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Transposable elements suppress recombination in all meiotic eukaryotes, including automictic ancient asexuals: a reply to Schön and Martens

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I discuss three problems with the argument that ancient asexual lineages lack meiosis and recombination resulting in a diminution of (potentially disadvantageous) transposable elements and other forms of repetitive DNA. First, all ancient asexuals are probably automictic, hence could retain meiotic recombination in the guise of gene conversion. Second, transposable elements alter recombination rates, not vice versa. Third, increasing the number of transposable elements and other forms of repetitive DNA reduces meiotic recombination rate. Ancient asexuals lack those transposable elements that are transmitted via outcrossing, and this has nothing to do with meiotic recombination.

KEYWORDS: Methylation, 5-methylcytosine, heterochromatin, parthenogenetic, bdelloid, darwinulid, *Darwinula*, *Artemia*, automixis.

Introduction

In their stimulating paper on the role of transposable elements in lineages of ancient asexual metazoans, Schön and Martens (2002) argue that lack of sexuality implies lack of meiosis and recombination, which in turn causes a diminution in the number of costly transposable elements and other forms of repetitive DNA. Although their paper is remarkably informative and provides a nice review of ancient asexual metazoans and the possible role of transposable elements and other forms of highly repetitive DNA in the continued existence and asexuality of these lineages, I wish to point out three crucial problems with their argument. First, there is growing evidence that all ancient asexuals are automictic, hence could retain meiotic recombination in the form of gene conversion even if chromosomes are no longer homologous. Second, transposable elements alter recombination rates, not vice versa. Third, a preponderance of transposable elements and other forms of repetitive DNA results in a reduced meiotic recombination rate, i.e. transposable element number and recombination rate are negatively correlated. I elaborate on all three of these problems below and, in so doing, introduce all known extant ancient asexual lineages and the possible evolutionary consequences of their peculiar mode of reproduction.

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Ancient asexual lineages are those that have survived without any outcrossing for tens of millions of years. There are probably only three such ancient asexual animal lineages: bdelloid rotifers, darwinulid ostracods, and one or a few lineages of parthenogenetic brine shrimp (Judson and Normark, 1996). Lack of outcrossing causes species concepts to be a bit muddled for asexual lineages. Nonetheless, these are well-defined lineages with facultatively or obligately outcrossing sister taxa.

Ancient asexuals are automictic

The term ancient asexual conveys a misleading connotation. All ancient asexuals probably underwent and still undergo meiosis, albeit automixis, either occasionally or obligately each generation. Automixis refers to production of functional gametes (eggs) via normal meiosis, but pairs of gametes from the same individual then fuse to form a zygote. Thus, antithetically, ancient asexuals are meiotic, but essentially self-fertilizing from pairs of eggs. They never outcross.

There are two forms of meiotic recombination: crossing over and gene conversion. In sexual lineages both forms of recombination are roughly equally prevalent (Carpenter, 1987; Carpenter, 1994). Lineages that have evolved without any form of meiosis for tens of millions of years are predicted to have widely divergent homologues (Welch and Meselson, 2000), to the point where each pair of chromosomes can scarcely be termed homologous. However, this prediction also applies to any lineage that has been without crossing over recombination for tens of millions of years, even if there is obligate or facultative automixis. Unlike crossing over, gene conversion can still occur on non-homologous pairs of chromosomes (Carpenter, 1994). Gene conversion is mediated by early recombination nodules, which can bind to non-homologous segments of DNA. Crossing over is mediated by late recombination nodules, which can only bind to homologous DNA strands (Carpenter, 1994). Therefore, gene conversion is far more likely than crossing over in ancient asexuals.

Parthenogenetic *Artemia* brine shrimp have been asexual for approximately 30 million years (Browne, 1992) and are automictic (Barigozzi, 1974, citing Stefani, 1960). Curiously, polyploid lineages of parthenogenetic *Artemia* are apomictic (Browne, 1992).

Freshwater ostracods in the family Darwinulidae have been asexual for at least the past 70 million years (Butlin and Griffiths, 1993). Darwinulid ostracods probably have occasional facultative automixis (Butlin *et al.*, 1998). This conclusion is based on lack of divergence of ITS1 (internal transcribed spacer 1) sequences, in which there is virtually no allelic divergence. Although this inference is based on a single locus, it runs completely contrary to expectations from any model of ancient obligate apomixes (e.g. Welch and Meselson, 2000). Butlin *et al.* (1998) suggest that darwinulid ostracods' lack of allelic divergence could be due to somatic recombination, gene conversion or automixis. Mitotic recombination is possible, but has never been regarded as an important evolutionary force in animals. The other two possibilities, gene conversion and automixis, are essentially synonymous. Gene conversion requires some form of meiosis, whilst automixis seems to be inevitable if there is any form of meiosis. Thus, it is highly likely, albeit not yet proven, that darwinulid ostracods are automictic.

Rotifers in the family Bdelloidea have been asexual for at least the past 35 million years (Welch and Meselson, 2000). Although there is no definitive report of automixis in bdelloid rotifers, wholesale reset of their epigenetic inheritance systems each generation (Ricci *et al.*, 1999) is completely commensurate with existence of

meiosis and syngamy. In eukaryotes, wholesale epigenetic resets are only associated with gamete formation and following zygote formation, i.e. meiosis and syngamy (Jue *et al.*, 1995; Lei *et al.*, 1996; Shemer *et al.*, 1996; Bestor, 1998; Mertineit *et al.*, 1998). Bdelloid rotifers have experienced huge divergences between formerly homologous alleles, indicating that they never undergo outcrossing meiosis with crossing over recombination. However, despite an early report that bdelloid rotifers are apomictic (Pagani *et al.*, 1993), epigenetic reset data strongly indicate that bdelloids are automictic. Discovery of gene conversion would corroborate this inference of automixis in bdelloids.

Transposable elements suppress crossing over

Schön and Martens (2002) are not alone in asserting that lack of sexuality and recombination results in a diminution in the number of (potentially costly) transposable elements and other forms of repetitive DNA. Virtually all literature on this subject ascribes causality from sexuality and recombination to numbers of transposable elements (e.g. Charlesworth *et al.*, 1994; Charlesworth and Charlesworth, 1995), based largely on population genetic arguments. However, there is a preponderance of molecular evidence showing that the causality is, in fact, reversed. Numbers of transposable elements and other forms of repetitive DNA affect rates of meiotic recombination, and not vice versa.

Transposable elements and repetitive DNA affect recombination because they are disproportionally heavily methylated compared with other portions of the genome. Duplicated, translocated, or inverted portions of the genome are highly methylated (Volpe and Eremenko, 1974; Holliday, 1984; Yoder *et al.*, 1997; Matzke and Matzke, 1998; Regev *et al.*, 1998; Jones and Takai, 2001). This is a consequence of methylation having probably evolved as a defence against parasitic insertion of foreign DNA into the genome (Bestor, 1990; Yoder *et al.*, 1997). In addition to suppressing transcription for genomic defence, methylation also suppresses recombination. Recombination and transcription are suppressed by methylation blocking binding sites for proteins that mediate recombination and transcription, respectively (Catcheside, 1986; Hsieh *et al.*, 1986; Rauth *et al.*, 1986).

Methylation blocks protein binding sites in several inter-related ways. Methyl groups are bulky and hydrophobic and can directly block protein binding sites. Heterochromatic proteins bind to methylated cytosines, which then occupy protein binding sites, an effect that is enhanced by conformational changes in methylated DNA. Methylation restricts the conformational space of the major groove of DNA because methyl groups are bulky (Derreumaux *et al.*, 2001). Protein binding is inhibited by methylation-induced conformational changes, sometimes even to the point of converting the normal right-handed DNA helix to a left-handed helix (Behe and Felsenfeld, 1981; Behe *et al.*, 1981; McKay and Steitz, 1981). Methylation alters the folding of DNA because methyl groups are hydrophobic (Derreumaux *et al.*, 2001) and affects base stacking ability (Norberg and Vihinen, 2001). Methylation alters interactions of histones with promoter regions by stimulating histone deacetylase activity (Davey *et al.*, 1997; Jones *et al.*, 1998; Nan *et al.*, 1998; Henry *et al.*, 1999) and the interactions of DNA with itself (Mayer-Jung *et al.*, 1997), such as supercoiling, thereby affecting protein binding, transcription, and recombination.

Data on methylation suppressing recombination appear to only exist for crossing over (Holliday, 1984; Holliday, 1988; Colot and Rossignol, 1999), and not gene conversion. Crossing over recombination is suppressed in portions of the genome

with transposable elements and with copious highly repetitive DNA, and these portions of the genome have relatively large amounts of heterochromatin (John and Miklos, 1979), which provides an implicit indication of methylation. Lack of data regarding suppression of gene conversion is understandable because there is much more interest in crossing over. Thus, we must rely on a theoretical argument. Proteins (two different forms of recombination nodules) mediate both forms of recombination, and their actions are probably suppressed by methylation. However, gene conversion lacks the chiasmata formed in crossing over (Carpenter, 1994), hence gene conversion requires a smaller set of proteins. Therefore, methylation probably suppresses both gene conversion and crossing over recombination, but possibly does not suppress gene conversion to as great a degree as it suppresses crossing over.

Portions of the genome with a preponderance of transposable elements and repetitive DNA are more highly methylated than the rest of the genome. Therefore, recombination rate is negatively correlated with number of transposable elements and other forms of repetitive DNA because methylation suppresses recombination.

Contrary to the above theory and data of a negative correlation between transposable elements and recombination rates, Schön and Martens (2002: 384) state that 'Density of selfish DNA correlates positively with rate of recombination'. They readily admit that their assertion is based on a subset of transposable elements. It seems, however, that the most likely explanation for their putative positive correlation is that several classes of transposable elements are only transmitted sexually, hence are absent from ancient asexuals (Hickey, 1982; Arkhipova and Meselson, 2000). Schön and Martens' (2002) putative positive correlation between recombination rate and numbers of transposable elements appears to be based on the fact that ancient asexual individuals do not outcross, hence do not transmit certain classes of so-called selfish DNA. A broader look at all transposable elements (including those that are transmitted horizontally by pathogens) and highly repetitive DNA sequences (such as microsatellites) would undoubtedly reveal this to be a negative correlation, commensurate with theory.

Schön and Martens' (2002) paper contains a short discussion of Duret *et al.*'s (2000) nematode work showing that certain transposons are located preferentially in regions of high recombination, focusing on correlations. Instead, I focus on the causality underlying Duret *et al.*'s results. There is persuasive evidence that mobile genetic elements, such as transposons and repeats, are preferentially inserted into highly hypomethylated portions of the genome (Waugh O'Neill *et al.*, 1998; Ehrlich, 2000; Tuck-Muller *et al.*, 2000; Waugh O'Neill *et al.*, 2001). However, once these nascent genetic elements are inserted, they then become highly hypermethylated (Volpe and Eremenko, 1974; Deumling, 1981; Matzke and Matzke, 1998; Regev *et al.*, 1998; Jones and Takai, 2001). If the newly inserted portions of the genome form a relatively small portion of the genomic region, then that region will still be comparatively under methylated, and therefore will experience high recombination rates. Note that, with this argument, it does not matter whether the DNA was inserted into coding or non-coding portions of the genome. Thus, the results of Duret *et al.* (2000) are entirely consistent with the claim that, *ceteris paribus*, transposons are disproportionally highly methylated and methylation suppresses recombination.

Evolutionary implications

For ancient asexual lineages, what are the consequences of lower numbers of transposable elements due to lack of sexual outcrossing and the consequent increase

in recombination rates? Transposable elements are generally considered deleterious. However, they can provide sources for heritable variation, such as somoclonal variation in plants (Kaeppler *et al.*, 2000), and hence possibly allow individuals to survive environmental exigencies better (Griffiths and Butlin, 1995). It is not obvious how this balance between deleterious and beneficial effects of the dearth of sexually transmitted transposable elements affects the evolutionary trajectory of ancient asexual lineages.

Automictic ancient asexual lineages retain the ability to reset epigenetic inheritance systems each generation and to engage in gene conversion, an oft-forgotten form of meiotic recombination. These are two potent evolutionary forces, both of which probably allow these lineages to survive without any outcrossing or crossing over recombination. Furthermore, the potential increased recombination rate in ancient asexuals due to decreased methylation may increase the probability of gene conversion. Compared with apomixes, automixis clearly confers an evolutionary advantage.

Are ancient asexuals less burdened? Perhaps Schön and Martens (2002) are correct that ancient asexuals have escaped the costs of many classes of transposable elements and repetitive DNA. Schön and Martens provide some fascinating and persuasive arguments. I only wish to temper their arguments by tinkering with the details. In particular, I wish to highlight that ancient asexuals probably do undergo meiosis and syngamy—albeit automixis—and their lack of certain classes of transposable elements is due to lack of vertical transmission via outcrossing, and not due to suppressed recombination.

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