Perspective: Genetic analysis of meiosis using the *asynaptic 1* mutant: A perspective on George W. Beadle and Barbara McClintock’s 1928 contribution.

by Wojciech P. Pawlowski, Cornell University


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Genetic analysis of meiosis using the \textit{asynaptic 1} mutant: A perspective on George W. Beadle and Barbara McClintock’s 1928 contribution.

Wojciech P. Pawlowski

Department of Plant Breeding and Genetics
Cornell University
Ithaca, NY 14853
E-mail: wp45@cornell.edu

Abstract

The 1928 paper by George Beadle and Barbara McClintock is the first description of a mutant defective in meiosis in maize, and one of the first meiotic mutants found in any species. It reports on a mutant later named \textit{asynaptic 1 (as1)} that lacks proper chromosome pairing and synapsis, and exhibits defects in recombination. These abnormalities lead to the presence of univalents (single chromosomes) at metaphase I, unequal chromosome segregation in anaphase I, and, eventually, to severe male and female sterility. In the several decades since this paper was published, much has been learned about meiotic processes. This paper is at the root of research on chromosome dynamics, which is now a large and thriving field with studies conducted in a large number of species. The \textit{asl} mutant has been used in many genetic studies since 1928, and its stock is maintained at the Maize Genetics Coop Stock Center (http://maizecoop.cropsci.uiuc.edu).

Meiosis

Many of Barbara McClintock’s early papers focus on linking chromosome behavior in meiosis to gene inheritance and segregation. In contrast, the 1928 paper by Beadle and McClintock (Beadle & McClintock, 1928) deals with processes that underlie chromosome behavior itself.

During the prophase of meiosis, homologous chromosomes pair, and exchange segments (recombine). Proper progression of both pairing and recombination are essential for correct segregation of chromosomes to daughter cells. Chromosome pairing includes chromosomes pre-aligning and attaining close proximity, and then undergoing the homology search, an intimate process in which homologous chromosomes identify each other based on their DNA sequence (Bozza & Pawlowski, 2008). The exact mechanisms of chromosome pairing remain poorly understood. However, it is known that in plants, as well as in mammals and fungi, homolog pairing is dependent on the initiation of meiotic recombination and successful progression through its early steps. Several pieces of evidence support a hypothesis that single-stranded DNA ends created during the early recombination stages are used as molecular “probes” to search for homology (Bozza & Pawlowski, 2008).

Homologous chromosome pairing is followed by installation of a proteinaceous synaptonemal complex structure between the paired chromosomes, which stabilizes the pairing interaction. The synaptonemal complex consists of three components: two lateral elements and a central element that connects them (Page and Hawley, 2004). Lateral elements, referred to as chromosome axes or the axial elements before chromosomes pair, form in very early meiotic prophase or even during the pre-meiotic interphase. Axial element installation is essential for establishing a specific meiotic chromosome structure, which is a prerequisite for all meiotic processes, including pairing, synapsis, and recombination (Zickler and Kleckner, 1999). After homologous chromosomes pair in zygotene, the two juxtaposed axial elements are joined together by installation of a coiled-coil protein that constitutes the central element (Page & Hawley, 2004). The installation of the central element of the synaptonemal complex is referred to as “synapsis.”

Meiotic recombination is initiated by formation of double-strand breaks (DSBs) in chromosomal DNA. DSB formation also takes place very early in meiotic prophase, in most species including plants before the onset of chromosome pairing. The DSBs are then processed and repaired by several recombination complexes. Eventually,
a small fraction of DSB repair products become crossovers. Barbara McClintock, in another milestone discovery of her early career (Creighton and McClintock, 1931), demonstrated that the sites of crossovers are chiasmata, cytological structures connecting homologous chromosomes that are visible during late stages of meiotic prophase. Crossovers have two critical functions: (i) they facilitate exchanges of genetic information between parental chromosomes and (ii) they play a structural role in keeping homologous chromosomes together until they segregate in anaphase I.

**Description of asynaptic 1 in the 1928 Beadle and McClintock paper**

The less-than-a-page-long paper by Beadle and McClintock contains a description of abnormal progression of the first division of meiosis in a mutant now known as asynaptic 1 (as1), although the name of the mutant does not appear in the paper. *as1* was first identified as a male sterile mutant and later also found to be female sterile. Presence of both male and female sterility is a strong indication of a meiotic defect. The paper brilliantly demonstrates that basic cellular processes, such as meiosis, are genetically controlled and can be understood by analyzing mutants defective in single genes.

The 1920s and 1930s were the period of the first modern studies focused on understanding meiotic processes. The knowledge about meiosis gained since that time, particularly molecular genetic studies of meiotic mechanisms conducted during the past twenty years, allow interesting insights into this early work of Beadle and McClintock.

Although cytological methods for studying meiotic mutants have become much more sophisticated since the 1920s, the overall methodology of the Beadle and McClintock investigation was very similar to what we would do today. One of the first analyses that they performed to elucidate chromosome behavior in the *as1* mutant was examining the presence of bivalents (chromosome pairs) and univalents (single chromosomes) at metaphase I. They found that most mutant meiocytes (pollen mother cells) exhibited exclusively or nearly exclusively univalents – a sign of a severe meiotic defect. Since the 1920s, the nomenclature of meiotic processes has changed radically. Beadle and McClintock wrote that the reason for the presence of univalents at metaphase I was “a complete failure of synapsis” in diakinesis. However in today’s meiotic literature, the term “synapsis” is reserved for installation of the synaptonemal complex in zygotene, something that the authors could not have examined because (i) the synaptonemal complex was not discovered until the 1950s (Fawcett, 1956; Moses, 1956) and (ii) the synaptonemal complex dissolves by the beginning of diakinesis. Moreover, the extent of synapsis would normally be examined during meiotic pachytene stage, which McClintock observed in maize for the first time only in 1929 or 1930 (Kass, 2003) and published in 1930 (McClintock, 1930; see also Phillips’ perspective, this volume). In today’s language, we would say that Beadle and McClintock observed that chromosomes in diakinesis failed to form bivalents due to the absence of chiasmata. The reasons for the lack of chiasmata may include defects in meiotic recombination and formation of crossovers, abnormal chromosome pairing, as well as defects in chromosome axis formation and synopsis.

**Eight decades of follow up studies of as1**

The subject of the 1928 paper became the bases of Beadle’s Ph.D. dissertation (Beadle, 1930a). Beadle’s research and other later studies (Beadle, 1930b; Beadle, 1933; Maguire, 1978a; Maguire, 1978b; Maguire and Riess, 1991; Miller, 1963; Pawlowski et al., 2003) showed that *as1* mutant meiocytes indeed exhibit defects in synapsis, chromosome pairing, and early steps of recombination. In 1930, Beadle published a detailed description of meiosis in *as1*, including a very interesting observation that the presence of univalents at metaphase I, instead of bivalents, led to formation of unreduced gametes (Beadle, 1930b). The latter feature continues to be very exciting today, as it could be used to engineer apomictic propagation (i.e., propagation without fertilization) in maize. In another follow-up paper in 1933, Beadle suggested that meiotic abnormalities in the *as1* mutant were due to a defect in meiotic recombination. He also reported mapping the *as1* locus to chromosome 1.

In 1963, Oscar Miller, a student of Charles Burnham (who like McClintock and Beadle was a member of the Cornell maize genetics group; see cover photo) again examined recombination in the *as1* mutant and concluded that distribution of recombination events found in some mutant meiocytes differed from those of normal maize plants (Miller, 1963). Marjorie Maguire, a student of L. F. Randolph at Cornell (Ph.D. 1952), conducted studies of chromosome synopsis in *as1* using transmission electron microscopy, and identified several types of synaptic
defects (Maguire, 1978a; Maguire, 1978b; Maguire & Riess, 1991). Her studies were followed by more detailed examinations of synaptonemal complex components by Golubovskaya et al. (2011), who showed that they were properly installed in the mutant, albeit not always between homologous chromosomes. Finally, Pawlowski et al. (2003) reported that as1 exhibited a defect in an early step of meiotic recombination.

The identity of the as1 gene still remains unknown. However, the meiotic defects observed in this mutant could suggest that the as1 gene plays a role in early steps of the recombination pathway. Abnormalities in chromosome pairing and synapsis are likely downstream results of recombination defects as homologous chromosome pairing and synapsis are tightly linked to recombination in maize (Pawlowski & Cande, 2005; Pawlowski et al., 2004).

**Early observations of meiotic spindle and superb technical abilities of Beadle and McClintock**

Another important observation reported by Beadle and McClintock (1928) is abnormal installation of the division spindle in as1 mutant meiocytes. They found that not all chromosomes attached to the spindle and there were often multiple spindles, some associated with just a few chromosomes. The spindle abnormalities were one of the causes of the unequal segregation of chromosomes in anaphase I, which led to male and female sterility. Even though little was known about chromosome segregation mechanisms in 1928, Beadle and McClintock recognized spindle installation as critical for this process. The insightful observations of spindle installation dynamics in the as1 mutant were made by Beadle and McClintock using very simple methods. Chromosomes were stained using the acetocarmine method, but spindle microtubules were just seen as shadows in the lightly staining meiocyte cytoplasm. To make drawings, a camera lucida was used, a device that employs a mirror to superimpose the image of a piece of paper on which the drawing is made onto the microscope image (see Sorrells’ perspective, this volume). Nevertheless, the drawing shows a high degree of accuracy. This paper superbly demonstrates not only the observation and critical thinking abilities of Beadle and McClintock but also their technical skills.

**References**


Phillips, R. (this volume). McClintock’s presence of mind and forward vision as illustrated in the analysis of an interchange in maize.


A GENIC DISTURBANCE OF MEIOSIS IN ZEA MAYS

During and following the summer of 1927, a collection of maize carrying factors for male sterility (Eyster) was made for the purpose of genetic and cytological investigation. The occurrence of male-sterile plants in material from thirty or more unrelated cultures suggests the possibility of several genetic factors causing such sterility.

In segregating material obtained from I. F. Phipps, it has been found that sterility is due to a recessive mendelian factor causing irregular meiosis. In a count of 144 plants the observed ratio was 109 normal to 35 sterile plants, a deviation from the calculated ratio of but one plant. The cytological behavior in these sterile plants has been determined by studies of the meiotic divisions in the microsporocytes.

Early stages of microsporogenesis in the male-sterile plants have not been extensively studied. During the stages just previous to and during diakinesis there is observed a partial or complete failure of synopsis. Because of this lack of synopsis and the consequent presence of a large number of univalents, metaphases are characteristically irregular. Microsporocytes most frequently show twenty univalents. Progressively fewer cells show one bivalent and eighteen univalents, two bivalents and sixteen univalents, and so on, cells with ten bivalents rarely being observed. Some anthers show a high percentage of sporocytes containing some bivalents while other anthers show a high percentage of sporocytes containing twenty univalents.

Irregularity in the appearance of metaphase I increases with an increase in the number of univalents. A microsporocyte with ten bivalents in metaphase I appears normal. When univalents are observed they do not always lie in one spindle. Usually there is one major spindle containing the several bivalents, when present, plus some of the univalents, and one to several minor spindles containing one or more univalents (Fig. 1). In consequence of the presence of several spindles, the sporocyte is divided into a number of unequal cells after the first meiotic mitosis. Each cell contains one or more nuclei and each nucleus contains one or more chromosomes. These cells undergo a second division to form microspores.

It is obvious that most of these microspores and the pollen grains formed from them do not contain a normal haploid set of chromosomes, and they are probably non-functional under ordinary conditions.

![Fig. 1](image)

This particular type of male sterility is accompanied by a certain amount of female sterility. Several pollinations have given only sparsely-filled ears. Female sterility has been observed in male steriles from a few of the other sources also. Megasporogenesis remains to be studied in these cases.

With regard to at least one male-sterile culture, it may be stated that male sterility is due to a simple mendelian gene affecting synopsis and consequent meiotic behavior, the result being the formation of gametes containing varying chromosomal complements, only a few of which are viable.

GEORGE W. BEADLE
BARBARA McCLEINTOCK

CORNELL UNIVERSITY

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2 Data partly from I. F. Phipps.