A Review of:

The Mechanism of Secondary Non-disjunction in *Drosophila melanogaster* Females

Youbin Xiang and R. Scott Hawley

By Ari Akerstein

The model for secondary non-disjunction has over the last 90 years since Bridges originally observed it, undergone several key modifications. The authors, Xiang and Hawley, provide a review of the history and, further, propose a model of their own.

Despite the numerous modifications research usually provides upon existing theories, this review will focus on the two central models of secondary non-disjunction as discussed by Y.Xiang and Hawley (2006) - that of Bridges and then Coopers – followed by a brief look at the investigators own research and what insights new technology has provided.

Bridges in 1916 based his model for secondary non-disjunction on the observation that XXY Drosophila females displayed a far higher frequency of X chromosome non-disjunction than did normal drosophila XX females, nearly all XXY female non-disjunctions included non-crossover X chromosomes. Of great insight was the realization that this could be a distinct mechanism than normal (primary) non-disjunction, leading him to further investigation.

The explanation Bridges proposed for this connection was that the Y chromosome pairs with only one of the X chromosomes – suggesting a competitive pairing situation between the two X and the Y chromosomes. As such the other (non-paired) X

chromosome is left to sort randomly. This predicts a 50% observed frequency for secondary non-disjunction (actually 33% when adjusted for inviability of alternatives (XXX, YO to XXY) (Zhang, Howley, 2006). Data obtained by 1936 by Sturdevant and Beadle (1936) still supported Bridges idea, showing that ~90% of secondary non-disjunction events involved non-exchanging X chromosomes. Further, analysis of XXY females showed that the X chromosomes that undergo non-disjunction were usually lacking chiasmata (Puro and Nokkola 1981). This provided convincing data that the two events are connected.

In contrast, the explanation Cooper provided was that of the XYX trivalent. Briefly, this hypothesizes that the each of the Y chromosome's 2 arms attaches to one arm of each of the X's. Keenly, Cooper recognized that "both arms of the Y chromosome share homology with the X and thus May conjoin with an X". As such the trivalent would orient along the metaphase plate with the two X's facing one pole and the Y towards the other, facilitating secondary non-disjunctive segregation. Further strengthening his argument, it was found that both the X and Y chromosome share sequence homology (Lohe et al., 1993). Cooper himself showed evidence that one-armed Y chromosomes showed a lowered ability to induce secondary non-disjunction (Cooper, 1948). Still further evidence for the model came from the observation that Y chromosomes that had deletions also showed a reduced ability to induce secondary non-disjunction, suggesting physical interaction as the cause (Chadov, 1981).

In hopes of elucidating the mechanism leading to secondary non-disjunction, the investigators, Y.Xiang and Hawley, utilize FISH (fluorescence in-situ hybridization) on XXY drosophila oocytes. It should be noted that Cooper's has been the de-facto model for 60 odd years and the author's motivation was to use new technology to test it.

The authors go on to provide strong evidence for the existence of the putative XXY trivalent, originally proposed by Cooper (1948) as the mechanism that can lead to secondary non-disjunction. In so doing, they disprove Bridges proposed mechanism of competitive pairing.

FISH (fluorescent in situ hybridization) is a technique used to examine the position of chromosomes lined up on the metaphase plate and is therefore particularly well suited to the task of targeting unusual chromosome formations. To test these hypotheses, XXY drosophila female oocytes were studied.

In a FISH experiment a particular region of DNA, in this case, the short sequence of shared homology between the Y and X chromosomes (where it was believed they connect) is amplified (PCR). The fluorescent probe was created to have affinity for this particular sequence. Cells are broken (in this case XXY Drosophila oocytes), exposing the chromosomes and the probe applied. Binding should only occur at the sites to which they have affinity. Excess probe is washed off and the complex is hybridized at a particular temperature.

A balancer chromosome was created (FM7) to fully suppress the ability of the X to crossover, therefore allowing the investigators to isolate the role of X chromosome crossing over upon secondary non-disjunction.

The direct FISH visualization illustrates that the centromeres of two X chromosomes that were free to cross over nearly always situated on the ends of the chiasmatic mass, facing either pole, with the Y chromosome situated between them. In contrast, X chromosomes that did not cross over, due to the insertion of the FM7 balancer chromosome displayed an orientation of the Y towards one pole and both X's facing the other. This provides convincing evidence not only for the formation of the trivalent, incidentally showing that XXY pairing events are common to the early meiotic cell irrespective of X cross over status, but also for that the ability of the X chromosome to cross over directly impacts secondary non-disjunction. FISH data also shows the frequency of secondary non-disjunction is proportional to the fraction of achiasmate X chromosomes, further implicating X crossing over as instrumental to the mechanism.

The authors suggest it is the non-crossing over X chromosomes (non-exchange X) that are responsible for maintaining the associations through the end of prophase, while normally crossing over X chromosomes seem to dissolve the association with the Y by mid prophase. Therefore, the authors propose a new model where XXY associations are prevalent in early prophase of meiosis, but only in non-exchange X-chromosomes do these associations persist beyond early stages of meiosis I – a modification to Coopers model.

Central to their hypothesis is that crossing over in some way affects chromosome associations – specifically between the sex chromosomes - during meiosis I. Therefore it makes sense, given this distinction, that X chromosomes that do cross over should behave differently than those that do not. The authors' explanation is satisfying given that

chiasmata hold the bivalent together during the later phases of prophase (diplotene , diakinesis) and that the mechanical transition toward the metaphase plate, and thus segregation, would seem to be impacted by the structure of these complexes.

Literature Cited

Chadov, B. F., 1981 Behavior of chromosomes during mitosis and meiosis and chromocentral organization of nuclei in *Drosophila melanogaster*, Proceedings of the XIV International Congress of Genetics, Vol. 3, Ithaca, Nym pp.343-356

Cooper, K.W., 1948 A new theory of secondary non-disjunction in female Drosophila melanogaster. Proc. Natl. Acad. Sci. USA **34**:179-187

Lohe, A.E., A.J. Hilliker and P.A. Roberts, 1993 Mapping simple repeated DNA sequences in heterochromatin of *Drosophila melanogaster*. Genetics **134**: 1149-1174

Puro, J., and S. Nokkola, 1981 Secondary nondisjunction in multiple inversion heterozygotes as revealed by direct cytological and genetic tests. Proceedings of the 7th European Drosophila Research Conference, p.87.

Sturdevant, A.H., and G.W. Beadle, 1936 The relations of inversions in the X chromosome of *Drosophila melanogaster* to crossing over and disjunction. Genetics **21**: 554-604

Zhang, P., and R.S.Hawley, 1990 The genetic analysis of distributive segregation in *Drosophila melanogaster*. II. Further genetic analysis of the *nod* locus. Genetics **125**: 115-127.