Perspectives

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Helen Crouse (1914–2006): Imprinting and Chromosome Behavior

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HELEN Virginia Crouse died May 25, 2006, at age 91 in her home in Hayesville, North Carolina. She was a cytogeneticist *par excellence* who in the course of her studies coined the term "imprinting." She also used the expression "controlling element," which was employed in a different context by Barbara McClintock, her Ph.D. advisor.

Helen Crouse was born September 12, 1914, to Charles C. and Margaret Ross Crouse. Her father was a train and trolley conductor; her mother was educated through the third grade. Helen grew up in Owings Mills, Maryland, together with her older sister, Marie Garrish. In 1935 she obtained her B.A. degree from Goucher College where she was elected to Phi Beta Kappa and her M.A. degree from Smith College a year later. While working on her Master's degree, Helen had obtained a fellowship for a brief stay at Cornell where she met Marcus Rhoades, who was raising corn there. After receiving her M.A., for the next 3 years she worked in the Department of Embryology at the Carnegie Institution of Washington in the laboratory of Charles W. Metz whom she had first met while a senior at Goucher College. In the Metz lab, Helen earned the grand sum of $1200/year! Metz was on the faculty at Johns Hopkins University, engaged in research on the lower dipteran fly, *Sciara coprophila*. In Metz's lab, Helen was introduced to this model organism and began her studies (Crouse and Smith-Stocking 1938; Crouse 1939; Metz and Crouse 1939), continuing to unravel the secrets of Sciara for the rest of her research career.

THE EARLY YEARS

Barbara McClintock visited Baltimore in 1936 and Helen was thrilled to meet her, having as an undergraduate read McClintock's (1934) classic article describing the nucleolus organizer region. Helen arranged to visit the University of Missouri, where McClintock was teaching and doing research, to explore doing her Ph.D. thesis research there. Although she would have loved to see McClintock during her visit, this was highly unlikely because it was rare for McClintock to entertain visitors. However, destiny was on her side because when she arrived at the University of Missouri she discovered that her host had fallen ill and had arranged for McClintock to oversee her visit. With trepidation, Helen climbed the stairs to the top floor where McClintock's lab was located, knocked on the closed door, and was greeted by the revered scientist. McClintock, who was smoking a cigarette in a cigarette holder and wearing a green visor over her eyes, said "I've been waiting for you." It was a wonderful visit, and the two remained in contact afterward. McClintock wrote to Helen that she would be attending the Genetics Society of America meeting in Woods Hole, Massachusetts (1938) and suggested that they meet there. During the visit, they developed a plan for Helen to begin work that fall with McClintock at Missouri. However, this was delayed for a year as Metz could not find a replacement for Helen in his lab.

When Helen arrived at Missouri, the chair of the department, L. J. Stadler, had recently departed for a temporary stay at Cal Tech and there were upheavals in the department. As a result, several faculty members announced that they would leave the University of Missouri. McClintock was among them, opting to move to the Cold Spring Harbor Laboratory. Stadler's return to Missouri did not dissuade McClintock and others from leaving. For details about McClintock's move from Missouri to Cold Spring Harbor, see Kass (2003). Having worked together for 1 year, McClintock remained Helen's Ph.D. advisor from a distance with Stadler serving as the local mentor. Helen Crouse was one of only three Ph.D. students trained by Barbara McClintock, who later received the Nobel Prize. Of these students, Helen was the only one to remain in

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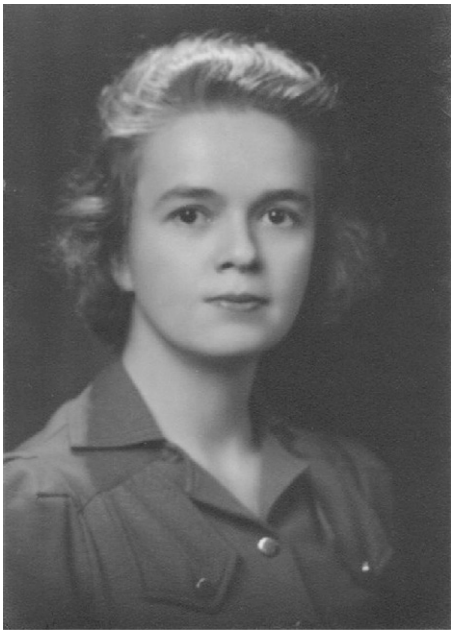

Helen Crouse as a young scientist.

Helen Crouse during retirement in North Carolina.

science. Spencer Brown, a classmate of Helen's, had also come to Missouri to work with McClintock, but when she left for Cold Spring Harbor, he transferred to Dan Mazia's lab. Helen said that Spencer was the smartest person that she knew. It is clear that they influenced one another through their scientific discussions. After joining the Berkeley faculty, Spencer published an article about the inactivation of the mammalian X chromosome (Brown and Chandra 1973), invoking the phenomenon of chromosome "imprinting" that had been described by Helen in Sciara (Crouse 1960b). It was with tears in her eyes that Helen related the great tragedy of Spencer's untimely death. He was murdered.

In 1941, slightly more than 2 years after enrollment (a record time by today's standards), Helen received her Ph.D. degree from the University of Missouri. Her thesis research dealt with the characterization of X-irradiation-induced translocations in Sciara (Crouse 1943), experimental material she had brought with her from the Metz lab. Through a study of 10 reciprocal translocation heterozygotes, 5 of which involved the X chromosome, Helen was able to conclude that the "precocious" chromosome behavior described previously by Metz *et al.* (1926) correlated with the translocation chromosome that contained the X centromere, as had been predicted by Metz (1936).

From 1941–1942 Helen was a postdoctoral fellow in the zoology department of Columbia University with Theodosius Dobzhansky. There, she crossed paths again with Marcus Rhoades and stayed with him and his family. Among her friends at Columbia were chromosome cytologists Franz and Sally Hughes-Schrader. Subsequently, Helen became a faculty member at Bennington College. But, after 3 years she decided that she missed having enough time for research. Therefore, in 1945 she rejoined Metz who had moved to the University of Pennsylvania where he became department chair, succeeding C. E. McClung. In this setting, Helen could pursue her research on Sciara (Crouse 1947). She met fellow biologist Jean Kerschner there and they became life-long friends, sharing summer homes in Swan's Island, Maine; Falmouth, Massachusetts; and their retirement home in Hayesville, North Carolina. In 1947 Helen joined the faculty of Goucher College, her alma mater, and remained there for 12 years. During that time she took two sabbatical leaves, spending the first at Harvard and the second at the Oak Ridge National Laboratory creating more translocations in Sciara by X-irradiation. Although chromosomal rearrangements can be induced by X-rays, visible genetic mutations are rare (Metz 1938a; Crouse 1949, 1950), a topic that Helen returned to later in her career (Crouse 1961a,b). The difficulty in obtaining visible mutations lessened the enthusiasm of the scientific community to adopt Sciara, which was competing with Drosophila as the model organism of choice. Helen was eager to devote her full time to study the new translocations, so she left Goucher in 1959 to join the laboratory of J. Herbert Taylor at Columbia University. Helen remained a research associate with Taylor for the remainder of her research career until she retired in 1974, moving with him to the Institute of Molecular Biophysics at Florida State University in Tallahassee in 1964. Helen's research in the Taylor lab revealed many important findings, summarized below.

SCIARA SEX DETERMINATION

Helen observed that reciprocal translocations in Sciara involving the X chromosome influenced the sex of the progeny (Crouse 1960a). Although several species of Sciara are digenic, *Sciara coprophila* is monogenic and mothers have only daughters or only sons.

Helen's observations on X-translocations suggested that the increased frequency in exceptional offspring (some sons from a mother who should have only daughters and vice versa) could be explained by the 3:1 disjunction during oogenesis so that the egg receives two copies or no copies of the X rather than just one copy (Crouse 1960a). Reasoning that the X-translocation had reduced synapsis in the heterozygotes, Helen found that exceptional offspring were not seen in X-translocation homozygotes where full synapsis was restored (Crouse 1960a).

Sciara lacks a Y chromosome, and sex is determined by the mother. Mothers in which one of the two copies of their X chromosome has a long paracentric inversion (X′), and whose breakpoints have been mapped (Crouse 1977), will have only daughters, whereas mothers that are XX will have only sons (Metz 1938b). It seems likely that the X′ chromosome may condition the ooplasm to eliminate only one and not two X chromosomes in somatic cells in early embryonic cleavage divisions (Gerbi 1986). Helen's observations of eggs that had nondisjunction of an X-translocation showed that, regardless of the number of X chromosomes in the egg and hence in the embryo, the X′ chromosome dictated that just one X chromosome would be eliminated in embryogenesis, whereas eggs from XX mothers would eliminate two X chromosomes (Crouse 1960a). Since unfertilized eggs can develop a bit (de Saint Phalle and Sullivan 1998), the system of sex determination in Sciara may be a predecessor for the evolutionary development of parthenogenesis.

GERMLINE-LIMITED L CHROMOSOMES

The germline-limited chromosomes (called L chromosomes) are eliminated from the soma by remaining on the metaphase plate and failing to segregate in the fifth or sixth cleavage division of the Sciara embryos. The number of L chromosomes is variable, and one to three L chromosomes are eliminated to reduce the germline complement to two L chromosomes (Rieffel and Crouse 1966). The function of L chromosomes is unknown, but Helen speculated that they may affect the sex ratio. This was based on her observations that a monogenic strain of *Sciara impatiens* that had L chromosomes became digenic when it lost these chromosomes (Crouse *et al.* 1971). However, half a year later the ratio of bisexual progeny shifted in the nullo-L strain and only sons were produced. The additional observation that all species of Sciara that are monogenic have L chromosomes but digenic species may have or lack L chromosomes (Gerbi 1986) weakens the correlation of L chromosomes and sex determination.

CHROMOSOME IMPRINTING

In a classic article published in Genetics (Crouse 1960b), Helen coined the term "chromosome imprinting" when she wrote: "the 'imprint' a chromosome bears is unrelated to the genic constitution of the chromosome and is determined only by the sex of the germ line through which the chromosome has been inherited." Today there are several examples of imprinted genes in mammals. At the time that Helen first used the term, chromosome imprinting was known to occur only in Sciara male meiosis. In the first meiotic division of Sciara spermatogenesis, a monopolar spindle forms upon which all the maternally derived homologs move to the single pole and all the paternally derived homologs move away from the single pole (reviewed by Metz 1938b; Gerbi 1986). The centromeres of the paternal chromosomes lag rather than lead their direction of motion away from the monopole (Abbott *et al.* 1981) to be discarded in a bud of cytoplasm. The "backward" chromosome movement coupled with electron microscopy data on the differential attachment of the homologs to kinetochore microtubles (Kubai 1982) challenges current ideas of mechanisms for how chromosomes move on spindles. Rieffel and Crouse (1966) surmised that both the maternal and paternal sets of homologs carried imprints. Moreover, they reasoned that the imprint must be reversible as the maternally derived set of chromosomes that is retained in the primary spermatocyte is later recognized as a paternal set of chromosomes after fertilization. Subsequently, Chandra and Brown (1975) suggested that it would be necessary to imprint only one of the two sets of homologs to fit the data. The mechanism for chromosome imprinting in Sciara remains enigmatic, although much headway has been made in mammalian systems.

In Sciara male meiosis I, all of the L chromosomes move to the single pole, apparently escaping the imprint mechanism (Metz 1938b). Helen proved the validity of this supposition by crossing an L-minus strain of *S. impatiens* with an L-plus strain and observed that the L chromosomes were never discarded in the bud with the paternal chromosomes, regardless of the parental origin of the L chromosomes (Crouse *et al.* 1971).

THE CONTROLLING ELEMENT OF THE "PRECOCIOUS" CHROMOSOME

In the second meiotic division of Sciara spermatocytes, the X dyad undergoes nondisjunction and is seen "precociously" at one spindle pole while the other chromosomes are still on the metaphase plate (Metz 1938b). The nullo-X product of this division degenerates, and all the resulting sperm have two copies of the X. The accumulation of X chromosomes in each generation is prevented by X chromosome elimination in the embryo, as discussed below. Metz called the X-dyad behavior "precocious" because he thought that it moved prematurely to one pole, while the other chromosomes were still on the metaphase plate. However, current studies (my unpublished results) suggest that the X dyad

is always adjacent to one pole (which was the monopole in meiosis I; Abbott and Gerbi 1981) and that it moves with that pole as the spindle elongates. Electron microscopy revealed that the X dyad lacks a visible kinetochore (D. F. Kubai, personal communication), although some kinetochore proteins are still localized there (B. de Saint-Phalle, personal communication). The nondisjunction of the X dyad appears to be a failure of its centromere function in meiosis II.

In her Ph.D. thesis, Helen had shown that "precocious" chromosome behavior correlated with the X centromeric region (Crouse 1943). She set out to subdivide this region, carrying out a beautiful set of experiments that demonstrated that the regulation of centromere function in the "precocious" chromosome resided not in the X centromere itself, but in chromatin nearby, which she dubbed the "controlling element." Somewhat earlier, McClintock (1956) had used the term "controlling element" with a somewhat different meaning. As a former graduate student trying to show independence from the mentor, Helen said in her independent-minded way: "McClintock can use the term as she wishes, but I will use it my way." Helen obtained translocation T1 with a breakpoint between the X centromere and the small region of heterochromatin between it and the end of the chromosome (Crouse 1960b). This was a reciprocal translocation between the X and chromosome II. In spermatocytes with the T1 translocation, the "precocious" chromosome was now chromosome II with the small region of X heterochromatin attached to it, and not the bulk of the X chromosome with the X centromere (Crouse 1960b). By analyzing translocations, Helen mapped the X centromere to a discrete polytene chromosome band (Crouse 1977) (Sciara lacks a chromocenter of centromeric heterochromatin). She obtained further translocations to subdivide the proximal heterochromatin on the X into three blocks and deduced that the "controlling element" resided in the middle block (Crouse 1979). Taken together, the data showed that the centromere of each of the autosomes as well as the X could be regulated by the "controlling element" in translocation stocks, giving rise to "precocious" chromosome behavior. Moreover, the "controlling element" did not have to be adjacent to the centromere that it regulated. Nevertheless, regulation always occurred in *cis* on the chromosome carrying the "controlling element."

How does the "controlling element" work? Collaborative studies on ribosomal RNA genes (rDNA) (Gerbi and Crouse 1976) led to *in situ* hybridization showing that all three blocks of proximal heterochromatin on the X contained rDNA (Crouse *et al.* 1977). However, at least 30 kb of non-rDNA sequence is embedded in the middle block and might contain the "controlling element" (A. W. Kerrebrock and S. A. Gerbi, unpublished results). Whether this region encodes RNA that mediates the "controlling element" regulation of the X centromere remains to be determined.

X CHROMOSOME ELIMINATION IN THE SCIARA EMBRYO

Since oogenesis is orthodox, giving rise to a haploid genome, fertilization of the egg by the double-X sperm results in a zygote with three X chromosomes. At the seventh or eighth cleavage division, one X chromosome is eliminated from the soma of flies that will be female and two X chromosomes are eliminated from flies that will be male (Du Bois 1933). Later in development, only one paternal X is eliminated in the germline of both sexes alike, producing the XX germline in both sexes (Berry 1941). The same X centromere region that governs nondisjunction in male meiosis also marks the chromosome for somatic elimination in early embryogenesis (Crouse 1943, 1960b, 1979). However, details of the regulatory action by the "controlling element" may differ in these two stages. In meiosis II, the "controlling element" inhibits kinetochore formation on the X chromosome. In embryonic chromosome elimination in the soma, the kinetochores presumably form as the X aligns on the metaphase plate but apparently the cohesins do not let go and the sister chromatids cannot complete their separation in anaphase (de Saint Phalle and Sullivan 1996).

Chromosome imprinting that occurs in Sciara male meiosis I also occurs in the embryo, as the X chromosome(s) to be eliminated are always paternal in origin. Therefore, the single X that remains in the male soma after chromosome elimination was maternally derived. Helen asked if a paternally derived rather than maternally derived X chromosome could be functional in the male. She found that the answer differed between the soma and the germline. In a set of experiments, a variety of X translocations were used for crosses of wild-type males with female-producing females that were heterozygous for the translocation (Crouse 1966). The patroclinous exceptional male progeny from these crosses appeared normal, demonstrating normal function of the paternal X when it is substituted in the soma for the maternal X (Crouse 1966). However, neither meiotic division could occur normally and these patroclinous exceptional male flies were infertile. Therefore, the imprinted patroclinous X chromosome could function in the soma but not in the germline (Crouse 1966).

The defect in the germline might have reflected the presence of one rather than two X chromosomes in the germline, but other experiments carried out by Helen showed this was not the case (Crouse 1965). Crosses of translocation males with male-producer females that were heterozygous for the translocation gave rise to sons in which some spermatocytes had only one instead of two X chromosomes. The single X was primarily maternally derived, with the segment of the X chromosome

distal to the point of translocation derived from their father (Crouse 1965). In this situation, meiosis proceeds normally and the semipatroclinous X translocation undergoes "precocious" behavior in male meiosis II, producing active sperm as is the case for wild-type flies (Crouse 1965).

POLYTENE CHROMOSOME DNA PUFF AMPLIFICATION

The beautiful polytene chromosomes in Sciara salivary glands were the first chromosomes to be used when the method of *in situ* hybridization was developed (Pardue *et al.* 1970). They are larger than their counterparts in Drosophila, having undergone a few more rounds of endoduplication (Rasch 1970b). As part of her Ph.D. thesis, Helen provided the first cytological maps of the four polytene chromosomes in the *S. coprophila* salivary glands (Crouse 1943). Later, she carried out a study on Sciara polytene chromosome structure with Hans Ris (Ris and Crouse 1945). At Columbia, Helen supervised the Ph.D. thesis research of Natalia Gabrusewycz-Garcia (1964) in which the developmental progression of *S. coprophila* polytene chromosomes was followed in late larval life. The appearance of large "DNA puffs" was described. Unlike the RNA puffs of Drosophila, the Sciara DNA puffs are sites of DNA amplification (Rasch 1970a; Wu *et al.* 1993). Microspectrophotometric data led Helen to suggest that this developmentally regulated, locus-specific amplification reflected stepwise doublings of the DNA (Crouse and Keyl 1968). Her suggestion is consistent with the current model of an onionskin of nested replication forks, emanating from an origin that had fired repeatedly. She injected the insect molting hormone, ecdysone, into young Sciara larvae and showed that it could prematurely induce DNA puffing and replication (Crouse 1968). Recent experiments have shown that ecdysone induces DNA amplification and transcription at DNA puff II/9A (Foulk *et al.* 2006). The Sciara II/9A locus has been characterized at the molecular level (DiBartolomeis and Gerbi 1989; Bienz-Tadmor *et al.* 1991; Mok *et al.* 2000; Urnov *et al.* 2002), and its origin of replication has been mapped prior to and during DNA amplification (Liang *et al.* 1993; Liang and Gerbi 1994; Bielinsky *et al.* 2001; Lunyak *et al.* 2002). It abuts an ecdysone response element, suggesting that the ecdysone induction of DNA puffing observed by Helen (Crouse 1968) may be a direct effect of the ecdysone receptor interacting with the replication machinery (Foulk *et al.* 2006).

EPILOGUE

It was a great honor for me to know Helen Crouse. I first encountered her when I was a student in J. Herbert Taylor's molecular genetics course at Columbia when Helen supervised my final exam. I heard about Helen from Reba Goodman with whom I carried out my senior undergraduate research on Sciara. I tried to correspond with Helen while I was a graduate student working on Sciara, but she directed her replies to my Ph.D. advisor, Joe Gall, rather than to me. It was only after I obtained a faculty position at Brown University that she began to interact with me directly. Visiting her in her summer home in Falmouth, Massachusetts, we collaborated on studies of Sciara rDNA. One year during her annual trek to Falmouth, she stopped by my lab at Brown with a surprise announcement. She had all of the Sciara stocks with her and said "Well dear, they are yours if you want them, as I am retiring." Although I was totally unprepared for this generous gift, I scrambled to accept the responsibility for their maintenance. I am grateful to Helen for her discoveries that provide a foundation for Sciara chromosome research and for her gift of the flies, providing me and future generations with a model organism whose unique biology serves to unravel fundamental questions. I encourage the scientific community to use this marvelous fly to full advantage to unravel questions of chromosome mechanics.

I thank Helen Crouse for allowing me to interview her a few years ago, providing the basis for the anecdotes related here. I thank Brigitte de Saint-Phalle, Stephen Doris, Michael Foulk, Janell Johnson, and Heidi Smith for their comments on this article and Jean Kerschner, Connie MacCorkle, and Jo-Ann Joyal for help with the photographs.

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