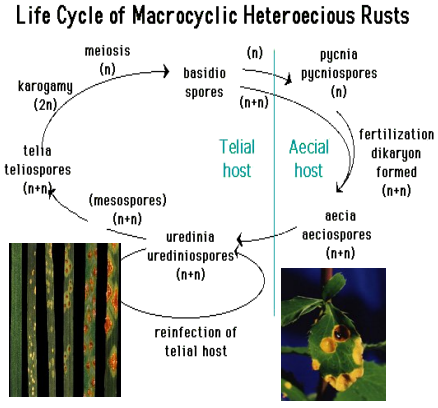
The Use of Epifluorescence & Transmission Electron Microscopy to Determine Chromosome Numbers in Rust Fungi

E.W.A. Boehm[1*], D.J. McLaughlin[2], W.R. Bushnell[1] & J.C. Wenstrom[3]

[1]USDA Cereal Rust Lab, [2]Department of Plant Biology & [3]Biomedical Image Processing Laboratory, University of Minnesota, St. Paul, MN. [*]Department of Biology, SUNY Potsdam.

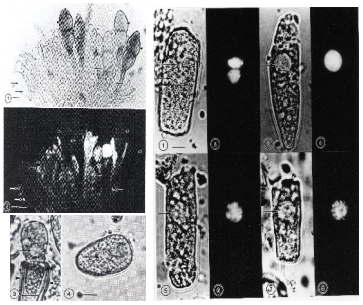
Background: Stem Rust of Wheat caused by the Rust Fungus *Puccinia graminis* Pers.:Pers. f.sp. *tritici* Eriks & Henn. (Uredinales, Basidiomycetes) has historically been the single most economically important disease of plants worldwide[1]. It is the only fungus mentioned in the Bible and has served as a paradigm for understanding host-parasite coevolution for plant pathologists over the last century and a half. The USDA Cereal Rust Lab (now the Cereal Disease Lab) was originally established at the University of Minnesota to deal solely with the challenges this disease presents. Large acreages of wheat preclude the use of fungicides and the only measure of control is that afforded by resistance breeding. After decades of research, the disease has effectively been controlled by deploying, on an annual basis, a multitude of resistant wheat lines to keep up with the continual evolution of new virulent races[1]. Each year these lines of wheat differentials are deployed from Mexico throughout the Midwest of the United States and into the central Canadian provinces (Saskatchewan and Manitoba) – along the prevailing spring winds which bear inoculum from the south to the north, along what has been termed the "Puccinia Pathway"[1].

The problem: Although a tremendous amount of effort has gone into the genetic characterization of wheat, only recently has attention shifted to the pathogen itself. Genome characterization, particularly the determination of the number of chromosomes or karyotype, is considered prerequisite for understanding any system in biology[2]. The estimated haploid chromosome number based on light microscopy for *P. graminis* f.sp. *tritici* is six[1]. However, earlier light microscopic chromosome counts in other fungi have often proved erroneous when reanalyzed[3, 4]. Fungal chromosomes lie at the limits of light microscopic resolution and so therefore can not be reliably counted using this method[2]. Alternatively, classical methods of linkage group determination using genetic crosses have not met with success in this system, as *P. graminis* f.sp. *tritici* is an obligate pathogen and can not be cultured. In addition, it has one of the most complex life cycles known in biology, involving five different spore states and two different plant hosts.



Above. The life cycle of *Puccinia graminis* f.sp. *tritici* is termed "macrocyclic" because it involves five different fungal spore states, most with a different nuclear constitution. It is "heteroecious" because it involves two different plant hosts: wheat and barberry. This is one of the most complex life cycles known in biology.

Methodology: In fungi, the preferred stage for determining the karyotype is pachynema of meiotic prophase I, in which in which chromosomes pair up and form synaptonemal complexes (SCs)[2]. In this stage, the chromosomes are present as paired homologues in SCs and achieve their greatest lateral condensation, staining readily under TEM. Karyotypes derived by these methods are considered definitive, because SCs account for all paired homologues and begin and terminate at the nuclear envelope, making reconstructions reproducible. Here[3], we reevaluate the karyotype for this important plant pathogen, using transmission electron microscopy of serially sectioned meiotic nuclei in pachynema. Nuclei were selected prior to TEM using DNA-binding fluorochromes and epifluorescence microscopy.



The problem was to determine at which point in the complex life cycle meiosis occurred and how to access pachynema of meiosis I for TEM. Using the DNA-binding fluorochrome DAPI and epifluorescence microscopy on mechanically released teliospore protoplasts, we were able to determine that mature teliospores are the site of nuclear pairing or karyogamy, a stage that immediately preceded meiosis[3]. We also determined that just prior to germination of the teliospore the early phases of meiosis I rapidly ensue. *The same nucleus selected under epifluorescence microscopy was then processed for serial sectioning and TEM*[2, 3].

Figs. 1 & 2. (above, far left). Teliospores of *Puccinia graminis* f.sp. *tritici* stained with DAPI reveal that mature spores do not stain due to the thick wall. Bars = 25um. **Fig. 3 & 4.** Only when the spore is cracked open to release the protoplast can DAPI stain the nuclei. Bars = 8um. **Figs. 1 – 8.** (above, near left). Pairs of micrographs: brightfield and epifluorescence (left & right pairs). Released teliospore protoplasts stained with DAPI reveal stages prior to nuclear fusion (Figs. 1 & 2), nuclear fusion (karyogamy) (Figs. 3 & 4), early pachynema (Figs. 5 & 6) and mid pachynema (Figs. 7 & 8), in which the chromosome complement reaches its highest level of condensation. Arrows denote nuclei. Bar = 5um.

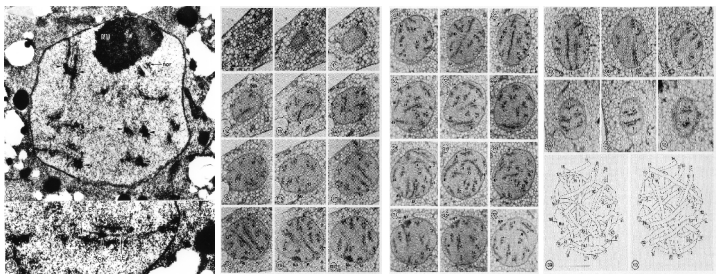
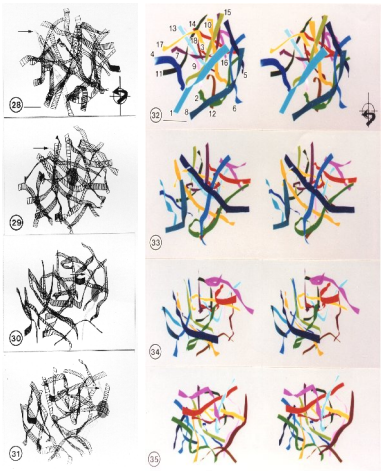
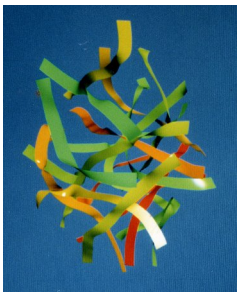


Fig. 6. (above left). Typical TEM section through a mid pachynema nucleus. Illustrated are examples of synaptonemal complexes (SCs) in differing orientations, some with centromeres visible (arrowheads), nuclear envelop attachment sites (*), and the nucleolus associated bivalent (nor) approaching the nucleolus (NU). Bar = 1um. **Fig. 7.** (above left). The tripartite, ribbon-like SC is composed of two lateral elements (LE), associated with condensed chromatin, and a central element (CE), which is anchored to the nuclear envelope (*). A recombination nodule (RN) is located on the central element. C = cytoplasm, N = nucleoplasm. Bar = 0.5um. **Figs. 9 – 40.** (above). An entire pachytene nucleus serially sectioned to display the continuity of the SC profiles from section to section. This series illustrates the process by which fragments of the tripartite SC, in consecutive sections, may be followed to determine the individuality of each bivalent. Figs. 39 & 40. Manual reconstruction, rear facing & forward facing views.





Figs. 28 – 31. TEM images of serially sectioned pachytene nuclei were scanned to form a composite image capable of rotation. An orthographic computer generated, 3D wireframe rendering of 18 bivalents of *Puccinia graminis* f.sp. *tritici*. **Figs. 32 – 35.** The same, as a space filling 3D model visible as stereo pairs rotating on the axis. (above). Enlarged view.

Literature cited:

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