hotel in Paris, where they had made a stop on their long journey to London. Mendel is in a jacket, light shirt and dark tie.

At the exhibition there was everything, from the perpetual engine to a mustache guard to eat broth safely. On display was the embalmed body of the famous Mexican Julia Pastrana, bearded (suffering from hypertrichosis), presented as "part human, part orangutan" (!) It was thought that this exhibition would have been as an ideal opportunity for Mendel to meet Darwin. However, Mendel had not read Darwin's *The Origin of Species*, which was published in1859, as he was not able to read English. He had also not yet read the German translation *Über die Entstehung der Arten* which appeared in 1860. His German copy was, in fact, the second edition dated 1863. It is worth noting, however, that when he did read it, the book had aroused a great interest in him judging from the parts he had underlined, sometimes even double underlined, and from the exclamation marks. Anyway, a meeting did not take place. Just in those days Leonard, Darwin's 12-year-old son, was in bed with scarlet fever. But one also needs to take into account that Mendel was an obscure Moravian monk who was shy and withdrawn, while Darwin was the most famous naturalist of the time, at the center of the scientific debate on his The Origin of Species. Furthermore, Mendel had not yet completed his experiments, although it was already clear to him that the traits do not mix but are passed on to subsequent generations as discrete elements. Most other naturalists, including Darwin and his cousin Francis Galton, believed instead that they blended, as if they were two tints of colors.

Upon his return to Brno in the fall, Mendel resumed teaching and returned to his experiments. This time he worked with the trihybrids, i.e. crosses between plants that differed in three distinct characteristics: pea shape, pea color, and seed coat color. The reason for choosing the color of the pod is not clear. This depended on the plant, not on the seeds, so this last feature would manifest itself in the next generation, about nine months later; this greatly complicated the getting of results. In this period Mendel had an aide, a fellow monk, Alipius Winkelmayer.

Then we come to the test of fire. In one experiment Mendel crossed the double hybrids *AaBb* (yellow and round) with the double dominant (*AB* for Mendel, *AABB* for us). No recessive traits appeared as expected. In the second test he crossed AaBb with the double recessive (similarly ab, i.e. *aabb*). Here he expected that the 4 possible types of gametes, *AB*, *Ab*, *aB*, *ab*, would result in 4 types of combinations (yellow and round, yellow and wrinkled, green and round and green and wrinkled) with a ratio of 1:1:1:1. Indeed the counts turned out to be 55, 44, 51 and 53 respectively. Triumph! Containing his excitement Mendel commented: "There could now be scarcely any doubt of the success of the experiment". It is now clear to us that Mendel had hit on the difference between phenotype and genotype. The result was so important that, over the next two years, Mendel checked that the self-pollination of each of the four categories of dihybrids gave the expected results; that was so. Mendel became so sure that he looked for confirmation in other plants (e.g. beans, snapdragons, the carnation of poets, corn). He concluded: "the law of development discovered for *Pisum* applies also to the hybrids of other plants."

The "Eureka!" of Archimedes is the experience of suddenly understanding a pre-

viously incomprehensible problem or concept. Darwin's Eureka moment was when, realizing the unity of life on earth, he drew that "1" at the base of the tree of life in his notebook, humbly cataloguing it with "I think". Surely, also in Mendel's life there must have been a moment when, from the bulk of his data, everything suddenly fell into place and the laws of segregation emerged. However, we have no hint of such a "Eureka" moment for Mendel. The reason may be quite simply the lack of notes on his experiments. All Mendel's possessions were gathered and burned in a bonfire when Anselm Rambousek, who, unlike Mendel, belonged to the Czech majority in the Brno region, took over as abbot after Mendel's death. It was not until the early 1900s when Mendel was rediscovered, about two decades after his death, that Information and anecdotes about his life began to emerge; this was mainly from his correspondence with others, for example, with Nägeli (see below).

Speaking of numbers, Ronald Fisher published an article in 1936 in which he insinuated that Mendel's numerical results were fraudulently adjusted to better adhere to the expected. The controversy has now been cleared. Noel Hellis et al. (Hereditas, *2019)* examined the data and concluded: "It seems clear from this re-examination of Mendel's data that the frequency distribution of genotypic classes is entirely as would be expected from his experiments..."

In the winter of 1864/65 Mendel was probably busy preparing the report to be presented to the Society for the Study of Natural Sciences of Brno, of which he was one of the founders. The report was scheduled for Friday 8 February 1865 to be held at the Realschule, where he had been teaching for several years. One can just imagine him going to the Institute with some brothers, holding his manuscript under his arm and some peas as an example of the varieties he had used. The audience was made up of about forty people coming mainly from the academic world, from Realschule itself and from other schools. His lawyer friend from Vienna, Johann Nave, who was passionate about botany, had died a year earlier and was missing. Perhaps he would have been the only one who fully understood his presentation. After the introduction by the vice-president of the Society, Mendel read his report. We can imagine him a bit awkward, with a somewhat monotonous voice, rattling off numbers. After about an hour he ended his lecture with an announcement that there would be a second presentation soon. He invited questions but there were none. Perhaps no one had understood. The second report would take place four weeks later, on 8 March. Also then there was no reaction. One cannot help comparing this with the meeting on 1 July, 1858, when the theses of Darwin and Wallace were read in front of the Linnean Society in London, that is, in front of the cream of the English scientific establishment. But even there no one realized that those concepts would be perhaps the most important cultural revolution of their time (to say the least).

Almost certainly, it was the mathematical calculations underlying the segregation of the gametes that proved difficult for the audience. One should also bear in mind that meiosis, as the bases of the segregations, was discovered only 25 years later. Furthermore, Mendel's second report with data on other plants was a bit problematic. While the

experiments on the beans (*Phaseulus vulgaris* x *Phaseulus nanus*) were confirmative, those for the colour of the *Penstemon* flowers were not. By reporting these results he was probably trying to invite other botanists to repeat and expand his experiments. But by doing so, he implied that his results were perplexing.

Finally, Mendel addressed the question of speciation. And here, for me, was the biggest surprise. I had always thought that Darwin and Mendel each had half of the treasure map and that the two parts had only come together in the early decades of the 1900s. However, the last part of Mendel's presentation said something different. They were Mendel's own words that made it clear, not those of benevolent interpretations of admirers. The speciation issues were not new to professors in Vienna (Unger in particular), and, after all, Mendel had read *The Origin of Species* very carefully and with great interest.

Discounting Lamarckism, Mendel claimed that the law is the same for everyone. "No one will seriously maintain that in the open country the development of plants is ruled by other laws than in the garden bed ... Here, as there, changes of type must take place if the conditions of life be altered, and the species possesses the capacity of fitting itself to its new environment". And "If adaptive changes occur in response to environmental influences they tend to be conserved and to be transmitted to subsequent generations" (... according to my laws, he might have added). This sentence reminds me of the title of the famous book by Jacques Monod *Le Hasard et la Nécessité* (Chance and Necessity). Mendel further went on to say: "Nothing justifies the assumption that the tendency to form varieties increases so extraordinarily that the species speedily lose all stability, and their offspring diverge into an endless series of extremely variable forms". No, indeed! The rule is stability (necessity). Here perhaps lies the biggest difference in perspective between Mendel and all those who had dealt with "transmutation." Many were looking for the cause of variability, the "force vitale". As a demonstration of the vagueness of ideas in biology at the time, one should bear in mind that the concept of spontaneous generation had been definitively buried by Pasteur only just a year earlier, in 1864. The laws that Mendel introduces instead speak of a stability that is expressed in precise mathematical ratios; a complete novelty in biology.

The Mendel's report was published by the Society for the Study of Natural Sciences of Brno in its proceedings, in 1866. The title was *Versuche über Pflanzen-Hybriden* (Experiments on Plant Hybridization), not a particularly exciting title, especially since there had been quite a few similar publications before. The Society sent 133 copies to various academic institutions.

Mendel was aware of the importance of his findings and ordered an unusually large number of reprints, 40, which attest to his desire to disseminate his data. In those days, the pages of books were printed on large sheets (parent sheets), then folded to form the book. The edges of the sheets were not always cut to separate the pages, and Mendel's reprints were not cut. This meant that an uncut reprint = an unread reprint. Of many reprints we do not know how they got to where they were later found. We know 12 certain recipients, and the history of some of these is important.

A reprint, it hurts to say, was received by Darwin but was found uncut. German was then a language widely used in the scientific field, especially for the natural sciences, and, according to Francis, son of Darwin (as reported on this page of the site The Friends of Charles Darwin), Darwin would not have had too much difficulty in reading it; but he did not. Darwin must have thought: Ah! One of the many and moreover by a stranger! Darwin had dismissed the work of Naudin who had done similar experiments, by saying "He cannot, I think, have reflected much on the subject." Darwin too had done some hybridization experiments, probably around the same time, as mentioned in one of his publications of 1868. He had crossed snapdragons, red flower x white flower, noting a "prepotency" of the red. But in the next generation obtained by self-pollination, he had found a red/white ratio of 88 to 37, which is 2.4:1, a ratio that was not reported by Darwin. So, that ratio was closer to a misleading 2:1 than Mendelian 3:1. Who knows, if the title of Mendel's paper had been clearer, such as: Die, bald berühmten, Mendelschen Gesetze (The, soon to be famous, Mendel's Laws 😂), it may have made an impact.

The story of two other reprints is also noteworthy. One was sent, no one knows by whom, to Martinus Beijerinck, a Dutchman, who surely read it. Beijerinck was aware that his young Dutch colleague De Vries was working on the hybridizations of *Oenothera lamarckiana* (evening primrose) and *Zea mays* (corn) and sent De Vries the reprint, saying that it was by a certain Mendel in 1866, and that it could be useful to him. To fully understand Mendel's influence on De Vries, one would need to know when exactly the reprint was sent to him, but that is not known. This story is dealt with in more detail below.

The third reprint landed on the desk of Carl von Nägeli, University of Munich, with a cover letter in which Mendel showed all his (excessive!) reverence towards the renowned luminary and asked for advice.

On 27 February 1867, Mendel received the answer from Nägeli, the only one to answer him. But he was not very encouraging, quite the contrary. Nägeli was skeptical of Mendel's assumption that the forms "A" and "a" are stable: "I expect that sooner or later they will change once again". From this and other comments it can be reasonably assumed that the luminary did not understand anything, probably conditioned by the fact that he had opposing views to what Mendel asserted. Nägeli believed in the blending of the parental traits, which was the common opinion of the time, of Darwin in the first place. Agreeing with Mendel would have meant recognizing that he, Nägeli, was wrong.

What a disappointment for Mendel! In his reply of 18 April 1867, Mendel explained again the experiments and the conclusions in greater detail: "The course of development consists simply in this: that in each generation the two parental traits appear, separated and unchanged, and there is nothing to indicate that one of them has either inherited or taken over anything from the other." To Nägeli's objection about the correctness of his deductions from the statistical data, Mendel replied: "…I have proved by previous experiments that the development of a pair of differing traits proceeds independently".

Nägeli did not reply to this second letter. It must be said that Nägeli had health problems at that time. Mendel then sent a third letter on 6 November 1867, always with the attitude of a schoolboy, avoiding the subject of peas (Nägeli obviously didn't like them) but talking instead about what Nägeli liked, that is, the (unfortunate!) *Hieracium*. He also said that he was waiting impatiently to see the results of the hybridizations he had done on this plant. Still silence from Nägeli! Mendel did not give up and sent a fourth letter on 9 February 1868, with a change of tactics. He tried to coax Nägeli by asking him to send him 12 samples of different species of *Hieracium* on which he would consider hybridizations. Mendel was a notorious expert in these things, and the *Hieracium* flowers were anything but simple to manage. Nägeli finally responded to this tempting offer, very briefly, by promising flowers and seeds.

On 4 May 1868, Mendel wrote again, but his life, in the meantime, had taken a radical turn. On March 30, 1868, Mendel was elected abbot of the monastery of St. Thomas as the successor of Napp, who died the year before. He would later receive plants and seeds from Nägeli but would be able to work on them only in his spare time. Slowly the hope that his extraordinary discoveries would be recognized faded away until it disappeared. The election as abbot, which he much appreciated, though he did not show it, partially rewarded him for these disappointments. As abbot he entered the "nomenclature" of Brno, with fairly regular meetings and receptions in the convent itself.

Nägeli did send him the requested material and Mendel, albeit at a much slower pace, got to work. But the *Hieracium* was really hard work. The manipulation was very difficult and had to be done with a microscope; Mendel's eyesight no longer allowed him such precision work. But the most unfortunate thing was not being aware that this kind of plants rarely undergoes sexual reproduction. In fact, apomixis (parthenogenesis in animals) is the rule. The results, therefore, contradicted his earlier results. Mendel honestly reported these new data in a further report to the Brno Society for the Study of Natural Sciences, which was regularly published in the Society's Proceedings. But this time Mendel did not ask for any reprints. One can just imagine how demoralized he was. There were other exchanges of letters with Nägeli, but were no longer of much importance for Mendel's scientific work. It seems that he did not talk about his experiments anymore. To a French visitor, a trader, who, intrigued by pea crops, had asked him questions, Mendel replied that it was a long story that would have taken too long to tell. Who knows, perhaps the answer was followed by a long sigh.

Mendel, however, continued to be interested in what was going on in the world of biology. He read the German translation of Darwin's second book *Variation of Animals and Plants Under Domestication* very carefully, perhaps hoping to find reference to his work but there was nothing. Until 1881, no one had heard of Mendel with the exception of Nägeli. In 1881 Wilhelm Olbers Focke published a book on Plant hybrids, *Die Pflanzen-Mischlinge*, and mentioned Mendel 15 times, but not in a flattering way. He said that “Mendel

believed that he found constant numerical proportions between the types of hybrids," (by *he believed*, did he mean *he was deluded*). He added, and it is not clear why, that the monk's work followed the tradition of the first hybridizers, that is, that hybrids tend to return to the parental form, a well-established preconception at the time (see Naudin). In practice, Focke had understood nothing of Mendel's work. However, Focke's views on Mendel were transferred to the Encyclopaedia Britannica.

In fact, there were others who mentioned Mendel while he was alive, but all without having understood much. Anyway, Mendel never learned of it.

Not understanding much of it has already been pointed out and will come back later. The reason must probably be sought in the fact that Mendel's mathematical-physical background, with particular reference to combinatorial calculus, was lacking, to say the least, in the botanists of his time. If we add to this the many prejudices, there is probably sufficient explanation for why most botanists could not understand Mendel.

Mendel, even as abbot, continued to be the official meteorologist of Brno, and described with detached accuracy, with humorous and witty passages, the tornado that hit the convent on 13 October 1870, which among other things destroyed a large part of the greenhouse. Mendel was shy, but humour, wit and jokes were also part of his character, and he was a regular reader of the humorous magazine *Die Fliegende Blätter* (The Flying Leaves).

It is worth noting his reaction, as an abbot, to the imposition of a heavy annual tax on the convent, in 1874, due to the disastrous situation of the imperial coffers. The shy monk appeared very determined, almost aggressive, in legally defending his convent. But this, perhaps, made him feel isolated. The only three people he trusted became his three grandchildren, the children of Theresia, who were in Brno for the Gymnasium. In Ferdinand, the youngest, he had found a capable chess challenger.

On 6 January 1884 Mendel died from a kidney disease. The obituaries spoke of his love for gardening, for meteorology and for beekeeping. No mention was made of his hybridization experiments. Gustav von Niessl, astronomer and mycologist, commemorated him during one of the sessions of the Society of Natural Sciences in Brno, but again without reference to his scientific achievements. Von Niessl lived long enough to see the rediscovery of Mendel. Only then did he confess to Hugo Iltis (Moravian, natural science teacher at the same Realschule where Mendel had taught, and the future first biographer of Mendel), that Mendel used to say to his friends "My time will come".

Many of his notebooks were burned in a large bonfire, as mentioned earlier. Mendel's correspondence with Nägeli, published by Karl Correns, had been obtained from Nägeli, who had been Correns teacher, and whose niece Correns had married.

Mendel rests in the Brno cemetery. The young Johann Mendel, as a student, in a poetic composition that was to celebrate a personality, imagined Gutenberg saying that his reward would be:

"That of seeing, when I arise from the tomb,
My art thriving peacefully
Among those who are to come after me."

1900, 34 years after Mendel's publication and 16 years after his death.

Anyone who has been preceded in the publication of an article by a colleague, perhaps can only guess how Karl Correns, professor of botany in Tübingen, felt on that Saturday, 21 April 1900 when reading the first version of the paper, the one in French (without the extra note as mentioned at the beginning) by his Dutch colleague Hugo De Vries. It had been published a month earlier in *Comptes Rendus de l'Academie des Sciences*, the official journal of the French Academy of Sciences. To understand the situation one needs to know that Correns was already aware of Mendel's publication. De Vries' article was also about the transmission of traits in plant hybrids. De Vries had narrowly preceded Correns in publishing research papers a few times, particularly one on the phenomenon called xenia, which was a hot topic at the time. But that was not the only reason for his irritation. According to Correns, De Vries understood the discreet nature of the elements that were transmitted from one generation to another, but, he had not fully understood the rules underlying these passages. Furthermore, Correns had a strong suspicion, which turned out to be justified, that De Vries already knew about Mendel's work but had intentionally ignored him in order to be able to prove later on that he had arrived there by himself. So, Correns decided to act. He immediately sent a note to the German Botanical Society.

De Vries

De Vries in 1889 had published a work that took up the hypothesis of Darwin's pangenesis. Towards the end of 1890 he started a project on mutations or "monstrosities", which he believed occurred randomly, for unknown reasons. De Vries regarded these monstrosities as the driving force of evolution. He had accidentally found a genus of local plants, *Oenothera*, which was particularly prone to monstrosities; he focused in particular on the *Oenothera Lamarckiana*, now *Oenothera glazioviana*. This work had increasingly convinced him of the correlation between the emergence of new species and mutations. De Vries had planned that at the beginning of the century, a series of publications would culminate in the release of the first of two volumes entitled *Die Mutationstheorie*, The Theory of Mutation. In 1890 he made hybrid experiments between *Lychnis diurna* (hairy) x *L. vespertina glabra* (smooth) which, at F_2 (self-pollination of hybrids) had given him a hairy/smooth ratio 99:54, obviously interpreted as 2: 1.

However, when in 1899 De Vries reported the above crossings at the First International Conference on Hybridization and Plant Breeding, held at the Royal Horticultural Society in London, he presented the 99:54 ratio as 3:1! Had he already read Mendel? However, he concluded in an interlocutory way that there was a lot of work to be done. Bateson, the eminent English zoologist (mentioned at the beginning) was also present. Bateson was the main supporter of the discontinuous variation of evolution, as opposed to the Darwinian line which saw evolution moving on a "continuous" line of variations, that is, with small imperceptible increases on which natural selection acts. Bateson's theories matched perfectly with De Vries' monstrosities. The two had made contact,

and De Vries had stopped at Bateson's house in Cambridge before they both went to London for the conference.

Bateson, in his speech, pointed out that in order to understand the results of the hybridizations in the context of evolution, it was absolutely necessary to examine the progeny of such crosses *statistically*. The word *statistically* is in italics in the written text of his speech. The question also applies to Bateson: had he already read Mendel? Other phrases that insist on the need for statistical analysis reinforce this assumption. R. A. Rolfe, a speaker that followed, summarized the history of the hybridization experiments, and in his summary the name Mendel appeared for the first time since his death. However, he referred to the experiments with *Hieracium*, which, as mentioned above, were problematic. Probably no one noticed the name of Mendel.

De Vries' work first appeared in French in the *Comptes Rendus*. On 27 April 1900, De Vries gave a seminar to the German Society of Botany; the Proceedings of the Society were published in German. It is here that De Vries had added a note, which was not very prominent, but which gave Mendel the rightful credit. He also added that indeed he had adapted his terminology to that of Mendel, but that he had learned of Mendel's work only after his experiments were practically completed and conclusions already drawn.

Correns

Correns' biting note to the German Society of Botany, entitled *The law of G. Mendel on the behavior of the progeny of hybrid varieties*, was sent on 22 April and published on 27 April, after the German version of De Vries' paper with the note on Mendel had already been published. Correns did not go so far as to use the word plagiarism but that was his message. He claimed that he too had come to the same conclusions, but that he had realized what Mendel had done so many years before him. This was to emphasize the difference in the sense of morality between himself and De Vries. He also remarked how the terms "active" and "latent" that De Vries had always used before, had suddenly become, in the *Comptes Rendus*, "dominant" and "recessive", terms which only Mendel had used. Correns also listed a number of other inconsistencies. He was, for example, surprised that De Vries had not reported any exceptions to these laws; exceptions that Correns had found.

Note that Correns used the terms *Merkmal* or *Elemente* (see above) instead of the word "anlage" which (they tell me) is closest to the term gene, in the sense that it refers, in a certain way, to the code that generates the characteristic, more than the characteristic itself. He added that the full set of these "anlages" could be located in the nucleus. Correns then expressed for the first time the notation 9:3:3:1 for dihybrids. It can be deduced from Mendel's work, but Mendel had never reported it in this way. Correns also introduced the terms "segregation" and "independent segregation" as Mendel's laws. Correns honestly acknowledged that recent advances in biology, unknown to Mendel, had been of great help to him in defining these laws, thus recognizing Mendel's remarkable scientific stature.

Correns, therefore, says that he had arrived at Mendel's laws on his own. Is that

believable? Correns, as mentioned, knew Nägeli very well. Could it be that Nägeli, who surely knew what Correns worked on, did not tell him about Mendel? And why had Correns focused on peas?

Von Tschermak

The third person to work hard to enter Mendel's club of rediscoverers is Erich von Tschermak, a Belgian, who first worked on peas and wallflowers in Belgium and then in Vienna. He was the grandson of Eduard Fenzl, one of the examiners who had failed Mendel on his second attempt. Von Tschermak's paper is dated June 1900. His results are preliminary, but it is very clear that he had not grasped Mendel's essential points, the combinatorial process of characters in particular. He struggled a lot to be among the Mendel's discoverers, but for many he is not.

Bateson (who in 1905 coined the term "genetics" from *genētikós, o*rigin; the term gene was coined later)

In 1899 Bateson met with De Vries and became enthusiastic about the theory of "monstrosities", which supported his ideas on the mechanisms of evolution, and set to work spreading these ideas in England. On 8 May he was on the train to London to give a seminar at the Royal Horticultural Society.

The "legend" mentioned at the beginning made this speech a milestone for the spread of Mendelism in England. But this legend is not convincing. The timing of getting hold of the German version of De Vries' work published in the *Proceedings of the German Botanical Society*, which carried the note on Mendel, does not add up. The timing of getting hold of Mendel's work (which was in the Cambridge library anyway) just before his trip to London on 8 May also does not add up. The timing fits in with De Vries' work in *Comptes Rendus*, but there the note on Mendel was not present. Finally, in the Proceedings reporting Bateson' intervention published in *Gardeners' Chronicle* on 12 May, there is no reference to Mendel. It is likely that the legend of the moment of epiphany on his way to London is a legend created by Bateson himself, to place Mendel in the Olympus of science but also, perhaps above all, to stand beside him as his prophet. In 1902 Correns published the English translation of Mendel's article titled *Mendel's Principles of Heredity: a Defense*. In the preface, Bateson explained how evolution proceeds by "discontinuous" changes and not by "continuous" passages. He coined the term "Mendelians" for himself and for the followers of the new theory, as opposed to the "biometricians", against whom he thundered in one of the passages: "Exactness is not always attainable by numerical precision, there have been students of Nature, untrained in statistical nicety, whose instinct for truth yet saved them from perverse inference, from slovenly argument, and from misuse of authorities, reiterated and grotesque".

It is amusing that Bateson's admiration for Mendel had led him to become, like Mendel, a chess player, a cigar smoker, and a reader of the humorous magazine *Die Fliegende Blätter* (The Flying Leaves) which, as mentioned, Mendel liked a lot. De Vries, on the contrary, moved further and further away from Mendelism. In his book *Pflanzenzüchtung* (Plant Breeding) Mendel is not mentioned, and in 1908 he refused to

sign the petition, with von Tschermak as the first signatory, to erect a monument to Mendel in Brno (see below).

Bateson became Mendel's bulldog just as Thomas Huxley was Darwin's. There was this famous debate of 19 August 1904 in Cambridge between him and the leader of the biometricians, Frank Raphael Weldon. Weldon was once a friend of his, but now there was a fight, no holds barred, as evidenced by reciprocal accusations contained in the letters and counter letters published in Nature. The debate started with a surprise: the biometrician Arthur Dukinfield Darbishire, a pupil of Weldon, changed sides to support the Mendelians (probably because Bateson had noticed errors, perhaps falsifications, in his work against Mendelism). In his turn Weldon vehemently accused Mendel's theory as "cumbrous and undemonstrable" and listed the exceptions that would refute Mendelism. Bateson spoke with equal vehemence. Karl Pearson, the famous biostatistician, considered a biometrician, proposed a three-year truce, but the chairman, the Reverend T. R. Stebbing, first hesitated, and then decided to let them fight. Bateson concluded his speech emphasizing that arguments based on exceptions reveal only the paucity of their own evidence. Incidentally, the debate was attended by Reginald Crundall Punnett, a collaborator of Bateson, who was the first to use a square matrix, called the Punnett square, to better visualize the intersections.

The battle between Mendelians and biometricians came to a close in 1905 with the cavalry charge of Colonel Charles Chamberlain Hurst. Hurst submitted a work to the Royal Society in which he described that, in horses, bay, brown and chestnut colours showed simple Mendelian segregation (bay and brown being dominant and chestnut recessive). Hurst had obtained these results by patiently sifting through the twenty volumes of racehorse pedigrees recorded in Weatherby's General Stud Book of Race Horses. Weldon, as chairman of the Zoological Committee, was one of the first to have access to the work, and immediately set about combing through the aforementioned twenty volumes in search of inconsistencies; he found them. In some cases, brown horses (dominant) were reported as foals of chestnut (recessive) parents. Weldon rejected the paper on the basis of these inconsistencies. However, after a few weeks Hurst resubmitted his work, this time spurred on by Bateson, who had not been so keen at first (was it envy?). Hurst explained the exceptions as clerical errors of transcription or errors of colour evaluation, which is not easy in foals. Colonel Hurst had gone on to look for the colour of "inconsistent" horses (foals with dominant colours born from recessive parents) in the racing news, but there he did not find any inconsistencies. Weldon did not take it well and started combing through the 20 volumes again, even taking them on a trip to Italy, but focusing much more on this than on the attractions of Italy. On his return, he visited various stables around Oxford hoping to find material to counter Hurst's data. In early April 1906 he visited a stable with Pearson and discussed with him a project for a book on the colour of horses. On 8 April he went to take some photos of horses that he had inspected for developing, but he felt very tired. A few days later he was hospitalized and died on 13 April, at the age of 46. Max

Planck has once said that a new scientific truth does not triumph by convincing its opponents and making them see the light, but rather because its opponents eventually die, and a new generation grows up that is familiar with it.

From then on there were only triumphs for Mendel but the battles between his various rediscoverers have a sequel.

Von Tschermak, the self-proclaimed discoverer of Mendel, had been the first and most active member of the committee set up to raise funds for the erection of a statue of Mendel in Brno. After all, not having many scientific merits of his own, he was the one who had the most to gain by showing off. The statue was inaugurated on Sunday 2 October 1910, in the renamed Mendelplatz (now Mendlovo Náměstí). The event was attended by the most illustrious scientists of the moment; Hugo Iltis introduced Correns for a short inauguration speech, in German. Correns expressed regret that Mendel had not had the recognition he deserved during his life. There are a few points worth noting: the inscription at the foot of the monument was in German as it was still the time of the Austro-Hungarian Empire, which disliked linguistic minorities; the abbot of the monastery, Franciscus Salesius Bařina, whom Abbot Mendel had accepted into his convent, was absent; the documents gathered for the occasion to form a small museum were not exhibited in the convent.

Hugo De Vries, who had not wanted to subscribe to the fundraising for the statue, did not attend the ceremony. He probably considered himself more than a Mendel rediscoverer. In fact, he concentrated on connecting Darwin's evolutionary theories with his monstrosities. He also did not participate in the celebrations of the first centenary of Mendel's birth in 1922.

In 1910 the Mendel statue stood at the center of Mendelplatz, but its story deserves to be told further because it traces the path of genetics in the maze of ideologies and politics of the twentieth century. One night in 1950, the Czechoslovakian army secretly removed the statue and placed it, without its pedestal, at the back of the courtyard of the convent, which in the meantime was being used as government offices. For the communist ideology, genetics was a bourgeois heresy to be fought. With the advent of Trofim Lysenko and his Lamarckian-like theories, the battle became physical; there were layoffs, deportations to Siberia and even executions. Genetics research was effectively destroyed until the death of Stalin in 1953.

In 1964 a group of geneticists had the statue moved to the main courtyard of the former convent of San Tommaso.

A final consideration on Mendel. Isaac Newton is quoted to have said “If I have seen further, it is by standing upon the shoulders of giants.” Mendel too had taken from others, from Gärtner and Kölreuter, for example. However, he used mathematics and statistics, tools that had never been used before in the field of botany and biology in general. Mendel was a giant with his feet on the ground!

I am very grateful to Kamlesh Madan for her help in editing this paper.

Mitelman Database Now Includes Genomic Coordinates

The information in the ***Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer*** (https://mitelmandatabase.isb-cgc.org/) relates cytogenetic aberrations and their genomic consequences, in particular gene fusions, to tumor characteristics. It has served the cancer research community since 1983, first in book form, and since 2000 as an online database hosted by the NCI.

The user queries the database by parameters such as topography, morphology, gene characteristics, cytogenetic aberrations, and journal references. Until now, the resulting genetic location information retrieved from the database was only displayed in karyotypes. As of June 2022, genomic coordinates are also displayed. Thanks to procedures incorporated from the web-based tool CytoConverter, karyotypes are converted to genomic coordinates and can be optionally viewed by the Mitelman Database user.

The user has the option of viewing the genomic coordinate information for either individual karyotypes or for multiple karyotypes in a search result. For individual karyotypes, the corresponding chromosome and its start and end position are given. In addition, the type of imbalance (gain or loss) is noted. For multiple karyotypes in the search results, net imbalances across the selected group are displayed in chart, ideogram or tabular format; information includes the chromosome affected, start and end positions, and whether the segment has been lost or gained.

Predatory Journals

Many of you will wonder why I bother to write about this subject saying 'after all, everybody knows about it, we are all inundated with emails inviting us to submit an article to one of the many hitherto unheard of journals'. Yet, I am amazed at the number of researchers that I have spoken to who have never heard about it.

For the past few years my email inbox is flooded with invitations to submit an article. I could not understand it until I read the text of the lecture given by the president of the E.C.A., Mariano Rocchi, titled 'But what does science say?' (For reference see page 9 of the E.C.A. Newsletter No. 46, July 2020). It was an eye opener! These journals are defined as predatory journals; lists of such journals can be found on the internet. I was not the only one with the problem, which has only become worse. A quick totting up for the first 4 months of 2022 shows that I receive an average of 8 such emails per week. I am invited to contribute regardless of the subject of the journal. In the last 2 weeks I have been invited to submit my articles to a journal about surgery, to join the editorial board of a journal concerned with diseases of the bone, and to give a lecture at a congress for ENT (Ear-Nose-Throat) specialists. We, who once felt so honoured if a manuscript was accepted for publication by a journal, are now beating off journals like flies. From the sublime to the ridiculous!

Besides 'predatory' journals there are also hijacked journals (that mimic reputable journals). Submitting an article to such journals could mean that you pay a lot of money, but it could also mean that you lose all your money as you may never see your paper in print; if it does get published you may lose your reputation.

There is a lot of information available on the internet on how to recognize predatory journals and how to avoid them. Below are a few links including two articles in Nature.

I am sure many of you will find my note superfluous but I am hoping it will be useful to some of you.

Kamlesh Madan

https://www.nature.com/articles/d41586-019-03759-y

https://www.nature.com/articles/d41586-021-02906-8?WT.ec_id=NATURE-20211028&utm_source=nature_etoc&utm_medium=email&utm_campaign=20211028&sap-outbound-id=4B20F4741C1B73223D8E9611DCEC6121EC582C0F

https://www.sciencedirect.com/science/article/abs/pii/S0165614716300037

https://doi.org/10.1016/j.heliyon.2022.e08999

https://journals.sagepub.com/doi/full/10.1177/0192623320920209

Literature on Social Media

E.C.A. is now also present on Social Media. Here are announcements of interesting articles that we have posted on Facebook. The articles and news items are related to cytogenomics or to biology in general. If you have relevant articles that you would like to share, please contact mariano.rocchi@uniba.it.

MCM9 POLYMORPHISMS AND REDUCED RECOMBINATION OF CHROMOSOME 21 DURING MATERNAL MEIOSIS I

The reduction in the number of exchanges in early female meiosis has been repeatedly considered as the cause of chromosome non-disjunction, particular of chromosome 21. See the earlier post "Recombination failure in human oocites".

Are gene variants involved in this reduction? The authors of an article in PLoS Genetics[1] have identified a variant of the MCM9 gene, which is certainly involved in the reduction of recombinations; they also identified minor genes that influence the reduction or increase of recombination.

[1] Pal U, Halder P, Ray A, Sarkar S, Datta S, Ghosh P, Ghosh S: The etiology of Down syndrome: Maternal MCM9 polymorphisms increase risk of reduced recombination and nondisjunction of chromosome 21 during meiosis I within oocyte.
PLoS Genet 17:e1009462 (2021)
https://journals.plos.org/plosgenetics/article?id=10.1371/journal.pgen.1009462

ANEUPLOIDY IN CANCER AND RESISTANCE TO CHEMOTHERAPY

Two articles in Developmental Cell[1,2] deal with cancer resistance to chemotherapy. Both underline the importance, in this regard, of the instability of the genome due to aneuploidies and variations in copy number, the latter being a consequence of the former. It is noteworthy that somatic aneuploidies are not subject to the highly selective filter of the complex process of embryonic development. Therefore, these events, when not detrimental, could fuel cancer evolution.
In this context it is interesting to keep in mind that the phenomenon of aneuploidy is exploited as fuel for species evolution by some organisms. Two examples:
A 2014 paper in PloS biology[3] states: "Candida albicans, the most prevalent human fungal pathogen, is generally diploid. However, 50% of isolates that are resistant to fluconazole (FLC), the most widely used antifungal, are aneuploid and some aneuploidies can confer FLC resistance". Another paper, in press in PNAS[4], suggests that Leishmania (a protozoan) exploits the frequent variations in chromosome and gene copy number to regulate gene expression levels for adaptation.

1- Chromosomal instability accelerates the evolution of resistance to anti-cancer therapies
Lukow DA, Sausville EL, Suri P, Chunduri NK, Wieland A, Leu J, Smith JC, Girish V, Kumar AA, Kendall J, Wang Z, Storchova Z, Sheltzer JM. Dev Cell. 2021 56:2427-2439.
https://www.sciencedirect.com/science/article/abs/pii/S153458072100592X?via%3Dihub

2- Gene copy-number changes and chromosomal instability induced by aneuploidy confer resistance to chemotherapy.
Ippolito MR, Martis V, Martin S, Tijhuis AE, Hong C, Wardenaar R, Dumont M, Zerbib J, Spierings DCJ, Fachinetti D, Ben-David U, Foijer F, Santaguida S. Dev Cell. 2021 56:2440-2454.
https://www.sciencedirect.com/science/article/abs/pii/S1534580721005621?via%3Dihub

3- Harrison BD, Hashemi J, Bibi M, Pulver R, Bavli D, Nahmias Y, Wellington M, Sapiro G, Berman J: A tetraploid intermediate precedes aneuploid formation in yeasts exposed to fluconazole. PLoS Biol 12:e1001815 (2014)
https://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.1001815

4- Bussotti G, Piel L, Pescher P, Domagalska MA, Rajan KS, Cohen-Chalamish S, Doniger T, Hiregange DG, Myler PJ, Unger R, Michaeli S, Spath GF: Genome instability drives epistatic adaptation in the human pathogen Leishmania. **Proc Natl Acad Sci U S A** 118 in press (2022)
https://www.pnas.org/content/118/51/e2113744118.long

A LARGE STUDY ON THE FREQUENCY OF SOMATIC CNV IN LYMPHOCYTES

The human genome project disclosed that up to 5.5% of our genome is composed of segmental duplications. These duplications started, obviously, from a single event which was then fixed in the population. In 2004 two simultaneous papers, in Nat Genet[1] and in Science[2], documented for the first time that Copy Number Variations (CNVs) are indeed present in the human population. This achievement was possible by exploiting the micro-array technology. Next step was the discovery, by the J.P. Dumansky's group[3], that CNVs can discriminate different tissues of the same individual. The possibility of analysing single cells further improved our knowledge of somatic mosaicism for CNV.

In this context, Liu et al., in a paper in Genome Res., have published a large-scale single-cell whole-genome profiling of normal human lymphocytes (20,000 lymphocytes from 16 individuals), allowing a detailed statistics on this topic. 7.5% of the cells had large-size copy number alterations. Trisomy 21 was the most prevalent autosomal aneuploidy. Monosomy X occurred most frequently in females older than 30 years.

1- Iafrate et al.: Detection of large-scale variation in the human genome. Nat Genet 36:949-51 (2004)
2- Sebat et al.: Large-scale copy number polymorphism in the human genome. Science 305:525-528 (2004)
3- Piotrowski et al.: Somatic mosaicism for copy number variation in differentiated human tissues. Hum Mutat 29:1118-1124 (2008)
4- Liu et al.: Low-frequency somatic copy number alterations in normal human lymphocytes revealed by large-scale single-cell whole-genome profiling. Genome Res (2021)
https://genome.cshlp.org/content/early/2021/12/28/gr.275453.121.long

COMPREHENSIVE REVIEW ABOUT THE EFFECTS OF ANEUPLOIDY

Aneuploidy is defined as the loss or gain of chromosomes, leading to a numerical deviation from multiples of *n*, the haploid chromosome complement (*n* being 23 in humans). The first aneuploidy identified in humans was trisomy 21 in 1959, the presence of an additional chromosome 21 in patients with Down syndrome.

Now, in the January 5th issue of Nature Reviews Molecular Cell Biology[1], Rong Li and Jin Zhu from the Mechanobiology Institute, National University of Singaporeand the Department of Cell Biology, Johns Hopkins University School of Medicine, Baltimore, published an extensive review about the consequences of aneuploidy. The review covers the natural presence of aneuploid cells in all humans, the causes of aneuploidy (chromosome missegregation, DNA replication stress, spindle defects) and the damaging effects of aneuploidy, e.g. related to cancer. Also, the mechanisms of compensation of these effects are described, as well as the relation between aneuploidy and ageing. The focus is on man, but examples from studies in model organisms such as yeast, fruitfly and mouse illustrate the universality of the consequences of aneuploidy.

A very useful reference that can be used for teaching basic knowledge about chromosome aberrations and their consequences.

[1] Li R, Zhu J: Effects of aneuploidy on cell behaviour and function. Nat Rev Mol Cell Biol *in press* (2022)
https://www.nature.com/articles/s41580-021-00436-9

MALE INFERTILITY

After decades of deadlock during which deletions of the long arm of the Y chromosome dominated the field, in just a few years exome sequencing and now trio-based Whole Exome Sequencing (WES) has provided a new picture of the genetics underlying the impairment of sperm production. Although WES already identified a number of male infertility-associated gene variants with autosomal recessive or X-linked inheritance, the etiology in approximately 40% of affected individuals remained unknown. "A de novo paradigm for male infertility[1]", Nat Comms, signed by Joris Veltman and over 40 co-authors, demonstrates how the search for rare de novo variant in trio-based exome sequencing data in a cohort of 185 infertile males detected and validated 192 rare mutations (MAF <0.1%), which altered 145 proteins. The *de novo* point mutations affected several genes, all autosomal except one on the X chromosome, none of which were already known to be involved in human male infertility with autosomal dominant inheritance. The variants were of the loss-of-function or missense type in genes intolerant to loss-of-function or missense variants, respectively. The situation, therefore, parallels what has been amply demonstrated in other conditions, such as neuro-developmental disorders. Furthermore, in rare cases, the de novo mutation, of paternal origin, was associated with a variant inherited from the mother. An analysis through the STRING database[2] suggested that the proteins affected by the de novo pathogenic variants share common biological functions with a possible link to mRNA splicing.

[1]- Oud MS, ..., Veltman JA: A de novo paradigm for male infertility. Nature Communications 13 *in press* (2022)
https://www.nature.com/articles/s41467-021-27132-8#auth-R__M_-Smits

[2]- https://string-db.org

TRANSCHROMOSOMIC RAT WITH HUMAN CHROMOSOME 21

In recent years, researchers have developed a mouse model with an extra-human chromosome 21. The aim was to study the biochemical, developmental, and behavioral consequences of this trisomy, in order to extrapolate the results to humans. This model, however, had limitations. For example, just as in somatic cell hybrids, mice tend to lose human chromosomes, thus creating mosaicism. A paper in press, in the Am. J. Hum. Genet.[1] now reports the creation of a transchromosomic rat model with human chromosome 21. TcHSA21, the transchromosomic rat, recapitulates the well-characterized brain defects of Down syndrome (DS) patients, including a smaller brain volume and a reduced cerebellar size. The authors are confident that the model will

facilitate basic DS research with respect, in particular, to drug development.
[1] https://www.cell.com/ajhg/fulltext/S0002-9297(21)00470-5

CELEBRATING A CENTURY OF STUDIES OF GENE BALANCE

James Birchler (University of Missouri, Columbia, USA) and Reiner Veitia (Institut Jacques Monod, Université de Paris, France) have been studying the phenomenon of gene balance for decades. In the January issue of Cytogenetics and Genome Research they have published a review on the occasion of a century of research on gene balance, dosage compensation and the effects of aneuploidy. In the early twenties of the previous century, Albert Blakeslee published his observation in the flowering plant *Datura stramonium* that adding an extra copy of a single chromosome has much more severe effects on the phenotype (i.e. the pigmentation of the flower) than the addition of an entire chromosome set. At the same time Calvin Bridges made similar observations in the fruit fly, *Drosophila melangaster*, namely that the addition of one autosome was lethal whereas three sets of chromosomes were viable in (triploid) females.
Now, after 100 years of genetic studies, including decades of molecular studies, in these and other model organisms (yeast, maize, *Arabidopsis*, the mouse), it has become clear that both additions and losses of chromosome segments disturb the normal regulatory processes of gene expression that operate all over the genome. One example is that both gains and losses of a gene encoding a single component of a multi-component system affect stoichiometric relationships in such a way that the amount of the complex becomes reduced. The gene balance concept also helps to understand how, during evolution, multiple rounds of whole genome duplication in the vertebrate lineage could have contributed to its evolutionary success.
https://www.karger.com/Article/FullText/519592

LONG LIFE TO THE Y-CHROMOSOME

The view of the Y chromosome as a functional desert has shifted in the last decades. Sex-chromosome evolutionary studies and the identification of Y-linked spermatogenic genes have revealed the importance of this tiny chromosome in testis differentiation and spermatogenesis. Nevertheless, the repetitive nature of the Y-chromosome involves several technical limitations that have restricted the number of studies devoted to uncovering the functionality of Y-linked genes. Accordingly, most of them have not yet been assigned to specific functions.
A recent review in eLife[1] summarized how evolutionary forces have led to the heteromorphic nature of the current mammalian X and Y chromosomes, and how these differences trigger two crucial events, namely, meiotic sex chromosomes inactivation (MSCI), and X-chromosome inactivation (XCi). Besides, the article summarizes the pros and cons of the different strategies for the study of the functionality of the Y-linked genes: from the limitations of classic gene targeting strategies to perform knockouts, to the relative usefulness of transgene complementation approaches. The authors highlight how new gene-editing techniques and advances in Y-chromosome sequence information have opened new avenues for the dissection of Y-gene functions.
The application of these new strategies for the individual study of Y-linked genes will be crucial to know which ones are essential for male fertility deepening the understanding of the link between Y genes and spermatogenesis.
https://elifesciences.org/articles/67345

PATHOGENICITY PREDICTION OF STRUCTURAL VARIANTS

Predicting the pathogenicity of structural variants (SV) is not an easy task, especially if they have been detected *de novo*. Several databases and software are available to help clinicians and researchers in this task. A paper in Am. J, Hum. Genet.[1] proposes a new supervised learning method, StrVCTVRE (available free at https://github.com/andrewSharo/StrVCTVRE).
The authors state that this tool allows clinicians to eliminate about half of the SVs from consideration while maintaining a sensitivity of 90%. The improvement is mainly due to the inclusion of information about expression and evolutionary conservation among the analyzed parameters of the gene in question.
[1] https://www.cell.com/ajhg/fulltext/S0002-9297(21)00462-6

KIFC1 AND SPINDLE INSTABILITY IN HUMAN OOCYTES

Human eggs, in contrast to those in many other mammalian species, are highly prone to aneuploidy. Recombination failure[1] (posted on December 25, 2020) is considered to be one of the major causes.
In an article published in Science[2], So et al. point to the lack of expression of the KIFC1 gene as another important cause of aneuploidy. This gene codes for a key protein involved in spindle stabilization in other mammalian oocytes and cancer cells. However, surprisingly, its expression is (almost) absent in human oocytes. The artificial depletion of this motor protein in mouse and bovine oocytes results in unstable spindles, as in humans. Conversely, the delivery of the KIFC1 protein into human oocytes reduces spindle instability.
One wonders: first, why is such a basic biological function, the assembly of the spindle apparatus, not well conserved in mammals. Second, why was this deficit in spindle assembly not rejected by natural selection?
[1]- https://www.cell.com/ajhg/fulltext/S0002-9297(20)30407-9

[2]- https://www.science.org/doi/10.1126/science.abj3944

CLINICAL DIAGNOSIS BY WHOLE GENOME SEQUENCING IN JUST ONE DAY

A new speed record for the time between arrival of the blood sample in the lab and the identification of the pathogenic variant by whole genome sequencing (WGS) has been entered into the Guinness Book of Records by a team from Stanford University School of Medicine. In a study published by Gorzynski et al.[1] in the February 17th issue of the New England Journal of Medicine, ultrafast WGS was performed for 12 critically ill patients. The shortest time between arrival of the blood sample to the initial diagnosis was 7 hours and 18 minutes. A diagnosis was found in 5 patients and had immediate clinical consequences, such as changes in medication. The speed record was possible by the application of the PromethION 48 sequencer from Oxford Nanopore that produces reads with a length of up to 1 Mb, but with higher error rates compared to the conventional short-read (up to 250 bp) sequencing-by-synthesis approaches. This was corrected by the use of the artificial intelligence-based PEPPER-Margin-Deep Variant software, developed by Google and the University of California Santa Cruz Genomics Institute, combined with NVIDIA Clara Cloud-based storage and real-time processing for base calling and alignment of the terabytes of raw data.
Is this giving us a glimpse of the future of clinical genetic diagnosis? Is this the way genome analysis will transform health care? A team led by Stephen Kingsmore of the Rady Children's Institute for Genomic Medicine in San Diego identified the cause of thiamine metabolism dysfunction syndrome 2 in a child with epileptic encephalopathy in 14 hours and 33 minutes. Thiamine and biotin medication were started, preventing permanent neurologic damage,and the child was discharged from hospital on the third day following admission (Owen et al.[2], New England Journal of Medicine June 3rd issue of 2021 for details).
1
https://www.nejm.org/doi/pdf/10.1056/NEJMc2112090?articleTools=true
2
https://www.nejm.org/doi/pdf/10.1056/NEJMc2100365?articleTools=true

LONG-READ SEQUENCING AND DE NOVO MUTATIONS

DNA sequencing has provided powerful tools for defining the *de novo* mutation rate per generation and for identifying pathogenic mutations. However, the short-read technology used so far has its limitations, as complex regions are almost intractable. An article published by the Eichler's group[1] shows that a combined approach based on complementary technologies, particularly long-read sequencing, can overcome these limitations. As a proof of principle, the authors sequenced a family consisting of parents and two daughters, one with autism, where short-read Illumina sequencing was unable to reveal any pathogenic variants. They validated 195 *de novo* germline mutations and 23 potential postzygotic mosaic mutations in both children, resulting in 1.41×10^{-8} substitutions per nucleotide per generation, which is a 25% increase over previous studies. Failure to identify potential pathogenic variants as the cause of the autism with certainty was likely due to the fact that, in this case, the pathogenicity was multifactorial.
The analysis was performed on primary tissue (peripheral blood lymphocytes). The authors point out that apparent variants were initially reported, using different technologies, in which transformed lymphoblastoid cell cultures were used instead of primary tissue.

[1]. https://www.cell.com/ajhg/fulltext/S0002-9297(22)00065-9

OLDER MEN PRODUCE MORE MUTATED SPERM THAN YOUNGER MEN DO

In older men, the frequency of germline mutations is higher than in younger men. This phenomenon has been linked to the accumulation of errors that occur during the lifelong replicative process of spermatogonia stem cells (SSCs), as well as the fact that some of these mutations confer a selective advantage to the SSCs promoting their clonal expansion. Accordingly, as men get older, the SSCs niche becomes a mosaic of mutations which in turn, increases the incidence of mutation carrying sperm, some of which may be damaging to the progeny.
A recent research paper in Genome Research1 has optimized the duplex sequencing (DS) methodology to discover ultra-low-frequency variants of the FGFR3 gene in spermatozoa from older and younger men. The product of this gene participates in the RTK-RAS signaling pathway and is highly expressed in SSCs. Several variants of this gene that accumulate in the SSCs have been described because the mutated protein confers a proliferative advantage.
The authors identified 34 never-before-reported variants out of a total of 75 annotated to the gene's coding regions. Some of these changes were only, or more frequently, identified in older sperm donors. Moreover, the distribution of changes was not uniform along the gene with some domains concentrating non-synonymous mutations, suggesting that these domains may be subject to stronger clonal expansions of pathogenic variants. Besides, the authors detected three amino acid substitutions associated with disorders that are thought to rise with paternal age.
This paper proves that the DS strategy is useful to uncover de novo germline mutations and their

association with paternal age-related congenital disorders. This is particularly relevant in western societies because postponed fatherhood is more and more frequent.

1. https://genome.cshlp.org/content/32/3/499

WHOLE-GENOME RISK PREDICTION IN HUMAN PREIMPLANTATION EMBRYOS

Sequencing technologies are progressing. Some private companies are claiming to be able to predict the susceptibility risks of some common conditions by analyzing the preimplantation embryos. In a paper that appeared in Nature Midicine[1], the authors "used a combination of molecular and statistical techniques to reliably infer inherited genome sequence in 110 embryos and model susceptibility across 12 common conditions".
The ethical implications are evident, and, in this respect, the subtitle of the editorial, which appeared in Nature[2] itself, is very clear: "Companies are marketing polygenic risk scores as a part of IVF well before the potential benefits - and dangers - are fully understood". It further highlights: "These tests demand a broader societal discussion", because these approaches "can open the door to evaluating not only disease risk, but also traits such as height or intelligence".
The problems are very clear. Less clear are the solutions.

1. https://www.nature.com/articles/s41591-022-01735-0
2. https://www.nature.com/articles/d41586-022-00787-z

SEGMENTAL DUPLICATIONS (SD) IN A COMPLETE HUMAN GENOME

On June 17, 2021, we published a post on "The complete sequence of a human genome", pre-published in BioRxiv. The paper is now out in a special issue of Science[1], which includes additional articles on the subject. From a cytogenetic point of view, the one on segmental duplications[2], by Eichler's group, is relevant.
SD and Copy Number Variations (CNV) are the major source of gene evolution and genome variations within and between species, especially the apes. Their precise identification and sequencing were a major technical problem when using the short reads, as second generation sequencing does. Third generation sequencing, as illustrated in the above-mentioned post, has completely overcome the problem and now a sequence of the entire human genome, from telomere to telomere (T2T), has been generated.
SDs were estimated to represent approximately 5% of the human genome. The percentage has now risen to 7%. Their precise definition in the 6 individuals studied allowed substantial progress in the understanding of expression and regulation through the methylation of the duplicated genes. For example, the resolved structure of lipoprotein A, including the expanded kringle IV repeat domain, showed that reduced copies of the latter domain are among the strongest genetic associations with cardiovascular disease.
Please note: the cytogenetics part of this work was carried out by an ECA cytogeneticist (Mario Ventura, co-author).

1. https://www.science.org/doi/10.1126/science.abj6987
2. https://www.science.org/doi/10.1126/science.abj6965

IN SITU SEQUENCING: TOWARDS A HOLISTIC VIEW OF GENOME ARCHITECTURE DURING INTERPHASE

Understanding the 3D arrangement of the 46 DNA molecules during interphase has been, and still is, a real challenge due to limitations of the available tools.
The use of Multicolor FISH and confocal microscopy led to the discovery of chromosome territories and large scale organization of DNA but the resolution was limited by optical constraints. Chromosome conformation capture technology, based on cross-linking of adjacent DNA sequences has been associated with massively parallel sequencing in Hi-C technique and allows one to understand the arrangement at the DNA loop level where interactions occur. However, this tool only gives an average view from the multiple cells used, which may be different from one cell to another.
Realizing that most modern sequencers are a kind of "microscope" reading a fluorescent signal emitted after each round of nucleotide incorporation, several teams started working on *in situ* sequencing, in order to obtain images of the chromosome conformation by imaging the position of the sequenced tags. Furthermore, these technologies can also image other nuclear components such as RNA, proteins, epigenetic markers etc…, giving a full comprehensive view of the genome, at the single cell level. Undoubtedly, exciting new data will emerge from these sequencing imaging technologies.
A paper[1] in Nature gives an overview of this emerging field and presents the most promising technologies to come.

1. https://www.nature.com/articles/d41586-022-00496-7

MUTATION RATE AND LIFESPAN

The relationship between DNA mutations and repair on the one hand and aging and lifespan on the other may seem somewhat intuitive but is nevertheless complex, as expressed, for example, by the Peto paradox (1977). Tumors are mainly due to DNA mutations occurring during DNA replication. If this is

true, Peto reasoned, large animals like elephants should be much more prone to cancer and aging than small animals like mice, which are home to far fewer cells, i.e. they undergo fewer cell doublings. But this is not the case. Some recent papers have added important pieces to the puzzle.

Kolora et al. (2021)[1] (see post dated 9 December 21) suggested that the large difference in lifespan between closely related rockfish species could be explained by the difference in DNA repair efficiency; in other words, better repair efficiency, fewer mutations, longer life.

Vincze et al. (2022)[2] reported a zoo survey showing that animals with larger and smaller bodies have a similar risk of dying from cancer.

Now, Cagan et al. (2022)[3] have filled an additional hole in this puzzle. They devised a clever way to measure the mutation rate in different animals. They then tried to correlate their results with different biological indicators. As Gorelick and Naxerova (2022)[4] comment in the related News and Views, "The most striking correlation was with lifespan. Longer-lived animals acquired few mutations every year and shorter-lived animals acquired many mutations, which meant that the total number of mutations at the end of an animal's life was roughly similar across species".

The puzzle isn't solved yet (for example, what about telomere shortening?), but the holes to fill are getting fewer.

[1] https://www.science.org/doi/10.1126/science.abg5332

[2] https://www.nature.com/articles/s41586-021-04224-5

[3] https://www.nature.com/articles/s41586-022-04618-z

[4] https://www.nature.com/articles/d41586-022-00976-w

BREAKAGE-FUSION-BRIDGE CYCLE

The breakage-fusion-bridge cycle was first described by Barbara McClintock in *Zea mays*. The paper was published 1941, in *Genetics*, more than 80 years ago.

An article in Trends in Genetics reviews this biological phenomenon and its implications in different biological fields, cancer in particular. It also deals with the genes involved and with the stages of the mechanism, some of which are still not well understood.

[1] https://academic.oup.com/genetics/article/26/2/234/5937137

[2] https://www.sciencedirect.com/science/article/pii/S0168952522000695?via%3Dihub

THE MYSTERY OF CHROMOSOME STRUCTURE

After more than a century of microscopic exploration, the intrinsic structure and chromatin organization of mitotic chromosome is still far from being solved. While we have an almost finished human genome map, from telomere to telomere (Nurk al. Science 2022)[1], it is still difficult to figure out the exact folding pathway of chromatin during chromosome condensation despite the fact that numerous models with several different approaches have been suggested and tentatively demonstrated.

Two recent papers add new proposals to the field.

Sedat et al. (Sedat J, McDonald A, Kasler H et al. A proposed unified mitotic chromosome architecture. PNAS 119 (2022)[2] rely on the exact sequence and nucleosome number of chromosome 10, and on electron microscopy observation of interphase chromatin as a starting point. They present a revival of the super coiled organization suggested several years ago. Their computational model is worked out so that it fits perfectly with the observed and calculated length and width of the mitotic chromosome 10 and with the required compaction level. They use a single unified mechanism of compaction during the whole cell cycle. However, further observations are needed to validate the model.

For this, new methodologies that avoid alteration of chromatin structure are essential. This is, for example, the case of the new approach which allows the analysis of mechanical properties of chromosomes in their native state (Meijering AEC, Sarlós K, Nielsen CF et al. Nonlinear mechanics of human mitotic chromosomes. (Meijering al. Nature. 2022)[3] and which could be a way of testing the above mentioned model (and probably many others).

[1] https://www.science.org/doi/10.1126/science.abj6987?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%20%200pubmed

[2] https://www.pnas.org/doi/full/10.1073/pnas.2119107119

[3] https://www.nature.com/articles/s41586-022-04666-5

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COMMITTEE

ECC SCIENTIFIC PROGRAMME COMMITTEE

E.C.A. News

- The 2022 General Assembly of the E.C.A. with Board elections will take place on 25 August 2022, at 6:30 pm at Goldrain Castle, Schlossstraße 33, 39021 Goldrain / South Tyrol, Italy.
- Elections 2022 – renewal or re-election of five board members: Heslop-Harrison, Lavabre-Bertrand, Madan, Pinto Leite, Rieder.
- The President has received only one list with the following candidates:
  Heslop-Harrison (UK), Lavabre-Bertrand (F), Madan (NL), Pinto Leite (P), Rieder (D).

E.C.A. Fellowships

- The **E.C.A.** offers two **Fellowships** for the following course:

  European Advanced Postgraduate Course in Classical and Molecular Cytogenetics

  to be held in Nîmes (France) March 2023 (see page 32).
- The fellowships **include the course fees and the accommodation** during the lectures in Nimes but **do not include travel expenses**.
- Applications with CV, list of publications and a letter of recommendation should be addressed to the course organizer (jean-michel.dupont@ aphp.fr).

EUROPEAN CYTOGENETICISTS ASSOCIATION (E.C.A.)

European Advanced Postgraduate Course in Classical and Molecular Cytogenetics

Director: Professor Jean-Michel Dupont, Paris – France

The course is scheduled to be held in Nîmes, France in March 2023. However, an online version will be organized, if the restrictions due to the pandemic are still in place.

2023 Course provisional programme

This approximately 55-hour theoretical part of the course attempts to cover the field of cytogenetics in the broadest sense. The topics can be divided into the following categories:

Technical aspects:

Classical Cytogenetics: Cell culture techniques; Chromosome staining methods (Q-, G-, C-, R-banding and high-resolution banding);

Molecular Cytogenetics: Methods and principles of Fluorescence In Situ Hybridization (FISH) and MFISH; Array CGH; Application of Massively Parallel Sequencing to Cytogenetics; Production and use of molecular probes; Database use in Cytogenetics;

Laboratory quality assessment.

Clinical cytogenetics:

Basics: Frequency of chromosome disorders; Cell cycle, mitosis and meiosis, gametogenesis; Heterochromatic and euchromatic variants; Numerical chromosome abnormalities; Structural abnormalities: translocations, inversions, insertions, deletions, rings, markers; Risk assessment for balanced abnormalities; X inactivation; numerical and structural abnormalities of the X and the Y; Mosaicism; Chimaeras; ISCN 2020.

Clinical: Phenotype of common autosomal and gonosomal aneuploidies; Chromosome abnormalities in recurrent abortions; Cytogenetics and infertility; Microdeletion syndromes; Uniparental disomy and its consequences; Genomic imprinting; Genetic counselling and ethical issues in cytogenetics.

Prenatal diagnosis: Indications, methods and interpretation; Risk assessment for chromosomal abnormalities; Non-invasive methods using foetal nucleic acids and foetal cells in maternal blood; Pre-implantation diagnosis.

Cancer Cytogenetics: Molecular approach to cancer cytogenetics; Predisposition to cancer, Chromosome instability syndromes; Chromosome mutagenesis; Solid tumors; Clinical application in onco-haematology.

Other:

Genome architecture; Structure of chromatin; Structure of metaphase chromosomes, Mechanisms of chromosome abberations; Origin of aneuploidy; Evolution and plasticity of the human genome; Animal cytogenetics; Plant cytogenetics.