

Sex Determination and Sex Change in Cycads: Tantalizing New Hints

Root Gorelick

At the 7th international meeting on cycad biology at the Institute of Ecology in Xalapa, Veracruz, Mexico in January 2005, Roy Osborne and I presented a hypothesis regarding sex determination and sex change in cycads (Gorelick & Osborne 2007; Osborne & Gorelick 2007). We proffered that sex is not determined by difference in DNA, such as what we have with mammalian X and Y chromosomes, but by different epigenetic marks on the two sexes (also Gorelick 2003; Gorelick 2005). More specifically, we hypothesized that one sex had more methyl groups (-CH₃) on cytosine nucleotides on certain genes than did the other sex. Both sexes may have the same genes, but have different patterns of methyl groups on to of those DNA nucleotides. 'Epigenetic' literally means 'on top of genetic.' Methyl groups, when attached to cytosines of regulatory genes, turn off downstream coding genes. Female structures (e.g., megastrobili and eggs) are encoded by a specific suite of genes, while male structures (e.g., microstrobili and pollen) are encoded by a different suite of genes. However, I readily concede that these two sex-specific suites of genes are closely related to one another, possibly produced by gene duplication events (orthologous) given that ancestral plants seem to have been unisexual or hermaphroditic. Thus, a female plant should have highly methylated regulatory loci for those genes that encode for male function. And a male plant should have highly methylated regulatory loci for genes that encode for female function. In theory, this should be readily testable. In practice, the problem is that we have no idea which genes encode for female or male function, hence do not know where to look for their regulatory loci, let alone for whether these regulatory genes are methylated or not.

In practice, researchers take a needle-in-haystack approach, looking for methylation differences between the two sexes, hoping that this might explain macroscopic sex differences. Ray Ming's group has led this effort for determining sex in papaya, *Carica papaya* (Ma *et al.* 2004; Zhang *et al.* 2008). Lou Guillette's group (Parrott *et al.* 2014) has done similar pioneering work for sex determination in temperature-dependent sex determination in alligators (*Alligator mississippiensis*), where different temperature seems to strip methylation from different genes (actually, new methylation is not added after gene duplication, but the effect is equivalent). What I just learned is that a group in Thailand tried the same approach in looking for methylation differences between the sexes in *Cycas* and *Zamia* species (Kanchanaketu *et al.* 2007).

Kanchanaketu *et al.* (2007) took the needle-and-haystack approach, called methylation-sensitive amplified-fragment-length-polymorphism (aka MS-AFLP or MSAP), which was the approach that Roy Osborne and I had advocated. Kanchanaketu *et al.* found some differences - albeit somewhat equivocal differences - between methylation patterns in the two sexes. For better or worse, their findings were published in the in-house journal of their home university, Kasetsart University, in Bangkok. While the *Kasetsart Journal* is needless-to-say cryptic, their results are tantalizing, making me hope that further research in this area will occur.

The primary horticultural motivation for this seemingly technical work on whether removal of methyl groups from cycad DNA determines or changes sex of individual plants. While sex is usually considered fixed in cycads (dioecy) and in the other living seed-bearing plant with swimming flagellated sperm, namely *Ginkgo biloba*, we now know that stressful conditions can occasionally alter the sex of an individual plant in both cycads (Osborne 1990; Osborne & Gorelick 2003, 2007; Crane 2013) and *Ginkgo* (Crane 2013).

There are certainly ways to strip away methylation (again, actually, keeping methyl groups from being replicated during mitosis), such as application of 5-azacytidine. But this is a nasty chemical that could cause all sorts of developmental abnormalities. When Mary Ann Fieldes first showed that methylation changes due to its application in one generation of flax (*Linum usitatissimum*) seeds could be inherited over many generations, she chose a dose that the plants could barely survive (Fieldes & Amyot 1999). Once she realized how

draconian these developmental effects were and that they were heritable, she conspicuously never worked with it again. Curiously, 5-azacytidine has been declared safe enough to be given to humans for various medical conditions, approved by the U.S. Food & Drug Administration in 2004, under the trade name Vidaza. With a prescription you can purchase it at your local pharmacy to treat blood diseases, cancer, and even mental disorders (Szyf 2003; Kan *et al.* 2004). But there is almost no doubt that significant amounts of this prescribed 5-azacytidine are urinated into the drinking water supply. Knowing that it fairly indiscriminately removes methyl groups from DNA, I would not want to work with this chemical nor taken it medicinally. Much of the methylation on our DNA is incredibly valuable, suppressing many viruses and stopping cells from rapidly dividing, i.e. taking 5-azacytidine might induce cancer.

What first got me interested in sex determination and DNA methylation (and working with the inimitable Roy Osborne) was the holy cycad grail of creating a female plant of *Encephalartos woodii* (Gorelick & Osborne 2002). Adding methylation back to DNA is not simple, but we could remove methylation from both female- and male-determining genes in the existing male plants, thereby creating a hermaphroditic *E. woodii*. While we do not have seeds to soak in 5-azacytidine, offshoots ("pups") or maybe tissue-cultured plants could, in theory, be soaked in a sub-lethal concentration of 5-azacytidine. Now that we have additional clues that this might work, including Kanchanaketu *et al.*'s (2007) tantalizing note, as well as the work on papayas and alligators, I hope somebody tries. But I also hope that this is done under highly controlled conditions, limiting 5-azacytidine exposure to humans and the environment. Please do not pour this chemical down the drain, even if it is legal.

References

- Crane, P. 2013. *Ginkgo*. Yale University Press, New Haven.
- Fieldes, M.A. & Amyot, L.M. 1999. Evaluating the potential of using 5-azacytidine as an epimutagen. *Can. J. Bot.* 77: 1617-1622.
- Gorelick, R. 2003. Evolution of dioecy and sex chromosomes via methylation driving Muller's ratchet. *Biol. J. Linn.Soc.* 80: 353-368.
- _____. 2005. Theory for why dioecious plants have equal length sex chromosomes. *Amer. J. Bot.* 92: 979-984.
- _____. & Osborne, R. 2002. Inducing sex change and organogenesis from tissue culture in the endangered African cycad *Encephalartos woodii* (Cycadales, Zamiaceae). *S. Afr. J. Sci.* 98: 114-117.
- _____. & _____. 2007. Evolution of dioecy and sex chromosomes in cycads. *Mem. N.Y. Bot. Gard.* 97: 326-334.
- Kan, P.X., Popendikyte, V., Kaminsky, Z.A., Yolken, R.H., and Petronis, A. 2004. Epigenetic studies of genomic retroelements in major psychosis. *Schizophrenia Res.* 67: 95-106.
- Kanchanaketu, T., Hongtrakul, V., & Sangduen, N. 2007. Analysis of sex determination in some cycads using methylation-sensitive amplification polymorphism (MSAP). *Kasetsart J.* (Natural History) 41: 641-650.
- Ma, H., Moore, P.H., Liu, Z., Kim, M.S., Yu, Q., Fitch, M.M.M., *et al.* 2004. High-density linkage mapping revealed suppression of recombination at the sex determination locus in papaya. *Genetics* 166: 419-436.
- Osborne, R. 1990. Two new reports of cycad sex changes. *Encephalartos* 23: 18-20.
- Osborne, R. & Gorelick, R. 2003. Sex change in cycads. *Palms & Cycads* 76: 10-15.
- Osborne, R. & Gorelick, R. 2007. Sex change in cycads: cases, causes, and chemistry. *Mem. NY Bot. Gard.* 97: 335-345.
- Parrott, B.B., Kohno, S., Cloy-McCoy, J.A., & Louis J. Guillette, L.J. 2014. Differential incubation temperatures result in dimorphic DNA methylation patterning of the SOX9 and aromatase promoters in gonads of alligator (*Alligator mississippiensis*) embryos. *Biol. Reprod.* (In press). DOI: 10.1095/biolreprod.113.111468.
- Szyf, M. 2003. DNA methylation and cancer therapy. *Drug Resist. Updates* 6: 341-353.
- Zhang, W.L., Wang, X.U., Yu, Q.Y., Ming, R., and Jiang, J.M. 2008. DNA methylation and heterochromatinization in the male-specific region of the primitive Y chromosome of papaya. *Genome Res.* 18: 1938-1943.