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Emil Heitz and the Concept of Heterochromatin: Longitudinal Chromosome Differentiation was Recognized Fifty Years Ago

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SUMMARY

The work of Emil Heitz (1892–1965) laid one of the keystones of cytogenetics. Using a new in situ method, he established between 1928 and 1935 the longitudinal differentiation of chromosomes in euchromatin (genetically active) and heterochromatin (genetically inert). He recognized the association of satellited chromosomes with the formation of the nucleolus, co-discovered the giant salivary chromosomes of diptera, and arrived at a cytological and genetic concept of chromosome structure that has been found essentially correct to date.

Yet, Emil Heitz did not gain due recognition by his contemporaries, suffered from the political disturbances of his time, and spent almost a lifetime in isolation, bolstered only by the conviction that his scientific work was significant.

Between 1888 and 1915 the work of Boveri, Sutton, and Morgan and his school established that Mendelian genes are arranged in linear, specific order along each chromosome [1–3]. In 1928, Heitz recognized cytologically detectable longitudinal differentiation of chromosomes which he correlated with their genetic linearity. He suggested the terms *euchromatin* and *heterochromatin* for differences detectable by suitable chromosomal stains [4]. From subsequent studies he clearly foresaw the development of cytological genetics—his term for cytogenetics—as a modern branch of genetics [5–9]. Although important recent developments in this field [10–11] can be traced directly to the work of Heitz, it is remarkable that the scope of his

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contributions and his biography have remained relatively obscure. This article provides an annotated bibliography of Emil Heitz (1892–1965) and shows why he must be regarded as one of the major pioneers of cytogenetics.

The early work of Emil Heitz held all the promise that should have made him one of the most renowned scientists of his time. As a young man he developed a new, inexpensive cytological method (the boiling technique, "Kochmethode") to obtain direct chromosome preparations in situ from moss [12] and applied it to a wide range of organisms (over 115 species of plants, and several species of *Drosophila* and other diptera). He recognized new cytological features and interpreted them correctly (euchromatin/heterochromatin [4]), discovered a new type of chromosome (giant salivary gland chromosomes of diptera [13]), described the relation of chromosomal constriction and satellite formation to the nucleolus (SAT chromosomes [6]), and arrived at a concept of genetic differentiation of chromosomes [8, 9] that, for the most part, has withstood the test of time.

In spite of this eminent work, Emil Heitz was not recognized in his time, never held an academic position commensurate with his scientific achievements, and never had a technician or other staff. He had only two doctorates (H. Jachimsky, in 1933–1935; F. Resende, in 1934–1936), and it was not until the late 1950's that he gained some recognition and found a reasonable basis to support his family.

BIOGRAPHIC DATA

Emil Heitz was born on October 29, 1892 in Strasburg, Alsace, to Paul Timotheus Heitz, a printer and editor, and Mathilde Heitz, née Schwalb, the daughter of a protestant clergyman of Jewish ancestry. Editing and printing had been a business of the Heitz family in Strasburg since 1720, and it was noted at the time for publishing inexpensive romanic classics and art work. Incidentally, they also printed the dissertation of Goethe.

After a short apprenticeship, Emil Heitz decided against his father's profession and went on to study sciences at Munich and Strasburg (1912–1914), which was interrupted by four years of service at the German front lines in World War I. He continued his studies in Basel (1919–1921) and obtained a PhD at the University of Heidelberg in 1921. Following brief stints at the University of Tübingen (1921), the Institute for Fermentation Physiology at Weihenstephan 1922–1924 (the scientific backbone of the Bavarian beer industry), and the University of Greifswald (1924–1926), he came to the Department of Botany, University of Hamburg, in 1926 following an invitation by H. Winkler.

In Hamburg (1926–1937), Heitz did his work on heterochromatin for which his name stands today. On account of his ancestry he was forced to leave in 1937 when the Nazi-ruled administration declined further salary and withdrew his appointment. Heitz and his family left for Basel, Switzerland, the hometown of his wife Elisabeth, née Staehelin. There he could still barely support his family. Earlier their home had not even included a kitchen. An invitation to the University of Missouri at Columbia by A. Stadler in 1939 had to be postponed due to the imminent war.

In 1947, after the war, Heitz did go to the University of Missouri. His family was to follow. However, this musical, sensitive man was too deeply rooted in central

European culture. He felt lonely in America's middle west and concluded that this could not become his new home. He returned to Basel in late 1947 forfeiting an opportunity to continue his work, and remained in Basel under still unfavorable conditions until 1955 when he was invited to join the Max Planck Institute for Biology at Tübingen by G. Melchers. After an interruption of nearly 20 years, he could now resume his profession. He began chromosomal studies using electron microscopy. He celebrated his 70th birthday in 1962 with several colleagues and the appearance of a Festschrift in his honor [14]. He then received honorary doctorates from the Universities of Cologne, Berlin, and Frankfurt. He had retired to Switzerland the previous year. He died on June 6, 1965, half a year before a major article in *Science* acknowledged Heitz's important role in the study of heterochromatin [15]. His portrait is shown in figure 1.

WORK ON HETEROCHROMATIN

Since all of Heitz's contributions are published in long, carefully produced articles which may not be easily read, his major works are summarized individually in the following section.

The 1928 paper on "The Heterochromatin of Moss." [4]

Heitz notes that in *Pellia epiphylla,* certain parts of five out of nine chromosomes remain condensed throughout interphase (figs. 2 and 3). Heitz recognizes them as new autosomal structures and derives the term *heterochromatin* from earlier designations heteropyknosis and heterochromosomes for comparable observations concerning sex chromosomes [16]. Heitz suggests the term *euchromatin* for the part of chromosomes that do become invisible at late telophase. He refers to the usage of the term heterochromosome and euchromosome by McClung in 1902, although his references do not include McClung's paper which reports the discovery of sex chromosomes [17].

Heitz redefines *heteropyknosis* as "the differential behavior of a whole or part of any chromosome at prophase and telophase during the entire development of an individual or during a certain stage of development" [4, p. 765]. Heterochromosome refers to an entire chromosome, heterochromatin to a part of a chromosome that remains

FIG. 1.—Emil Heitz, about 1950 (photograph courtesy of Mrs. Emil Heitz).

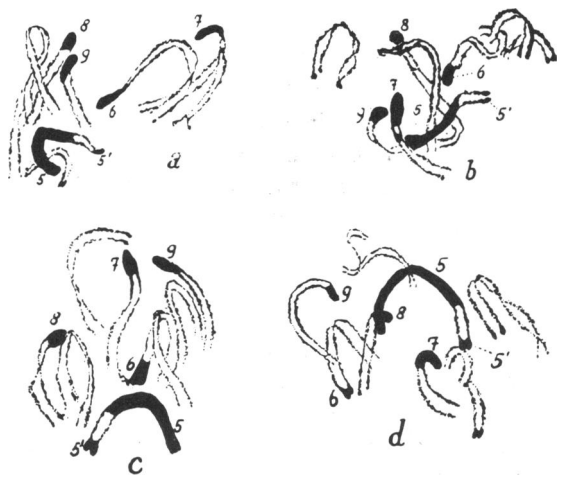

FIG. 2.—Darkly stained heterochromatin and lightly stained euchromatin in *Pellia epiphylla* (from Heitz, 1928) [4].

heteropyknotic after telophase and thus behaves opposite to euchromosomes/ euchromatin. Heitz recognizes what later became known as constitutive and facultative heterochromatin [15, 18–20].

Heitz follows the heterochromatic parts of autosomes through the cell cycle and concludes: (1) three of the six symmetric chromosomes (his nos. 1–6) contain heterochromatin in specific areas, one of these almost in its entirety, (2) the three

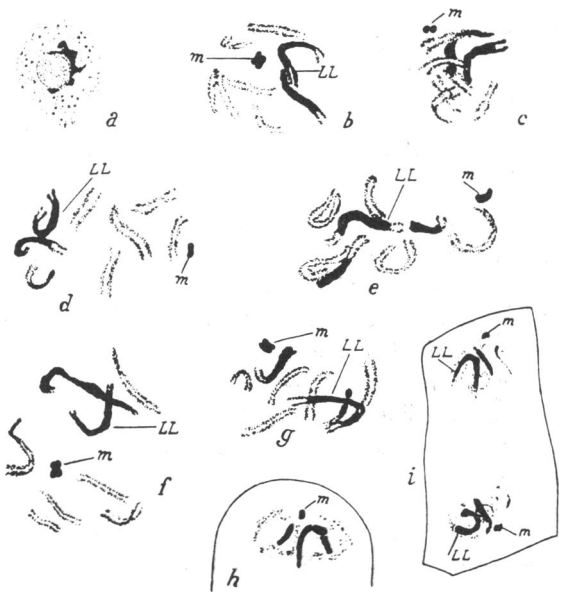

FIG. 3.—Heteropyknosis in *Plagiochila asplenioides*. One chromosome, *m*, is totally heteropyknotic and considered a sex chromosome (from Heitz, 1928) [4].

asymmetric chromosomes 7, 8, and 9 contain heterochromatin in the short arm, and (3) position and number of heterochromatin blocks are specific and reproducible by his in situ method (boiling in carmin acetic acid followed by staining under the microscope). Heitz then describes heteropyknosis in a different moss, *Pellina neesiana* which contains an unusually large X chromosome. He attributes its great length of a larger block of heterochromatin (accounting for about one-sixth of the chromosome in *P. neesiana* vs. one-fifteenth in *P. epiphylla*). Heitz notes that most of the heterochromatin eventually becomes invisible prior to the euchromatin. This functional aspect is included in his definition of heteropyknosis and elaborated further in his first paper of the series, published in 1933 (see below).

In another moss, *Pellia fabbronnina,* Heitz found satellites (trabants in his terminology) of heterochromatin on the distal short arm of one of the asymmetric chromosomes and observed an approximation to the nucleolus. Heitz regards the absence of both a satellited chromosome and a clearly discernible nucleolus in *P. epiphylla* as evidence that the satellite would not correspond to heterochromatin in the usual sense, but rather be a manifestation of nucleolus formation ("vom Nukleolus abgeschieden," [4, p. 790]).

Heitz points out that his preparation must reflect actual conditions and not result from artifacts because of his ability to produce his findings with great consistency. In addition, he describes observations on living cells which also show heterochromatin.

We recognize here the first application of an in situ method to produce C-bands (constitutive heterochromatin) in metaphase chromosomes, comparable to those discovered more than 40 years later in mammalian chromosomes [19–22]. In the 1928 paper, Heitz also presents data on the number of chromosomes and the distribution of autosomal heterochromatin in more than 70 species of leaf moss from 26 families and 47 genera. In each case he finds at least one heterochromosome which remains visible at interphase as a chromocenter. This is considered a sex chromosome consisting mostly of heterochromatin (fig. 3).

The 1929 Paper on: "Heterochromatin, Chromocenters, Chromomeres. Preliminary Communication." [5]

Heitz extends his study to 115 species of phanerogametic, monocotylic, and dicotylic plants. The heterochromatin of parts or, rarely, entire chromosomes remains visible as chromocenters in interphase in 86 (75%) of the species examined. Chromocenters occur mainly in plants with small chromosomes and are unrelated to the total number of chromosomes. Heitz argues that chromosomes consist of material with longitudinal differentiation in both eu- and heterochromatin. Different chromosomes would thus differ in the distribution of the two types of chromatin and the genes contained therein. The less stained euchromatin is considered genetically more important than heterochromatin.

The 1933 Paper on: "Total and Partial Somatic Heteropyknosis and Structural Sex Chromosomes in Drosophila funebris. Cytological Studies in Diptera. II." [6]

In his search for the expected longitudinal differentiation of visible chromosomal structures as revealed by eu- and heterochromatin, Heitz turns to *D. funebris* because

its chromosomes (one larger pair) are more favorable than those of *D. melanogaster*. The upper pharyngeal ganglion of larvae is prepared and stained with carmin acetic acid. An analysis of about 200 larvae reveals that the large chromosome in this species occurs in three forms: (1) as uninterrupted rod, as reported by other investigators, (2) with a secondary constriction in its middle part as "SAT chromosome" ("sekundäre Einschnürung," p. 730), and (3) with the same kind of constriction in the lower third of the rod. Heitz determines that this latter chromosome occurs only in males, whereas either of the other two types occurs in females. Thus the chromosome with a constriction in the middle was considered to be an X chromosome.

These findings demonstrated for the first time the existence of partial somatic heteropyknosis in animals. In addition, sex chromosomes showed longitudinal differentiation in heterochromatin and euchromatin: total heteropyknosis in the Y chromosome, half euchromatin and half heterochromatin in the X chromosome. Heitz notes that these structurally different sex chromosomes must have functional significance. In a later paper [7], he relates this observation to the expression of X-linked genes.

The 1933 Paper on: "Somatic Heteropyknosis in Drosophila melanogaster and Its Genetic Significance. Cytological Studies in Diptera. III." [7]

Heitz now resumes his previously (1930) unsuccessful studies in *D. melanogaster* in order to exploit its well established genetics. He points out that secondary constrictions (called achromatic gaps by Navashin) in the chromosomes of this species have been previously described. Heitz suggests a definite location of these constrictions at the junctions of the proximal to the middle third of the X chromosome (contradicting Dobzhansky), and a previously unrecognized constriction at the junction to the proximal fifth of one pair of the V-shaped autosomes. He concludes that it should now be possible to map linkage groups II and III more clearly.

From these observations at meta- and anaphase, Heitz moves on to *D. melanogaster* studies in prophase. (The chromosomes at telophase were too small to be studied in either *D. melanogaster* or *D. funebris*.) Heitz describes a clear-cut heterochromatin pattern as previously seen in the liver moss *Pellia* and the X chromosome of *D. funebris*. Size, position, and distribution of heterochromatin areas are recognized as distinct features of each of chromosomes II and III, and the X chromosome. Heitz recognizes one-half of the X chromosome as euchromatin and the other, somewhat longer half also containing the secondary constriction, as heterochromatin. Chromosomes II and III consist of euchromatin except for a small heterochromatic area at the centromere. The small autosomes are regarded as completely euchromatic, while the Y chromosome is completely heterochromatic. In one larva, Heitz finds two heteropyknotic Y chromosomes. Metaphases of the same animal revealed an XYY male.

Heitz details the findings of the linear arrangement of genes on the chromosomes of *D. melanogaster,* being fully aware of the work of Morgan's group [3]. He points out that the "genetically inert region" of the X chromosome is the proximal, heterochromatic half where only 9 genes are localized, whereas the other euchromatic region of the X contains 46 genes known at that time. Heitz provides a cytological genetic map of the chromosomes of three *Drosophila* species (*melanogaster, funebris,* and *virilis*). *D. virilis* does not have a sex chromosome dimorphism, but Heitz recognizes a

sexual dimorphism with respect to the heterochromatin–euchromatin pattern. He notes that the euchromatin portions are comparable to those of other species. From Heitz's statements and figures the picture emerges that chromosomal differences between the related species rest primarily in the amount and position of heterochromatin. Modern cytogenetics arrived at similar conclusions just during the past eight years.

Heitz then moves on to *D. simulans* and *D. Hydei*, and several other species of diptera. His hypothesis of chromatin structure-gene relation states that the "density" (Dichte) of genes is closely related to the longitudinal differentiation in euchromatin and heterochromatin. (Obviously he refers to the number of genes per chromatin segment.) Euchromatin is "rich," heterochromatin is "poor" in genes. He considers the possibility that during embryonic development a heterochromatic region could become euchromatic and vice versa.

The 1933 Paper on: "Evidence for the Chromosomal Nature of Nuclei in Bibio hortulanus L. Cytological Studies in Diptera. I." [13]

This is the first description of the giant chromosomes from larval salivary glands and other organs. Arguments are advanced not only for their chromosomal nature but for individual morphological differences. The nucleolus, terminal disc, and terminal clubbing are specific features of each of the five pairs. Quantitative differences in the amount of heterochromatin at the same site are recognized. The same discovery was reported by Painter a few months later in the same year [23].

The 1935 Paper on: "Chromosomal Structure and Genes." [8]

This extensive review, based on a presentation before the German Society of Genetics at Jena 1935, summarizes Heitz's views, taking into account the work of Muller, Painter, Bridges, Muller and Gershenson, Muller and Prokofyeva, McClintock, and others [24–29]. It can be summarized as follows: (1) All chromosomes show a longitudinal differentiation into euchromatin and heterochromatin that relates to the genetic properties of each chromosome. (2) The differentiation is specific for each chromosome and is different in the karyogram of each animal and plant species. (3) Each chromosome has a primary constriction (now called centromere) for the insertion of the mitotic spindle. In this region, the number of genes and genetic recombinations appears to be reduced. (4) Some chromosomes contain a secondary constriction which sometimes leads to the formation of satellites (trabants in Heitz's terminology) which are associated with the formation of the nucleolus. (5) Heterochromatin formation and the degree of chromosomal contraction is genetically determined, and heterochromatin is located at corresponding positions of homologous chromosomes. (6) Chromocenters of interphase nuclei result from equilocal positioning of heterochromatin of different chromosomes. (7) Species can be distinguished by their size and pattern of chromatin distribution (shown for several species of moss by Heitz's student Jachimsky in 1935, [30]). (8) Euchromatin is closely connected to gene activity during interphase; heterochromatin corresponds to genetically inert regions. (9) The X chromosome must contain fewer genes or fewer active genes than expected from its length. (10) Supernumerary X chromosomes are genetically inert. (11) Sex chromosomes are frequently subject to heterochromatin formation. (12) Dimorphic heterochromatin

formation of the autosomes occurs in species where a sex-chromosomal dimorphism has not yet evolved. (13) Chromosome inversions play a role in speciation.

Heitz goes on to develop a general concept of chromosomal structure using terms considered strange today, such as kalymma, stromatin, genoplasma, etc., which reflect the lack of knowledge of the role of DNA and its structure in the transmission of genetic information.

SOME PERSONAL TRAITS AND PROBLEMS OF HIS TIME

Emil Heitz's profound understanding of the relationship of cytology and genetics (cytological genetics in his terminology) allowed him to develop a concept of chromosome structure based on the evidence available to him. Much of this was from his own cytological work. He was firmly convinced of the significance of his work and felt bitter about the lack of recognition it received.

What were the reasons for the failure of his contemporaries to recognize the impact his work should have had? First of all, to be sure, the adverse political development in Germany was a major factor which directly forced Heitz out of the country in 1937. Heitz had an upright, uncompromising personality which precluded any sort of cooperation with the new political system, perhaps saving him from a worse fate later on.

However, there must have been other reasons as well. Heitz was a hard worker demanding a high quality of work, be it his own or that of others. At scientific meetings he was critical, if not aggressive, frequently engaging in debates and head-on collisions with other workers. He never held back on his superior technical abilities and theoretical understanding of the problems of interest to him. Still he was viewed too specialized for a chair in botany or genetics. Later, when he was considered eligible, the political situation became increasingly adverse and hindered any success. In addition, his sensitive personality must have been in his way. He did not lecture well, but was a very stimulating (albeit exhausting) teacher to work with. He did not accept any findings he could not confirm himself. It was his good fortune to be strongly supported by his wife, Elisabeth. She came from a wealthy Basel family and married Heitz against the advice of her family. He was considered by his in-laws as an unsuccessful, unpromising young man. The Heitz family has three sons and one daughter, none of whom has gone into science.

His personal situation was complicated by the location of his birth place. When his home town became French after World War I in 1918, he could not return for 12 years on account of his German citizenship. His family's business was confiscated. Temporarily he became a French citizen, but this made it difficult to continue his studies in Germany. Thus he shared the fate of people in a borderland between once hostile countries. Emil Heitz would have been happy to see this dispute settled today and the impact of his work recognized. But he was destined to carry his task in loneliness at a difficult time, supported only by a loyal wife, yet convinced to be ahead of most of his contemporaries, and eventually to be proven right in the annals of science.

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