THE SPECIAL CHARACTER OF BARBARA MCCLINTOCK'S NOBEL PRIZE ADDRESS[i]

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INTRODUCTION

Her choice of content makes Barabara McClintock's 1983 Nobel Prize address an extraordinary scientific document. Despite the fact that she received the most prestigious of scientific honors "for her discovery of mobile genetic elements,"[ii] her speech reviews her career since 1931 (McClintock 1931; Birchler, this volume) without describing any of the evidence for transposition, the genome restructuring process cited as her major achievement. She simply tells us that many of her observations involving transposable controlling elements have been repeated in numerous organisms, but she does not explain how she demonstrated their genetic mobility in maize. Her text treats the underlying mechanisms as well-established science not requiring specific review.

Why did McClintock relegate what the Nobel Prize committee considered her crowning discovery to so few words? The answer, I believe, is that she had something she considered far more important to discuss. In conversation, McClintock frequently said that regulation, not transposition, was the major focus of her research from the 1940s onward. She did not understand regulation simply as a molecular mechanism. Rather, she thought of regulation as a deeply biological phenomenon, exemplifying the vital processes of sensing, evaluating, responding, repairing and adapting.

The Nobel Prize address contains words professional scientists rarely apply to living cells and organisms, like "thoughtful" and "wise." As the neurobiologist Dennis Bray points out in the introduction to his recent book, *Wetware: A computer in every living cell*,[iii] McClintock was the first modern biologist to ask that future research "determine the extent of knowledge the cell has of itself, and how it utilizes this knowledge in a "thoughtful" manner when challenged."[iv]

Since we recognize McClintock as among the 20th Century's greatest biologists, we need to ask how she validated and clarified her use of such unabashedly anthropomorphic language in a speech about the genome. This question leads us immediately to the heart of her true subject matter and also to the special problems a 21st Century reader faces in understanding this paper.

McClintock viewed her 60+ year career studying the maize genome as a never-ending series of lessons about the amazing sophistication of cellular cognition and control. At the same time, she felt that others could only appreciate what she had learned if she described each lesson in detail. Because of McClintock's need to be straightforward about the import of her conclusions and also precise in her descriptions of the underlying observations, her papers in general (and this one in particular) make special demands on the reader, who frequently has to shift back and forth between detailed accounts of maize cytogenetics and the broadly stated implications of her findings.

One further feature of McClintock's thinking and writing has proven challenging for contemporary readers of her work. Today, we are accustomed to

papers that conclude with summarizing models and explanatory schemes. McClintock, on the other hand, was deeply skeptical of what she called "the NOW explanation." Her long career exposed her to many concepts and ideas that were inevitably superseded by later discoveries and theoretical formulations. She expressed how her own experience in the founding years of cytogenetics had provided her "the pleasure of witnessing and experiencing the excitement created by revolutionary changes in genetic concepts that have occurred over the past sixty-odd years. I believe we are again experiencing such a revolution." Given this background, she preferred to state the meaning of her observations without attempting to provide explanations that would ultimately prove inadequate.

McClintock was comfortable in saying, as she did in this address, that certain well-documented phenomena "are beyond our present ability to fathom." This was not a statement of futility but rather a well-founded recognition that science had to progress both technologically and conceptually before certain problems could be fruitfully investigated. Remember that McClintock received the Nobel Prize over a decade before the scientific community started to become aware of the importance of RNA-directed regulation in general (and of epigenetic states in particular; see Fedoroff 2013 & this volume). The revelation of this unexpected layer of complexity in cell control systems would not have seemed unusual to her because she had already lived through six decades of comparable revelations. To her, new and surprising shifts in thinking were recurring, inevitable and enjoyable.

THE COGNITIVE LESSONS MCCLINTOCK LEARNED FROM MAIZE

McClintock organized her presentation in the chronological order in which key observations and realizations occurred. She left out studies on the mechanics of transposition because she was interested in explaining how her maize plants responded to "shocks" experienced in the course of genetic experimentation. Her main discoveries concerned the ability of plant cells to sense and repair genome damage. The major surprise in her work was the totally unanticipated discovery that maize genomes contain latent elements that can be activated to alter patterns of genome expression and restructure chromosomes. Recognizing that her focus was on how maize cells sense damage and respond appropriately makes the paper easier to follow and helps illuminate the logic of her narrative.

McClintock's introduction gives the reader an overview of her argument. She uses the contemporary examples of heat shock and "SOS" responses to remind us that we take certain programmed genome adaptations to damage stimuli for granted, but she immediately places them in the cognitive context she will employ throughout ("Some sensing mechanism must be present in these instances to alert the cell to imminent danger…"). Then she moves on in a very condensed fashion to mention less programmatic responses to damage, her own surprising experience "in the mid-1940s" and the study of X-ray mutagenesis, before she reminds us that we are in the midst of an upheaval in our "views of components of cells and how they operate…" This section brings in many relevant topics but only makes sense once the reader has become familiar with the whole story McClintock is telling (see Kass & Chomet 2009).

Following the introduction, McClintock moves to a brief and technical description of the 1944 experiment that led to her discovery of mobile genetic elements (Kass & Chomet 2009, p. 27). These findings led to her Nobel Prize, which may be the reason she brings them in outside of the historical sequence that would

have made them easier to follow. She tersely explains the crosses that she designed to use her previously acquired knowledge of chromosome breakage and repair to isolate deficiencies (deletions) removing segments from the short arm of chromosome IX. Instead of the expected deficiencies, she recounts how she was surprised to obtain genetically unstable plants that produced variegating sectors in the leaves and other parts of the plant. Noting that these sectors often appeared in pairs ("twin sectors") gave her the idea that allowed her to track down the source of variegation as transposable controlling elements "that could regulate gene expressions in precise ways."

But McClintock does not really explain how she solved this intricate puzzle of genetic mobility. That story is recounted at length in the 1987 collection of her papers.[v] She had a different point to make about cell sensitivity, which required a historical narrative of her studies on responses to chromosome breakage. Accordingly, the next three sections of the address tell the story of how McClintock learned that maize cells sense the presence of broken chromosome ends and activate latent transposable elements when they cannot easily repair them.

LESSON ONE

Historically, the first lesson about genome repair came from her experience beginning in 1931 with Stadler's mutant X-irradiated maize stocks (McClintock 1931b, Birchler this volume). This was a time, she noted, when "our knowledge of chromosomes and genes was limited. In retrospect we might call it primitive." Nonetheless, she was eager to study them, being "delighted to do so, as this would be a very new experience." She realized that the altered phenotypes obtained with X-rays were not due to the expected "gene mutations" but resulted from deficiencies and other rearrangements. These changes were explicable as the results of fusions of two chromosome ends following X-ray induced breakage events. This was McClintock's first demonstration of genome repair capabilities, and she confirmed her ideas by studying the behavior of ring chromosomes; these were subject to forming double dicentric rings by recombination that ruptured to produce two broken ends in each daughter cell that then resealed after cell division.

Summarizing this first lesson about break repair from her cognitive perspective, McClintock writes: "The conclusion seems inescapable that cells are able to sense the presence in their nuclei of ruptured ends of chromosomes, and then to activate a mechanism that will bring together and then unite these ends, one with another. And this will occur regardless of the initial distance in a telophase nucleus that separated the ruptured ends. The ability of a cell to sense these broken ends, to direct them toward each other, and then to unite them so that the union of the two DNA strands is correctly oriented, is a particularly revealing example of the sensitivity of cells to all that is going on within them. They make wise decisions and act upon them."

LESSON TWO

A second major lesson came when McClintock decided to find out what happened when there was only a single broken chromosome end in a cell. Again, in this section of the address, she explains the experimental procedures and chromosome events in terse, technical language and provides three figures to illustrate her experiments.

She discovered that chromosomes with one broken end replicate, fuse their ends to form a dicentric chromosome and then undergo breakage again during mitosis (the "breakage-fusion-bridge" or BFB cycle) in haploid microspore and triploid endosperm cells. However, in diploid zygotes and embryo cells, the broken end is quickly "healed" so that no further fusions or breaks occur. McClintock mentions that she found a recessive mutation (now lost) that prevented the healing process. These experiments showed that a broken end could be sensed and capped with a telomere in embryo cells but that the repair process is not expressed in other cell types.

LESSON THREE

The most important lesson about sensitivity to broken ends was the surprising outcome of McClintock's "failed" experiment looking for chromosome IX deficiencies that unexpectedly led to the discovery of transposable controlling elements.

Where did these previously unknown elements come from? McClintock reasoned that they must have been latent in the genome and become active in response to the sensing of an uncapped broken end in the microsporocyte divisions before fertilization. To confirm this hypothesis, she looked for activation of the *Dotted* (*Dt*) element that Rhoades had shown to generate variegated expression of the standard recessive *a* allele of the *A* locus.

Because homozygous *a/a* plants produced colorless kernels that reverted to dark spots in the presence of *Dt*, new activations should be easy to detect. McClintock found such activations in endosperm nuclei fertilized by plants that had undergone the BFB cycle in the previous haploid generation. Doerschug later confirmed this result by using the same method to obtain active, transposable *Dt* elements in embryos.[vi]

McClintock's conclusion was: "Activation of potentially transposable elements, as well as other structural modifications of the chromosomes not considered here, are recognizable consequences of the cell's response to the continuing trauma." In other words, genome monitoring does not only involve turning on repair functions; it also activates systems that create new genome configurations. In McClintock's perspective, hereditary change is a cognitive response to damage.

HOW WIDELY APPLICABLE ARE THE OBSERVATIONS AND CONCLUSIONS DRAWN FROM MAIZE?

After laying out the experiments that convinced her of maize cell sensitivity to "genome shocks," McClintock addresses the extent to which her findings have parallels in other organisms. In keeping with her treatment of transposition as a well-established phenomenon not needing fuller explanation, she only makes a few terse references to the detection and application of mobile genetic elements in a wide variety of other organisms.

Instead of discussing the mechanisms of genome restructuring, McClintock places the emphasis on "the numerous homeostatic adjustments required of cells." She takes a very broad view of this subject and links it to developmental genome control during the morphogenesis of multicellular organisms. Here she brings in a whole new series of stimuli that induce complex but programmatic responses: the formation of insect-induced leaf galls and bacterially-induced root nodules in plants. She links these "reprogrammings" to the ability of "a single genome" to encode "two brilliantly designed organisms, the caterpillar and the moth."

Her point is that we are still at the beginning of understanding how cells extract information from their genomes: "…we know little of the potentials of a genome. Nevertheless, much evidence tells us that it must be vast." Since McClintock often suggested insect galls as potential material for molecular study, her goal seems to be to set out key elements of a future research agenda on whole-genome regulation.

After pointing out how much remains to be learned about realizing "the potentials of a genome," McClintock shifts the focus to genome modification and restructuring "when confronted with unfamiliar conditions." She cites examples of genome-wide changes that scientists in the early 1980s rarely thought about (but which we currently view from an epigenetic perspective): nuclear reprogramming of tissue culture cells and the shift from somatic to flower (germinal) development in plants.

McClintock links these genome-wide changes to the need for whole genome regulatory modifications during normal sexual reproduction (gamete formation, fertilization, and zygote development) as well as during vegetative plant reproduction from cuttings. Then she moves on to the abnormal generation of whole plants from tissue culture cells. It is in this abnormal situation that phenotypic and genomic changes arise (a process often called "somaclonal variation"[vii]). McClintock emphasizes that such changes "could be potent sources for selection by the plant breeder, and incidentally, for theoretical ponderings by the biologist."

From tissue culture and plant regeneration, McClintock moves on to other unpredictable but nonetheless common events that lead to genome restructuring, such as the activation of transposable elements by RNA virus infection and interspecies crosses. She places particular emphasis on the role of interspecific hybridization in formation of a new plant species, *Triticale* (x *Triticosecale* Wittm.), and how important genome restructuring has been in animal evolution, citing the chromosome fusions in Muntjak deer and movements of heterochromatic blocks in copepods of the *Cyclops* group.

Considering unusual events triggering genome change leads McClintock to propose a connection between "shocks," chromosome restructuring, and species change. Clearly, she ranks evolution among the areas subject to "theoretical ponderings by the biologist." In her concluding summary she returns to this point when she says that "illustrations from nature are included because they support the conclusion that stress, and the genome's reactions to it, may underlie many species formations."

HOW HAS MCCLINTOCK'S COGNITIVE PERSPECTIVE HELD UP SINCE 1983?

It is now over 27 years since McClintock received the Nobel Prize. In that interval, there has been tremendous progress in our knowledge of molecular mechanisms of sensing, regulation, DNA repair, mobile genetic element activities and genome rearrangements. In addition, whole genome sequencing has provided unimpeachable documentation of all these classes of genome restructuring events involved in evolutionary transitions (Shapiro 2011). Altogether, these developments have reinforced McClintock's views on genomic change in response to stress and begun to answer the challenge she posed at the very end of her address, when she said, "We know nothing, however, about how the cell senses danger and instigates responses to it that often are truly remarkable."

Among the many developments in the molecular cell biology of the genome

that have emerged since 1983, the following are particularly relevant to McClintock's observations and the conclusions she drew from them:

(i) The importance of **genome monitoring and checkpoint execution** in maintaining genome stability. The articulation of the checkpoint concept by Weinert & Hartwell in 1988[viii] introduced a cognitive element (*viz*. damage sensing, signaling and adaptive regulatory response) to the molecular biology of cell cycle control. Our knowledge of the molecular components of checkpoint monitoring and signaling systems has increased tremendously since then[ix].

(ii) The discovery of **DS break repair centers in eukaryotic nuclei**. Key among genome monitoring modalities in eukaryotes are detection of broken DNA molecules by ATM-related proteins, their marking with a complex of proteins unique to broken ends, transmission of information about the presence of the break, and active transport of the broken ends to subnuclear repair centers for correction by homologous recombination (HR) or non-homologous end joining (NHEJ).[x] NHEJ of broken ends from different sites and chromosomes is fundamental to the kinds of genome restructuring McClintock described in her maize plants.[xi]

(iii) The documentation of **whole genome** duplications **(WGDs) at key points in evolution**. McClintock's view that shocks such as interspecific hybridization play a key role in formation of new species fits well with sequence data documenting the occurrence of WGDs in evolution of new species, genera and families ranging from yeasts[xii] and protozoa[xiii] to vertebrates[xiv] and many groups of flowering plants[xv] (whose rapid diversification seemed to be "an abominable mystery" to Darwin[xvi]). WGDs are commonly observed in the allotetraploid progeny of interspecific hybrids, and their occurrence is widely seen as a source of genome instability and rapid phenotypic diversification.[xvii]

(iv) The elucidation of **RNA-directed epigenetic control regimes for silencing mobile genetic elements**. McClintock's address places great emphasis on the activation of silent mobile elements in the genome as a totally unpredictable cognitive response to the detection of a single broken end that cannot readily be repaired. Although quite a few distinct control mechanisms have been documented for individual mobile elements, the most general regulatory mode for transposons and retrotransposons in eukaryotes involves their incorporation into silent heterochromatin.[xviii] This epigenetic control is directed by small siRNAs transcribed from specialized DNA segments that preserve a genomic memory of earlier element invasions.[xix]

It is therefore of the highest significance that we are learning of how sensitive genome stability and epigenetic chromatin configurations are to many kinds of stimuli, or "shocks" (to use McClintock's term).[xx] A tabulation of these stimuli can be found online at http://shapiro.bsd.uchicago.edu/TableII.7.shtml and http://shapiro.bsd.uchicago.edu/TableII.10.shtml. In other words, the molecular study of mobile genetic elements has brought us to see them as reflecting a cell's ability to sense disturbances and modify the epigenetic regulatory status of its genome.

Considering our growing knowledge of sensory processes in cell cycle regulation, DNA repair, and the control of genome stability, there can be no question that molecular biology is bringing us ever closer to McClintock's cognitive view. In addition, genome sequencing is confirming her insight that cellular responses to

shocks of all kinds have played key roles at major steps in evolution (Shapiro 2011).

Since 1983, we have made major advances towards deciphering "how the cell senses danger and instigates responses to it that often are truly remarkable." Nonetheless, our knowledge remains fragmentary; it is composed more of a long and often confusing parts list than of functional insight into how cellular cognition operates. If we are to succeed in formulating a comprehensive picture that integrates our molecular discoveries with the adaptive survival capabilities of living organisms on all time scales, we will certainly need to ponder deeply why McClintock calls cells "thoughtful" and "wise" when it comes to using their genomes. In practical terms, this means developing novel information-processing concepts and experimental models to explore the extraordinarily sophisticated capabilities that living cells apply to extracting and writing genomic data and inherited biological programs.

REFERENCES

Birchler J. this volume. Summary and Analysis of "Cytological observations of deficiencies involving known genes, translocations and inversions in *Zea mays*."

Fedoroff, N (ed.). 2013. *Plant Transposons and Genome Dynamics in Evolution*. Wiley-Blackwell.

Fedoroff, N. this volume. McClintock and Epigenetics: Insights from Changes in the Phase of Transposon Activity.

Kass, LB and P Chomet. 2009. Barbara McClintock. Pp. 17-52. *In:* J. Bennetzen and S. Hake (eds.), *Handbook of Maize: Genetics and Genomics.* Springer.

McClintock, B. 1931b. Cytological observations of deficiencies involving known genes, translocations and an inversion in *Zea mays*. *Missouri Agricultural Experiment Station Research Bulletin* 163 [December, authorized 23 December 1931]: 1-30.

Shapiro, JA. 2011. *Evolution: A View from the 21st Century*. Upper Saddle River, NJ, FT Press Science.

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[i] Published as McClintock, B. 1984. The Significance of responses of the genome to challenge. *Science* 226:792-801. http://www.ncbi.nlm.nih.gov/pubmed/15739260

[ii] http://nobelprize.org/nobel_prizes/medicine/laureates/1983/

[iii] Bray, D. 2009. *Wetware: A computer in every living cell*. Yale University Press, New Haven. (May 04, 2009) 280 pp.

[iv] All quotations are taken directly from McClintock's text.

[v] McClintock, B. 1987. Introduction. Pp. vii-xi. *In:* J. A. Moore (ed.) *Discovery and characterization of transposable elements: the collected papers of Barbara McClintock.* New York: Garland.

[vi] Doerschug, EB. 1973. Studies of dotted, a regulatory element in maize**.** *Theoretical and Applied Genetics* 43: 182-189. http://www.springerlink.com/content/vrutl67k54p47410/

[vii] http://en.wikipedia.org/wiki/Somaclonal_variation

[viii] Weinert, TA and LH Hartwell. 1988. The RAD9 gene controls the cell cycle response to DNA damage in *Saccharomyces cerevisiae*. *Science* 241: 317-22. http://www.ncbi.nlm.nih.gov/pubmed/3291120.

[ix] Hartwell, L. 1992. Defects in a cell cycle checkpoint may be responsible for the genomic instability of cancer cells. *Cell* 71**:**543-546. http://www.ncbi.nlm.nih.gov/pubmed/1423612.

Zhou, J, J Yao, HC Joshi. 2002. Attachment and tension in the spindle assembly checkpoint. *Journal of Cell Science* 115: 3547-3555. http://www.ncbi.nlm.nih.gov/pubmed/12186941.

Harrison, JC, JE Haber. 2006. Surviving the breakup: the DNA damage checkpoint**.** *Annual Review of Genetics* 40: 209-235. http://www.ncbi.nlm.nih.gov/pubmed/16805667.

Ishikawa, K, H Ishii, et al. 2006. DNA damage-dependent cell cycle checkpoints and genomic stability. *DNA Cell Biology* 25(7): 406-411. http://www.ncbi.nlm.nih.gov/pubmed/16848682.

Chen, CC and J Tyler. 2008. Chromatin reassembly signals the end of DNA repair. *Cell Cycle* 7: 3792-3797. http://www.ncbi.nlm.nih.gov/pubmed/19066448.

Barlow, JH, M Lisby, et al. 2008. Differential regulation of the cellular response to DNA double-strand breaks in G1. *Molecular Cell* 30(1): 73-85. http://www.ncbi.nlm.nih.gov/pubmed/18406328.

Fuller, BG and PT Stukenberg. 2009. Cell division: righting the check**.** *Current Biology* 19: R550-553. http://www.ncbi.nlm.nih.gov/pubmed/19640489.

Kang, J and H Yu. 2009. Kinase signaling in the spindle checkpoint. *Journal of Biological Chemistry* 284: 15359-15363. http://www.ncbi.nlm.nih.gov/pubmed/19228686.

Voineagu, I, CH Freudenreich, SM Mirkin. 2009. Checkpoint responses to unusual structures formed by DNA repeats. *Molecular Carcinogenesis* 48: 309-318. http://www.ncbi.nlm.nih.gov/pubmed/19306277.

Nezi, L, A Musacchio. 2009. Sister chromatid tension and the spindle assembly checkpoint. *Current Opinion in Cell Biology* 21: 785-795. http://www.ncbi.nlm.nih.gov/pubmed/19846287.

[x] McGowan, CH and P Russell. 2004. The DNA damage response: sensing and signaling. *Current Opinion in Cell Biology* 16: 629-633. http://www.ncbi.nlm.nih.gov/pubmed/15530773.

Czornak, K, S Chughtai, et al. 2008. Mystery of DNA repair: the role of the MRN complex and ATM kinase in DNA damage repair. *Journal of Applied Genetics* 49(4): 383-396. http://www.ncbi.nlm.nih.gov/pubmed/19029686.
.

Lisby, M, A Antunez de Mayolo, et al. 2003. Cell cycle-regulated centers of DNA double-strand break repair. *Cell Cycle* 2(5): 479-483. http://www.ncbi.nlm.nih.gov/pubmed/12963848.

Lisby, M and R Rothstein. 2004. DNA damage checkpoint and repair centers. *Current Opinion in Cell Biology* 16(3): 328-334. http://www.ncbi.nlm.nih.gov/pubmed/15145359.

Lisby, M and R Rothstein. 2005. Localization of checkpoint and repair proteins in eukaryotes. *Biochimie* 87(7): 579-589. http://www.ncbi.nlm.nih.gov/pubmed/15989975.

Kidane, D and PL Graumann. 2005. Dynamic formation of RecA filaments at DNA double strand break repair centers in live cells. *Journal of Cell Biology* 170(3): 357-366. http://www.ncbi.nlm.nih.gov/pubmed/16061691.

Plate, I, SC Hallwyl, et al. 2008. Interaction with RPA is necessary for Rad52 repair center formation and for its mediator activity. *Journal of Biological Chemistry* 283(43): 29077-29085. http://www.ncbi.nlm.nih.gov/pubmed/18703507.

Wood, JL and J Chen. 2008. DNA-damage checkpoints: location, location, location. *Trends in Cell Biology* 18: 451-455. http://www.ncbi.nlm.nih.gov/pubmed/18760607.

[xi] Pastwa, E and J Blasiak. 2003. Non-homologous DNA end joining. *Acta Biochimica Polonica* 50(4): 891-908. http://www.ncbi.nlm.nih.gov/pubmed/14739985.

Lisby, M, UH Mortensen, et al. 2003. Colocalization of multiple DNA double-strand breaks at a single Rad52 repair centre. *Nature Cell Biology* **5**(6): 572-577. http://www.ncbi.nlm.nih.gov/pubmed/12766777.

Lupski, JR, P Stankiewicz. 2005. Genomic disorders: molecular mechanisms for rearrangements and conveyed phenotypes. *PLoS Genetics* 1(6):e49. doi:10.1371/journal.pgen.0010049 http://www.ncbi.nlm.nih.gov/pubmed/16444292.

Weterings, E, DJ Chen. 2008. The endless tale of non-homologous end-joining. *Cell Research* 18(1, Jan.): 114-24. http://www.ncbi.nlm.nih.gov/pubmed/18166980.

[xii] Wolfe, KH, DC Shields. 1997. Molecular evidence for an ancient duplication of

the entire yeast genome. *Nature* 387: 708-713. http://www.ncbi.nlm.nih.gov/pubmed/9192896.

[xiii] Aury, JM, O Jaillon, L Duret, B Noel, C Jubin, BM Porcel, B Segurens, V Daubin, V Anthouard, N Aiach, *et al*. 2006. Global trends of whole-genome duplications revealed by the ciliate *Paramecium tetraurelia*. *Nature* 444: 171-178. http://www.ncbi.nlm.nih.gov/pubmed/17086204.

[xiv] Nakatani, Y, H Takeda, Y Kohara, S Morishita. 2007. Reconstruction of the vertebrate ancestral genome reveals dynamic genome reorganization in early vertebrates. *Genome Research* 17: 1254-1265. http://www.ncbi.nlm.nih.gov/pubmed/17652425.

[xv] Cui, L, PK Wall, JH Leebens-Mack, BG Lindsay, DE Soltis, JJ Doyle, PS Soltis, JE Carlson, K Arumuganathan, A Barakat, *et al*. 2006. Widespread genome duplications throughout the history of flowering plants. *Genome Research* 16: 738-749. http://www.ncbi.nlm.nih.gov/pubmed/16702410.

[xvi] Darwin, C. July 22, 1879, Letter to Joseph Hooker. P. 20-21, Letter 395. *In:* F Darwin and AC Seward (eds.), *More letters of Charles Darwin; A record of his work in a series of hitherto unpublished letters,* Vol. II, 1903, London: John Murray. http://darwin-online.org.uk/EditorialIntroductions/Freeman_MoreLetters.html.

[xvii] Table 3. Genomic responses to changes in ploidy and interspecific hybridization in plants; published in Shapiro, JA. 2010. Mobile DNA and evolution in the 21st Century. *Mobile DNA* 1(1): 4. http://www.ncbi.nlm.nih.gov/pubmed/20226073.

[xviii] Slotkin, RK and R Martienssen. 2007. Transposable elements and the epigenetic regulation of the genome. *Nature Reviews Genetics* 8(4): 272-285. http://www.ncbi.nlm.nih.gov/pubmed/17363976.

Lisch D. 2009. Epigenetic regulation of transposable elements in plants. *Annual Review of Plant Biology* 60: 43-66. http://www.ncbi.nlm.nih.gov/pubmed/19007329.

[xix] Brennecke, J, AA Aravin, et al. 2007. Discrete small RNA-generating loci as master regulators of transposon activity in Drosophila. *Cell* 128(6): 1089-1103. http://www.ncbi.nlm.nih.gov/pubmed/17346786.

Brennecke, J, CD Malone, et al. 2008. An epigenetic role for maternally inherited piRNAs in transposon silencing. *Science* 322(5906): 1387-1392. http://www.ncbi.nlm.nih.gov/pubmed/19039138.

Thomson, T and H Lin. 2009. The biogenesis and function of PIWI proteins and piRNAs: progress and prospect. *Annual Review Cell Developmental Biology* 25: 355-376. http://www.ncbi.nlm.nih.gov/pubmed/19575643.

[xx] O'Neill, RJ, MJ O'Neill, JA Graves. 1998. Undermethylation associated with retroelement activation and chromosome remodelling in an interspecific mammalian hybrid. *Nature* 393: 68-72. http://www.ncbi.nlm.nih.gov/pubmed/9590690.

Dong, YZ, ZL Liu, XH Shan, T Qiu, MY He, B Liu. 2005. Allopolyploidy in wheat induces rapid and heritable alterations in DNA methylation patterns of cellular genes and mobile elements. *Genetika* 41: 1089-1095. http://www.ncbi.nlm.nih.gov/pubmed/16161630.

Tanurdzic, M., M. W. Vaughn, et al. 2008. Epigenomic consequences of immortalized plant cell suspension culture. *PLoS Biology* 6(12): 2880-2895. http://www.ncbi.nlm.nih.gov/pubmed/19071958.

Wang, H, Y Chai, et al. 2009. Molecular characterization of a rice mutator-phenotype derived from an incompatible cross-pollination reveals transgenerational mobilization of multiple transposable elements and extensive epigenetic instability. *BMC Plant Biology* 9: 63 (12 pages) . http://www.ncbi.nlm.nih.gov/pubmed/19476655.