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Origin and maintenance of sex: the evolutionary joys of self sex

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Sex is generally thought of as meiosis, conjugation, and syngamy, with the primary function of sex believed to be genetic mixing. However, conjugation does not occur with complete automixis, whereas syngamy does not occur with restitutional automixis. Self sex in the forms of automixis and autogamy does not include genetic mixing. Yet sex, including self sex, is necessary for most eukaryotic lineages. What is the purpose of sex without genetic mixing? Obligate self sex is not an evolutionary dead end, but holds the key to understanding the evolutionary origin, function, maintenance, and ubiquity of sex. We extend the rejuvenescence hypothesis that sex provides a necessary developmental reset for multicellular eukaryotes and even many unicellular eukaryotes. Sex reduces additive genetic variance of epigenetic signals, especially cytosine methylation, and of ploidy levels. Furthermore, we argue that syngamy is a modified form of meiosis that maintains ploidy and resets epigenetic signals. Epigenetic resetting is consistent with sex being induced by starvation or desiccation. Diminution of additive genetic variance is consistent with the origin and maintenance of an adaptive trait, sex, that has been present for approximately two billion years. © 2009 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2009, **98**, 707–728.

ADDITIONAL KEYWORDS: asex – automixis – cannibalistic oral sex – Emile Maupas – endomitosis – endoploid – Hayflick limit – methylation – methylcytosine – parthenogenesis – premeiotic endomitosis – restitutional automixis – restitutional meiosis.

'I would like to see sex kept not only for our recreation but also, for a long while, let it retain its old freedom and danger, still used for its old purposes.' Hamilton (1988: 90)

INTRODUCTION

'Despite some ingenious suggestions by orthodox Darwinians, there is no convincing Darwinian history for the emergence of sexual reproduction' (Kitcher, 1982: 54; see also Hamilton, 1999) The current paradigm, which has persisted for over a century, is that sex functions to increase heritable variance in a population, speeding up evolution (Weismann, 1882; Weismann, 1889–1891; Bell, 1982; Kirkpatrick & Jenkins, 1989; Burt, 2000; Lustig, 2000; Gillespie, 2004; Keightley & Otto, 2006). Because of unrelenting directional selection, increased additive genetic variance is the exact opposite of what we expect from an adaptive trait, regardless of the two-fold cost of sex (Maynard Smith, 1978). This paradox was highlighted by Graham Bell, who asserted that 'If the immense genetic variation of natural populations is maintained by selection, then sex must represent an advantage because it *slows down* evolution' (Bell, 1982: 99; emphasis in original; for similar notions, see also Shields, 1982; 1988). Likewise, 'the initial function of chromosome pairing [in meiosis] was to *limit*, not enhance, recombination' (Wilkins & Holliday, 2009: 3). To rectify this conundrum, we provide a new Darwinian theory for the origin of sex by arguing that sex is beneficial via reducing additive genetic variance.

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The current paradigm that sex functions to increase additive genetic variance has taken several forms, which have relied on crossing-over recombination, independent segregation, ecological variation, and coevolution. Hypotheses that fall under this umbrella of sex increasing genetic variation include the red queen, Vicar of Bray, tangled bank, genetic hitchhiking, plastic recombination, Muller's ratchet, Kondrashov's hatchet, and the advantage of meiotic segregation (Weismann, 1889–1891; Bell, 1982; Kirkpatrick & Jenkins, 1989; Gillespie, 2004; Keightley & Otto, 2006). Each of these hypotheses may help explain the evolutionary maintenance of sex, where a plurality of mechanisms are undoubtedly at work (West, Lively & Read, 1999), but none have ever been convincingly implicated in the evolutionary origin of sex, which is our focus. Nonetheless, if sex reduces additive genetic variance, then we no longer have any difficulty in explaining maintenance and ubiquity of sex.

We hypothesize that eukaryotic sex is not important because it creates genetic variation, but rather because it diminishes heritable variation in epigenetic signals and maintains ploidy. Epigenetic signatures, including cytosine methylation, change irreversibly each generation to control development. The resetting of epigenetic signatures each generation allows development to proceed in a conservative fashion in both haploid and diploid stages. These resets remove almost all intra-generational epigenetic variation via resetting cytosine methylation and chromatin marks to levels that successfully weathered previous generations, whereas reduction division diminishes heritable variation in ploidy. We propose that both meiosis and syngamy begin with a chromosomal duplication and end with a division coupled with an epigenetic reset. Maintaining ploidy via meiosis and/or syngamy plus epigenetic resetting are the *sine qua non* of sex. The key to understanding sex is to examine lineages with either obligate complete automixis or obligate restitutional automixis, for which genetic mixing is irrelevant and for which true gametes and/or syngamy may be lacking. Because sex is almost certainly monophyletic (Cavalier-Smith, 1995; Redfield, 1999), the first sexual eukaryote almost certainly had complete automixis or, more likely, restitutional automixis. Who else could it have had sex with? Diminution of additive genetic variance is consistent with a phenomenon such as sex that is conservative across approximately two billion years of eukaryotic evolution.

DEFINITIONS

There are a large number of nuanced definitions of sex in the biological literature. For example, in contrast to the present study, some authors insist that sex requires outcrossing (amphimixis) or even that genital contact constitutes sex. The largest area of contention is whether self sex, including many forms of parthenogenesis, constitutes sex. We equate the term sex with any process in which meiosis occurs, including self sex. We also contend that syngamy is a modified form of meiosis, hence sex becomes any process encompassing meiosis and/or syngamy. This definition captures the kernel of what most biologists would deem sex to be. Definitions are arbitrary and only as good as the conceptual simplifications that derive from them. By equating sex with meiosis, we hope to demonstrate better understanding of why sex originated, persists, and is so ubiquitous.

Table 1. Glossary

Amphimixis	Outcrossing Reduction division + gametes + syngamy
Complete automixis	Both gametic nuclei are products of the same meiotic division Reduction division + syngamy (either with or without gametes)
Restitutional automixis	Meiosis, but no gametes and no syngamy Endomitosis in lieu of syngamy Reduction division, but no syngamy
Parasex	No discrete reduction division nor gametes Syngamy + reduction
Apomixis	No meiosis nor syngamy
Autogamy	Self fertilization
Endomitosis	Duplication of chromosomes without nuclear division (aka endoploidy or endoreduplication)
Fertilization	Fusion of gametic cell membranes (aka plasmogamy)
Syngamy	Fusion of gametic nuclei/pronuclei and subsequent mixing (i.e. decondensation and un-pairing) of homologous chromosomes

Definitions of specific forms of sex garner greater consensus than the definition of sex itself (Table 1). Amphimixis is outcrossing sex, where eggs and/or sperm are from different diploid individuals. Autogamy is self fertilization (although Cleveland, 1947 used the term autogamy to refer to what we now call complete automixis). Complete automixis is where two products of a single meiotic division fuse with one another to restore diploidy, which is an extreme form of self fertilization. Fusion could be between two nuclei/pronuclei, even if the one meiotic mother cell has not itself divided to form true gametes. We consider automictic parthenogenesis to be a form of sex (Ghiselin, 1988; Kondrashov, 1997; Haccou & Schneider, 2004). Restitutional automixis (also known as premeiotic endomitosis or restitutional meiosis) is

alternation of meiosis and endoploidy. Endoploidy, also called endomitosis and endoreduplication, is duplication of all chromosomes without a mitotic division of the nucleus. With restitutional automixis, syngamy never occurs. We consider all forms of meiosis, including restitutional automixis, to be forms of sex. We also count facultatively sexual lineages as being sexual. We use the terms asexual and apomictic to mean obligate asexuality without any meiosis (i.e. only mitotic divisions).

Syngamy (aka karyogamy) is the fusion of pronuclei and the subsequent mixing of chromosomes. By contrast, fertilization (plasmogamy) is the fusion of cells (i.e. gametes; Maupas, 1890; Kondrashov, 1997). Because we show below that syngamy is a modified form of meiosis, we will consider any organism that has syngamy to be sexual.

Prokaryotic and eukaryotic sex are fundamentally different. Prokaryotic sex involves horizontal gene transfer, usually of only a small fraction of the genome, with the gene transfer usually being unidirectional. Eukaryotic sex involves reduction division (halving) of entire nuclear genomes and bidirectional exchange of genomes, conceding that there is some limited unidirectional exchange with gene conversion and genomic imprinting and conceding that, in organisms with self sex, the bidirectional genetic exchange is trivial. Even the highly derived parasex in some fungi consists of halving the complement of chromosomes, retaining one copy of each homologue. It seems unwarranted to use the same word 'sex' to describe such disparate phenomena as prokaryotic and eukaryotic sex but, unfortunately, we are saddled with that nomenclatural legacy. However, we confine attention exclusively to eukaryotic sex.

THEORY

The shift in thinking that we are proposing is that the essence of eukaryotic sex is to reset epigenetic signatures (Gorelick & Osborne, 2002). Epigenetic signatures such as cytosine methylation allow eukaryotes to develop normally (i.e. stably and conservatively). Eukaryotes, including unicellular eukaryotes, require an epigenetic reset because they are developmentally more complex than prokaryotes. Stable development of complex organisms means that epigenetic signals could be considered as adaptive, although intra-generational changes in these same epigenetic signals also cause senescence. Individual organisms senesce as a result of unidirectional ontogenetic changes caused by epigenetic signals, including telomere degradation (Bacchetti, 1996; Howard, 1996; Russo, Martienssen & Riggs, 1996; Blackburn, 2000; Lushai & Loxdale, 2007). Without sex, developmental errors will accumulate as an asexual lineage evolves, eventually leading to senescence of each individual. Senescence can be 'reversed' by resetting epigenetic signatures to their original gametic, gametophytic, zygotic, or embryonic configuration. We use the phrase 'senescence can be reversed' liberally insofar as the rejuvenescence only occurs in a new organism in the next haploid or diploid generation. Changes in epigenetic signals cause both developmental changes (adaptive) and senescence (maladaptive), thereby necessitating sex in order to reset epigenetic signals. Development, senescence, and sex are all pleiotropic effects of intra-generational unidirectional epigenetic changes. We are reviving the notion of sex causing rejuvenescence (Braun, 1853 [1851]; Maupas, 1886; Geddes & Thomson, 1889; Maupas, 1889; Maupas 1890; Bell, 1988). Epigenetic resetting must occur after a finite number of successive mitotic divisions, otherwise individuals run up against the Hayflick limit (Hayflick & Moorhead, 1961). Because offspring benefit by inheriting an epigenetic reset that allowed their ancestors to survive development and reproduction in previous generations, this is a form of individual-based selection, which can occur during both haploid and diploid stages.

Although development is an essential part of this theory, we really only examine it from an evolutionary perspective. What matters here is inter-generational inheritance, in this case of the mechanisms that control development. Therefore, when discussing epigenetic signals, such as cytosine methylation, we mostly examine those signals that get passed to subsequent generations (Gorelick & Laubichler, 2008). Zoologists will thus typically confine attention to the germ-line, although epigenetic changes can sometimes be transmitted from somatic to germ cells (Jablonka & Lamb, 2005). The germ–soma dichotomy is fuzzy in most organisms, even including metazoans; hence we will generically examine epigenetic signals throughout the body of multicellular organisms.

MEIOSIS

How did the first meiotic eukaryote evolve? There is little doubt that meiosis arose from mitosis. It is generally held that all extant eukaryotes evolved monophyletically, from a single sexual ancestor (Cavalier-Smith, 1995; Redfield, 1999; cf. Margulis & Sagan, 1988), although asexual eukaryotes may have secondarily evolved from this sexual lineage. 'There are many good reasons for considering asexual reproduction to be not primitive but secondary in Metazoa' and other eukaryotes (Montgomery, 1906: 82). The few theories previously put forward to explain the origin of sex have focused on correction of DNA point mutations (Bernstein, Byers & Michod, 1981) and

reduction division (halving of chromosomes number, by parsing one of each homologous chromosome into each daughter cell or nucleus; Cleveland, 1947; Cavalier-Smith, 1995). Despite a renaissance in evo-devo approaches to explaining evolutionary novelty, it seems that nobody has proposed an epigenetic theory for the origin of sex.

All cells in a multicellular eukaryotic organism contain virtually the same DNA sequences, although cells within an individual can vary greatly in ploidy levels from their base haploid or diploid conditions. For example, endoploidy is rampant, especially in cells that have high energetic demands, such as the heart muscle, flight muscle, and liver cells of many metazoans, salivary cells of dipterans, as well as in various tissues in many plants and even in protists (De Rocher *et al.*, 1990; Cavalier-Smith, 1995; Palomino *et al.*, 1999; Anatskaya & Vinogradov, 2004). Differentiation in space and time primarily occurs by epigenetic mechanisms, especially changes in cytosine methylation and chromatin marks (Holliday, 2006; Allis, Jenuwein & Reinberg, 2007). These epigenetic signals change irreversibly as cells and tissues differentiate and age (Finnegan, 1996; Li & Bird, 2007). Regulatory loci gain cytosine methylation (Ruiz-García, Cervera & Martínez-Zapater, 2005). Telomeres gradually lose cytosine methylation as an individual develops and ages (Howard, 1996). Thus, epigenetic mechanisms controlling development must be reset each generation. 'This suggests a supplementary definition of epigenetics to include transmission from one generation to the next, other than the DNA sequence itself' (Holliday, 1994: 454) and suggests that reset of epigenetic signatures may be the essence of meiosis and syngamy. Although epigenetic resets accompanying meiosis and syngamy are not well-known, there appears to be no controversy that they occur (Surani, 1998; Simon *et al.*, 1999; El-Maarri *et al.*, 2001; Santos & Dean, 2004). What is novel here is that an epigenetic reset (i.e. not recombination or independent segregation) is the crux of meiosis (i.e. epigenetic resetting is necessary with meiosis).

Epigenetic resetting is a conservative mechanism. What worked for resetting development in previous generations should be good enough to work in present and future generations. Epigenetic resetting is adaptive. 'Inbreeding sex, could have originated and still be maintained in many organisms because it conserves adaptation by reconstituting parental [epi]genomes in the face of damage or [epi]mutation or both' (Shields, 1988: 268). Although we do not fully understand the molecular mechanisms by which epigenetic resetting of cytosine methlyation and chromatin marks occur during gamete formation and after syngamy, we know that these epigenetic signals are highly heritable, albeit not quite as heritable as DNA nucleotides (Gorelick, 2005; Gorelick & Laubichler, 2008). In organisms with meiosis and/or syngamy, epigenetic resetting once or twice each generation greatly reduces additive genetic variance in epigenetic signals.

The first sexual eukaryote must have been automictic. We make this bold statement because the origin of sex is a major transition (Maynard Smith & Szathmáry, 1995) and so the chance of it evolving twice in one location and time is virtually inconceivable (Cavalier-Smith, 1995; Redfield, 1999; for a contrasting view, see also Margulis & Sagan, 1986b; 1988). By automictic, we mean that the first sexual organism must have had sex with itself, in which case no genetic mixing could have been involved (Cleveland, 1947). Furthermore, autogamy, complete automixis, and restitutional automixis result in extreme homozygosity of both genetic and epigenetic loci. With extreme homozygosity, neither crossing-over recombination, gene conversion, nor independent segregation has any effect upon the frequencies of genotypes or epigenotypes. Therefore, an increase in genetic variance or epigenetic variance could not have been the original function of sex.

A quintessential aspect of sex is reduction division to restore chromosome numbers after fertilization or endomitosis (Cleveland, 1947; Cavalier-Smith, 1995). Our hypothesis is that sex equals maintenance of ploidy via a cell division plus epigenetic resetting (Jablonka & Lamb, 1995; Simon *et al.*, 1999; Whitelaw & Martin, 2001; Santos & Dean, 2004). Recombination is an epiphenomenon of mitosis (Burt, 2000), whose biochemical machinery was present in the common ancestor of eukaryotes and archaea (Cavalier-Smith, 2002). Recombination is required in most taxa to effectuate pairing of homologous chromosomes and prevent aneuploidy during meiosis (and mitosis?) (Mell *et al.*, 2008; Wilkins & Holliday, 2009; also hinted at by Holliday *et al.*, 1976). Recombination is not necessarily functioning as a means of genetic mixing (Page & Hawley, 2003), but rather recombination is required for proper reduction division. A beneficial side-effect of the machinery of recombination, however, is that it can stop Muller's ratchet, even in automictic or obligately selfing organisms (Shields, 1982; Haccou & Schneider, 2004). Our hypothesis is that an epigenetic reset is needed in all sexual lineages, including automictic ones, whenever there is a reduction division or syngamy.

SYNGAMY

We hypothesize that all sexual eukaryotes undergo two rounds of meiosis per generation, except for those with restitutional meiosis and possibly parasexual fungi (the latter of which are highly derived; Forche

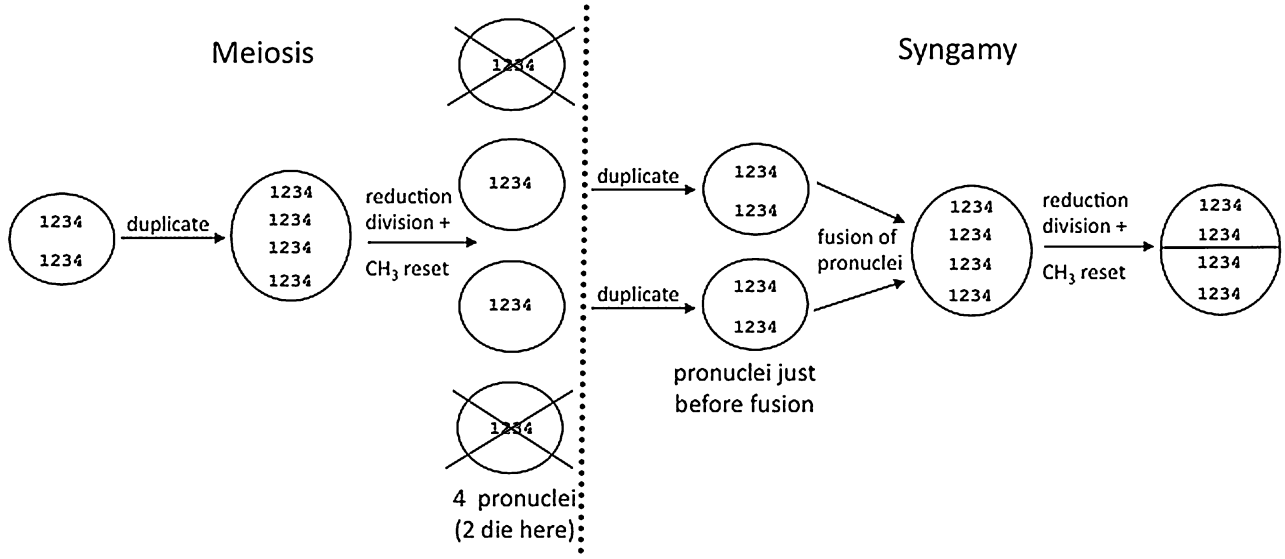

Figure 1. Sex = (Ploidy Maintenance) + (Epigenetic Reset). This cytological view of automictic and amphimictic sex depicts one generation of the lifecycle of an organism with complete automixis and six pairs of diploid chromosomes. There can be both independent segregation and crossing-over recombination, although neither is depicted here. Both meiosis and syngamy begin with a chromosomal duplication and end with a change in ploidy level through a cell division plus cytosine methylation reset. By the addition of a second parent and/or second meiosis, this figure can easily be generalized to amphimixis and autogamy.

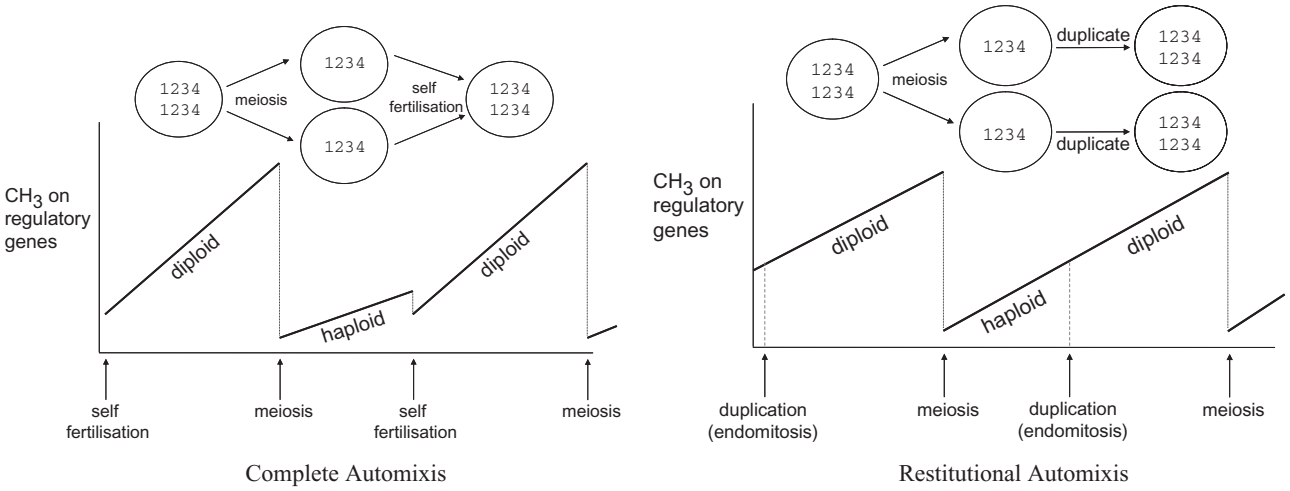

Figure 2. Cytological and molecular views of complete and restitutional automixis over 1.5 generations. Empirical evidence exists for the epigenetic resets (vertical drops in the graph) and for the monotonic increase in cytosine methylation levels during the diploid stage. Cytosine methylation levels are not reset to zero because many methylated cytosines are needed to silence foreign genes, such as transposons. Increases in methylation may not be linear; decreases during the epigenetic reset may not be instantaneous. For organisms with distinct germ lines (e.g. many metazoans), this figure applies only to germ line cells. The panel on the left also depicts the pattern of cytosine methylation levels that occurs with amphimixis (outcrossing) and non-automictic self fertilization (autogamy). Time histories of cytosine methylation levels (with positive slopes) are for regulatory loci. Time histories of cytosine methylation at other loci, such as telomeres, will also be sawtooths, but with negative slopes.

et al., 2008). One meiotic division is associated with gamete formation and is a reduction division, and the second one is associated with syngamy and ultimately results in chromosome doubling (Figs 1, 2). Two epigenetic resets will restore proper development to both diploid and haploid individuals (i.e. considering gametes and/or gametophytes to be individuals); hence, the possible need for a second reduction divi-

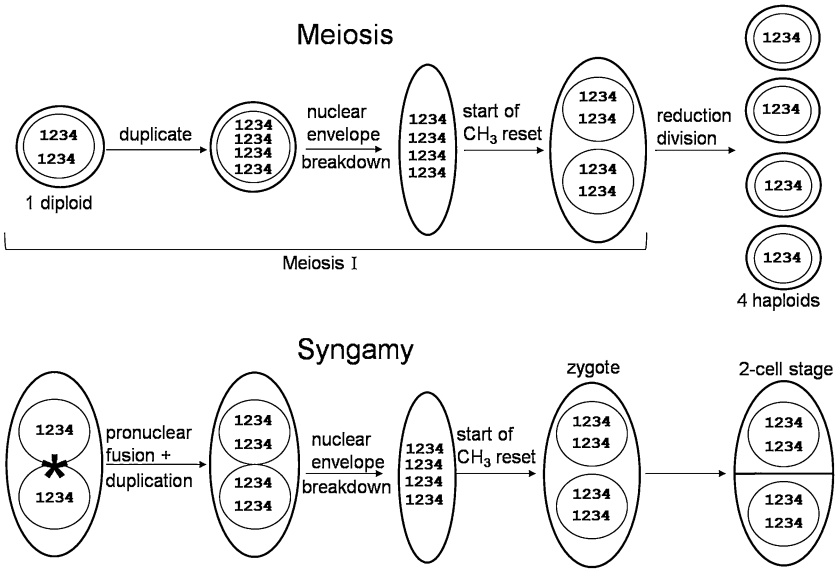

Figure 3. Syngamy parallels meiosis because it is a cell division that restores ploidy level. In meiosis, a diploid cell with one set of chromosomes undergoes a chromosomal duplication followed by segregation and two reduction divisions, ultimately resulting in four haploid cells. A depiction of crossing-over recombination is omitted. In syngamy, maternal and paternal haploid pronuclei undergo a chromosomal duplication, which we hypothesize is triggered by initial contact and fusion of pronuclei (represented by an asterisk), followed by segregation and cell division. In *Ascaris*-type syngamy, depicted herein, which occurs in most animal taxa that have been studied, chromosomes pair up on the cleavage spindle during metaphase of meiosis. Syngamy is identical to meiosis I in terms of absolute number of chromosomes ($2C \rightarrow 4C \rightarrow 2 \times 2C$). Syngamy differs from meiosis I in that homologous chromosomes are compartmentalized into two pronuclear membranes and, rather than reducing ploidy by half, syngamy doubles ploidy level. Bold ovals depict cell membranes; light ovals depict nuclear/pronuclear membranes.

sion per generation with large or long-lived haploid stages and large or long-lived diploid stages.

Syngamy is a modified form of meiosis. We hypothesize that the contact of two pronuclei induces duplication of all chromosomes in both gametic pronuclei. A similar induction exists in almost all metazoans with respect to meiosis in eggs; almost all metazoans require fertilization to either initiate meiosis in eggs (i.e. to initiate chromosomal duplication) or to induce the continuation of egg meiosis after meiotic arrest at a particular stage. Subsequent to this endomitosis, the pair of 2C gametic pronuclei fuse to form a tetrad that is equivalent to what is observed in mitosis and meiosis. Similar to meiosis, the final steps in syngamy are cell division restoring ploidy levels and an epigenetic reset. However, with syngamy, there is only a single division, with the entirety of syngamy converting a cell with two haploid nuclei into a pair of cells each with a single diploid nucleus. A single generation of any autogamous, completely automictic, or amphimictic organism therefore goes through a reduction division to form haploids (aka meiosis), which is followed by fusion of pronuclei (aka syngamy). There may be a long-time span between meiosis and syngamy with lots of intervening mitotic divisions, such as in cycads and pteridophytes, or a long-time span between syngamy and meiosis with lots of intervening mitotic divisions, such as in yeast.

Although not well known, there exists unequivocal evidence (Veeck, 1999) that a second meiotic division occurs each generation and that male and female pronuclei undergo chromosomal doubling (endomitosis) prior to syngamy. In most animals (the only possible known exception being sea urchins), intermixing of maternal and paternal chromosomes does not occur via pronuclear fusion and a restoration of diploidy at the one-cell stage. Instead, intermixing of maternal and paternal chromosomes occurs at the boundary of the one- and two-cell stage, during a cellular division in which a cell containing two separate haploid pronuclei gives rise to two cells, each containing a diploid nucleus. To ensure correct ploidy levels are achieved during this division, a doubling of chromosomes must occur prior to the first cleavage of the cell. Thus, syngamy is characterized by maternal and paternal pronuclear duplications followed by a cell division (Fig. 3), which results in two diploid cells.

Syngamy in which diploidy is restored not at the one-cell stage, but at the two cell stage, is known as *Ascaris*-type syngamy. It is considered to be very

widespread (Wilson, 1925), including most animals (Kawamura, 2001). In humans, where fertilization has been extremely well studied, the single-celled zygote stage is characterized by maternal and paternal chromosomes positioned on the cleavage spindle, lacking pronuclear membranes. Therefore, syngamy in humans is not characterized by pronuclear membrane fusion but rather by the pairing of two sets of maternal and two sets of paternal chromosomes (2C + 2C = 4C) in the zygote after pronuclear membrane breakdown (Austin, 1965; Schatten, 1994; Veeck, 1999). Thus, humans undergo a round of chromosomal duplication followed by pronuclear membrane breakdown and the first cell cleavage in order for diploidy to be restored in the two-cell stage of the developing embryo. This type of syngamy has been documented in mammals (Longo, 1973; Gwatkin, 1977; Schatten, 1994; Veeck, 1999), gastropods (Zissler, 1992), nematodes, annelids, mollusks, and crustaceans (Wilson, 1925).

Gonomery, which probably occurs in all insects, is very similar to *Ascaris*-type syngamy (Kawamura, 2001). Gonomery has been observed in *Drosophila* (Metz, 1916; Huettner, 1924; Sonnenblick, 1950; Callaini & Riparbelli, 1996), *Bombyx* (Kawamura, 1978), and *Gryllus* (Sato & Tanaka-Sato, 2002). With both gonomery and *Ascaris*-type fertilization, the diploid zygote first comes to be surrounded by a nuclear membrane at the two-cell stage. In *Ascaris*-type fertilization, the male and female pronuclei come into close proximity with each other (probably into contact) prior to the disappearance of the pronuclear membranes, at which point the chromosomes duplicate. After the pronuclear membranes disappear, the paternal and maternal chromosomes intermix on the spindle during metaphase of the cell division (Kawamura, 2001). The zygotic cell cleaves for the first time and diploidy is restored. By contrast, in gonomery, the pronuclear membranes come into contact with each other prior to breaking down, at which point a chromosomal duplication occurs. Maternal and paternal chromosomes then line up independently (i.e. on separate spindles) throughout the cleavage until spindles disappear in telophase (Kawamura, 2001).

Chromosomal duplication in the two gametic pronuclei is probably induced by contact of pronuclear cell membranes prior to pronuclear fusion. In several organisms, such as humans, fusion of egg and sperm cell membranes induces completion of meiosis in the egg: meiosis II occurs in the human egg after the sperm cell has fused with it (Austin, 1965). Indeed, in almost all animals, fertilization (plasmogamy) occurs before female meiosis is complete, with the only known exceptions being echinoderms and cnidarians (Austin, 1965). In many ciliates, endomitosis and then meiosis of micronuclei is induced by conjugation, a conjugation event that is homologous to syngamy (Raikov, 1982 [1978]). In *Paramecium*, there are actually a pair of successive endomitotic divisions of the micronuclei prior to meiosis (Lustig, 2000). Thus, it is not too great a stretch to consider that pronuclear fusion may induce an endomitotic division in all pronuclei. We are essentially proposing that all eukaryotes with syngamy (i.e. all forms of meiosis except restitutional automixis) undergo Haig's 'microsporidial shuffle' (Haig, 1993). This pronuclear chromosomal duplication may have evolved as a simple heterochronic shift, where a nuclear division was suppressed after chromosomal duplication. Consequently, we consider both meiosis and syngamy to begin with a mitotic chromosomal duplication and end with a cell division (Fig. 1) that has an associated epigenetic reset (Fig. 2).

Epigenetic resetting and reduction division need not occur every generation, realizing that the term 'generation' is ambiguous. Facultatively sexual organisms, which we refer to here as being sexual, such as aphids, should only undergo methylation reset and reduction division during the sexual phases of their lifecycle (Lushai & Loxdale, 2007). We are only hypothesizing that an epigenetic reset and reduction division occur prior to a lineage reaching the Hayflick limit (Hayflick & Moorhead, 1961), and not necessarily during intervening apomictic stages. Indeed, unicellular eukaryotes, protists, can go many generations without meiosis. Oligocellular eukaryotes must engage in meiosis a bit more often than unicellular ones. And multicellular eukaryotes need meiosis most often, being able to weather fewer rounds of truly clonal reproduction before needing to engage in meiosis, which could be self sex.

Having a pair of divisions that maintain ploidy plus the associated epigenetic resets per generation has possibly been overlooked because of zoological biases in which only the diploid stage is thought to matter. For example, Nei's model of Muller's ratchet with recombination implicitly assumes that selection is nil or negligible in the haploid stage (Nei, 1970; Gorelick, 2003a). Yet, development must occur in the haploid stage in any organism having alternation of generations. Haploid stages are massively multicellular in all plants, except angiosperms. Furthermore, even flowering plant female and male haploid stages undergo several mitotic divisions with cells differentiating. Metazoan haploid stages develop and many have long-lived gametes, such as stored sperm in insects or long-lived human ova. Sperm cells in most eukaryotes develop from spherically symmetrical products of meiosis to specialized cells with long tails and a nucleus at the opposite end (Conklin, 1917). Whatever argument is posited for the origin of sex, it should be applicable to both haploid and diploid stages, as is the case with a pair of epigenetic resets per generation.

Meiosis can occur without syngamy (i.e. restitutional automixis) (Fig. 2). A lack of syngamy translates into lack of the second ploidy-restoring division per generation. This may result in the lack of an epigenetic reset during the diploid stage, which may not be problematic for the individual if there is limited development in either the haploid or diploid stage. Such a situation may occur in the putatively basal eukaryote *Giardia* (diplomonad), which presumably undergoes premeiotic restitution and meiosis while encysting (Bernander, Palm & Svärd, 2001; Ramesh, Malik & Logsdon, 2005; Cooper *et al.*, 2007).

Chicken and egg, part 1: which came first, meiosis or syngamy?

Which evolved first, meiosis or syngamy? Maynard Smith (1978), Margulis & Sagan (1986b, 1988), and Hickey & Rose (1988) have argued that syngamy evolutionarily preceded meiosis and that incomplete digestion was the progenitor of syngamy. Supposedly, starvation and desiccation induced one protist to engulf another in order to imbibe necessary food. It could digest most of the imbibed cell, but not the chromosomes and possibly not the nucleus. To deal with the extra chromosomes, the satiated protist merged its nuclear genome with that of its meal. Extra chromosomes are a metabolic burden to a cell, as can be seen with the much slower mitotic cycle of polyploid cells. Therefore, supposedly, a method of reduction division (i.e. meiosis) later evolved to halve the number of chromosomes. This is why the original sexual cell had to eat a close relative. Otherwise, according to the paradigm, meiosis could not evolve because of differing numbers of chromosomes between the two cells. Even with identical chromosome numbers, there still had to be quite a bit of homology between the chromosomes. What started as a meal, wound up being the origin of sex. In other words, the paradigmatic view suggests that sex originated as partially cannibalistic oral sex.

There are three substantial problems with cannibalistic oral sex as the origin of sex. First, chromosomes with all their nitrogen and phosphorus are probably the most nutritious part of the cell (Sterner & Elser, 2002). Cannibalistic oral sex only appears to be adaptive if the organism eats the good stuff, especially because nitrogen and phosphorus are limiting nutrients in most ecosystems. Second, the imbibed cell is most likely to be a close relative, which may not be adaptive if accounting for kin selection (Hamilton, 1963, 1964a, 1964b). Third, cannibalistic oral sex does not explain how restitutional automixis might have evolved.

Although we concur with the proponents of partially cannibalistic oral sex that sex arose to halve chromosome numbers, we differ from them in proffering that the initial chromosomal doubling was a result of endomitosis, and not syngamy or protosyngametic imbibition. Although cannibalistic oral sex would undoubtedly sell at the cinema, in real life, and for almost all organisms, self sex and endoploidy are much more common and plausible. Chromosomal duplication (endomitosis) probably first arose via a heterochronic 'error', such as pre-meiotic restitution, especially with the ubiquity of endoploidy throughout multicellular eukaryotes (Maynard Smith & Szathmáry, 1995). Margulis & Sagan (2002) consider that changes in developmental timing during meiosis are crucial for understanding macroevolution, although they were referring to kinetochore reproduction theory (karyotypic fission; chromosomal fission), not endomitosis. However, earlier, they suggested that such heterochronic shifts were responsible for the evolution of meiosis from mitosis (Margulis & Sagan, 1986a), citing Cleveland's (1947) classic work. Endomitosis often necessitates a subsequent reduction division, which could be provided by meiosis. Not only does fusion of two genetically distinct cells and their nuclei (approximately syngamy) require a cell division to restore previous ploidy levels, but so does endomitosis. Endomitosis probably provided the evolutionary stimulus underlying reduction division.

Supporting the argument that the first sex was restitutional automixis, we know that, in all eukaryotes environmental stress, such as starvation or desiccation, inhibits maintenance methylation (Gorelick, 2005; Richards, 2006). During subsequent mitotic divisions, such cell lines incur methylation-losing epimutations at those loci. Because meiosis and syngamy are the only ways to induce a wholesale reset of this lost cytosine methylation, starvation and desiccation provide a selective advantage to those taxa that can undergo meiosis and/or syngamy shortly after being ravaged by environmental stresses. Sex had nothing to do with genetic mixing if meiosis was the evolutionary predecessor of syngamy and/or if the primary functions of sex were and still are to correct environmentally-induced epimutations.

The extraordinary similarity and symmetry between meiosis and syngamy (i.e. the two sides of the coin known as sex in completely automictic and amphimictic organisms) virtually guarantees the rapid evolution of either meiosis or syngamy from the other. However, the existence of taxa with obligate restitutional automixis tip the balance in favour of meiosis evolving before syngamy, with syngamy merely being a form of meiosis that occurs sometime after an endomitotic event, especially if basal eukaryotes such as *Giardia intestinalis* or even heliozoans prove to be restitutionally automictic. Restitutional automixis occurs in a wide variety of taxa in all

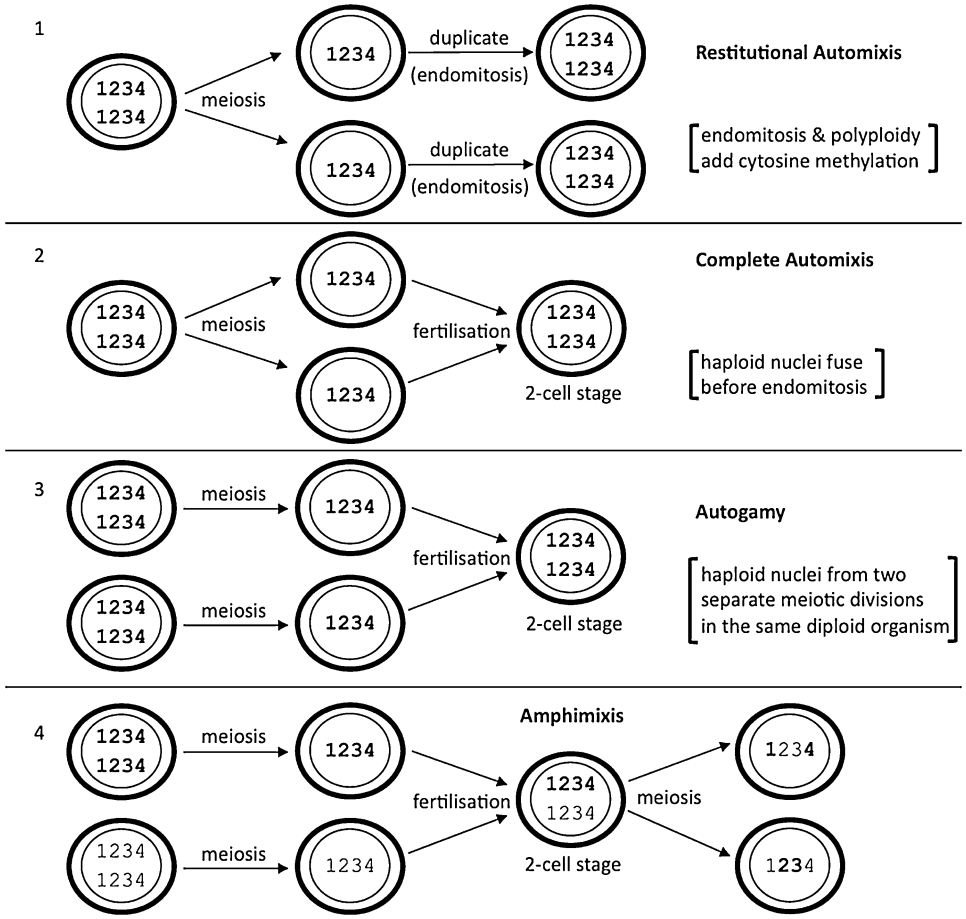

Figure 4. Evolutionary sequence from restitutional automixis to amphimixis. This vertical sequence depicts our hypothesis concerning how eukaryotes evolved sex, from restitutional automixis, to complete automixis, to autogamy, to amphimixis, in which (for conceptual simplicity only) we have not shown the endomitosis at the start of syngamy. Bold ovals depict cell membranes; light ovals depict nuclear/pronuclear membranes. Note also that, in many completely automictic taxa, both pronuclei are contained in a single cell, not two cells, as depicted. In the fourth frame, amphimixis, maternal and paternal chromosome are depicted by bold and normal fonts to demonstrate independent segregation. Crossing-over recombination could also be depicted as chimaeric chromosomes (partly bold and partly normal font).

four eukaryotic kingdoms. By contrast, with parasex (syngamy without meiosis), one chromosome is jettisoned at a time until one copy of each homologous chromosome remains. Tellingly, parasex only occurs in a few fungi (Pontecorvo, 1956; Cristina, Becker & De Castro-Prado, 2006) and possibly a few protists such as the slime mould *Acrasia* (Margulis & Sagan, 1986b). In addition, we now believe that parasex is simply a highly modified form of meiosis (Forche *et al.*, 2008). Thus, it appears that meiosis is generally much more crucial than syngamy.

Figure 4 depicts our hypothesized sequence from restitutional automixis, to complete automixis, to autogamy, to automixis. Complete automixis evolved from restitutional automixis via the fusion of two meiotic products prior to endomitosis. Autogamy evolved from complete automixis via the fusion of two pronuclei that were derived from two different meiotic divisions in the same diploid organism. Amphimixis evolved from autogamy via the fusion of two pronuclei that were derived from meiotic divisions of two different diploid organisms. Independent segregation, crossing-over recombination, and gene conversion only become important in amphimictic taxa.

Chicken and egg, part 2: which evolved first, euploidy or epigenetic resetting?

There exists a second vexing chicken-and-egg question: which evolved first, conservation of ploidy (euploidy) or conservation of cytosine methylation signatures (epigenetic reset)? Epigenetic regulation appears to be necessary for proper meiosis, especially segregation including in taxa without crossing-over

recombination (Karpen & Hawley, 2007). Cytosine methylation is a much more ancient phenomenon than meiosis or mitosis. Cytosine methylation in the form of 5-methylcytosine is not only ubiquitous in eukaryotes, but also in all bacteria and archaea, many of which also have N^4-methylcytosine (Noyer-Weidner & Trautner, 1993; Sartori, Fitz-Gibbon, Yang, Miller & Jiricny, 2002). In both eukaryotes and prokaryotes, cytosine methylation is involved with silencing regulatory genes (Boyes & Bird, 1992; Noyer-Weidner & Trautner, 1993). Deamination of 5-methylcytosine into thymine is a major source of point mutations in virtually all living organisms (Yang, Jones & Shibata, 1996). Although, naively, this may lead to the idea that epigenetic resetting evolutionarily pre-dated euploidy, there are problems with this argument. First, why is it that bacteria and archaea have not evolved wholesale epigenetic resets? Although morphologically not necessarily as complex as eukaryotes, bacteria can form large structured colonies (e.g. stromatolites). Second, even in eukaryotes, epigenetic resetting is only ever associated with meiosis or syngamy. Third, most instances of deamination of 5-methylcytosine are corrected by mismatch repair (Brown & Jiricny, 1987). We therefore argue that euploidy preceded wholesale reset of cytosine methylation, but only for a very short while.

Organisms could and still can weather small-scale additions of cytosine methylation. Over evolutionary time, 5-methylcytosine relatively quickly deaminates to the enol form of thymine (Shen, Rideout & Jones, 1994; Norberg & Vihinen, 2001), which is often subsequently converted to unmethylated cytosine via mismatch repair (Brown & Jiricny, 1987; Gorelick, 2003a). The problem for most organisms is when a sizeable fraction of the genome instantly becomes methylated (Gruenbaum, Naveh-Many, Cedar & Razin, 1981; Adams, Cronn, Percifield & Wendel, 2003), as occurs with polyploidy and endomitosis.

The key to understanding the relationship between halving chromosomes and epigenetic resetting is to examine what happens when chromosomes are duplicated. Newly-inserted genes, including duplicated genes are highly methylated (Yoder, Walsh & Bestor, 1997; Jones & Takai, 2001). The largest such gene duplication occurs with polyploidy, in which an enormous proportion of the genome quickly becomes highly methylated (Matzke & Matzke, 1998). Hypermethylation not only occurs when polyploids are created by fusion of unreduced gametes/pronuclei (Comai, 2000; Adams *et al.*, 2003), but, more importantly for this argument, hypermethylation occurs after artificially induced endomitosis when the cell cycle is interrupted with the application of colchicine (Martelotto, Ortiz, Stein, Espinoza, Quarin & Pessino, 2007). (Admittedly, the cell cycle is also interrupted using colchicine for production of unreduced gametes). Although meiosis can reverse the escalating effects of polyploidy or endomitosis, something clearly needs to be done to remove the nascent cytosine methylation when restoring the haploid or diploid condition via reduction division or syngamy. Therefore, we hypothesize that wholesale epigenetic resetting is a consequence of polyploidization and its associated hypermethylation. We hypothesize that, in evolutionary history, reduction division came first, but, out of necessity, was almost immediately followed by an epigenetic reset.

Sex is an evolutionarily stable strategy

Although couched in terms of origin of sex, epigenetic resetting also explains the maintenance of sex. There is enormous selective advantage to inheriting a developmental reset that was successful in previous generations. Obligately asexual lineages senesce as they irreversibly lose cytosine methylation from telomeres and gain cytosine methylation from regulatory loci. An asexual invader has relative fitness that converges to zero relative to sexual individuals. Therefore, the only way that an asexual individual can invade a sexual population is to occasionally (facultatively) engage in some form of sex in order to rejuvenate its epigenetic signals.

'Why are many somatic cells destined to self-destruct after a finite number of divisions, [in contrast with germ-line cells that] minimize the number of mutation-producing cell divisions? To prevent cheating strategies from evolving over a period of many cell divisions' (Wilson, 2007: 136). Wilson thereby provides a brief evolutionarily stable strategy argument linking meiosis and the Hayflick limit.

PREDICTIONS

Table 2 provides 11 predictions that arise from the above theory, falling into the four categories of chromosomal duplication, epigenetic resetting, evolutionary origins, and senescence. The two most crucial and testable predictions are that syngamy will begin with a chromosomal duplication as depicted in Figures 1, 3 and the time history of cytosine methylation at regulatory loci will follow the saw-tooth pattern of Figure 2.

Chromosomal duplication

We predict that chromosomal duplication will occur before pronuclear membrane breakdown in all sexual eukaryotes (Figs 1,3). This will be induced by contact of pronuclei (i.e. partial fusion of pronuclear membranes). This chromosomal duplication sets the stage

Table 2. Predictions

Chromosomal duplications
1. Chromosomal duplication occurs at the start of both meiosis and syngamy (Fig. 1)
Epigenetic resets
2. CH_3 reset occurs during or immediately after meiosis and syngamy and at no other times (Figs 1, 2)
3. With restitutional automixis and apomictic gynogenesis, there is only one CH_3 reset per generation
4. With amphimixis, complete automixis, automictic gynogenesis, and hybridogenesis, there are two CH_3 resets per generation
5. Obligately asexual (apomictic) lineages have much greater within-lineage epigenetic variation than sexual (amphimictic, completely automictic, restitutionally automictic) lineages
6. The only organisms completely lacking meiosis are unicellular eukaryotes completely lacking cytosine methylation
7. Epigenetic resetting erases the effects of meiotic crossing-over recombination, mitotic recombination, and gene conversion on the nuclear epigenome
Evolutionary origins
8. Restitutional meiosis is ancestral in eukaryotes; complete automixis, autogamy, and amphimixis are derived
9. Ancient asexual eukaryotic lineages must be unicellular and highly derived; all putatively asexual multicellular eukaryotes must undergo some form of meiosis
Senescence
10. The more cell types in a eukaryote, the fewer consecutive generations of asexual (apomictic) reproduction can occur before meiosis is required
11. physiological stress induces meiosis in facultatively sexual lineages by inducing epimutations

for the first cell cleavage, which restores previous ploidy levels, and the associated epigenetic reset at the end of syngamy.

Epigenetic resetting

We predict that levels of cytosine methylation at regulatory loci will gradually increase over ontogeny (Ruiz-García *et al.*, 2005), except for precipitous decreases shortly after meiosis and syngamy (saw-toothed time history; Fig. 2). Genomic levels of cytosine methylation at regulatory loci can be estimated using methylation-sensitive amplification polymorphism (Frommer, McDonald, Millar, Collis, Watt, Grigg, Molloy & Paul, 1992; Cervera, Ruiz-García & Martínez-Zapater, 2002).

We predict that the saw-tooth pattern in the time history of total cytosine methylation of regulatory loci throughout the genome will occur with any form of meiosis and syngamy, including amphimixis, autogamy, complete automixis, and restitutional automixis. Resets of cytosine methylation levels and other epigenetic signals will occur during or immediately after meiosis and syngamy, and at no other times. However, the pattern will look slightly different for cytosine methylation at other loci or for other epigenetic signals. For example, the time history of cytosine methylation at telomeres also has a saw-tooth pattern, although the methylation levels decrease during ontogeny and undergo a step increase during the resets associated with meiosis and syngamy, which is the mirror image of the time history of cytosine methylation at regulatory loci (Lushai & Loxdale, 2007). Epigenetic resets will not occur on organelle (prokaryotic) genomes. With mitotic apomixis, the intra-individual changes in genomic cytosine methylation at regulatory loci will have a much smaller range and have an aperiodic (stochastic) time history.

We predict that obligately apomictic lineages will have much higher heritable epigenetic variation, including of cytosine methylation, than will sister taxa with facultative or obligate meiosis each generation. Epigenetic resetting is a conservative force, which diminishes additive genetic variance and is presumably only associated with meiosis and syngamy.

Alternation of generations is not always so simple as the period-two cycle of reduction division and chromosomal doubling, possible confounding cases being parasex, haplo-selfing, gynogenesis, and repeated endomitosis. With parasex, we predict that an epigenetic reset will occur after syngamy, but we make no prediction about a second epigenetic reset per generation following return to a haploid state. The jettisoning of chromosomes, one at a time, in parasex may or may not have been too highly derived to have preserved the associated epigenetic reset. Haplo-selfing is syngamy in which a haploid cell and its nucleus fuse with one of its mitotic daughter cells, a phenomenon thus far only documented in fungi (Knop, 2006) and possibly heliozoans (Margulis & Sagan, 1988). At least one epigenetic reset should occur with haplo-selfing: the reset associated with

syngamy. There are two forms of gynogenesis: one with an apomictic female, the other with a completely automictic female. In both instances, a haploid sperm nucleus enters the egg cell (fertilization), but never fuses with the egg nucleus or nuclei (no syngamy). For apomictic gynogenesis, we predict only one epigenetic reset per generation: the reset associated with meiosis. For automictic gynogenesis, we predict two epigenetic resets per generation because both meiosis and syngamy occur. Similarly, with hybridogenesis, which involves meiosis and syngamy, we expect two epigenetic resets per generation. A few protists, including the red algae *Polysiphonia* (Goff & Coleman, 1986) and the flagellate *Pyrsonympha* (Hollande & Carruette-Valentin, 1970; Hurst & Nurse, 1991), increase their ploidy to many times the diploid level via repeated endomitosis. They subsequently undergo repeated successive reduction divisions to return to the haploid or diploid state. The brown algae *Ectocarpus siliculosus* undergoes syngamy of haploid cells followed by endomitosis to form a tetraploid, which subsequently undergoes two successive meiotic divisions to restore haploidy (Müller, 1967; Kondrashov, 1997). We predict that epigenetic resets will occur during the cavalcade of consecutive reduction divisions. We predict that epigenetic resets will occur during the chromosomal duplications if they are a result of the fusion of pronuclei (akin to complete automixis), but not as a result of endomitosis (akin to restitutional meiosis; Margulis & Sagan, 1988). Epigenetic resets occur if and only if there is an increase or decrease in ploidy as a result of meiosis or syngamy. Thus, we predict that *Ectocarpus siliculosus* will have three epigenetic resets each generation, one associated with syngamy and two associated with meiosis.

If meiotic crossing-over recombination was co-opted from mitosis and is not an integral part of meiosis (Engebrecht, Hirsch & Roeder, 1990; Page & Hawley, 2003; Mell *et al.*, 2008), then the epigenetic reset associated with syngamy will reset recombined epigenetic signatures. When cytosine methylation patterns undergo crossing-over recombination during meiosis, which they will do when their associated cytosines are recombined, these recombined epigenetic patterns will be erased during the epigenetic reset following syngamy. Epigenetic resetting also erases the effects of mitotic recombination and gene conversion. Unlike the nuclear genome composed of adenines, cytosines, guanines, and thymines, which is greatly affected by recombination, the effects of recombination on the nuclear epigenome are more ephemeral (intragenerational) as a result of epigenetic resets once or usually twice per generation.

When might epigenetic resetting and consequently facultative meiosis not be present? Cytosine methylation, in the form of 5-methylcytosine, is used to control development in virtually all eukaryotes. Only a few derived eukaryotes have abandoned 5-methylcytosine and all of these unusual eukaryotes have replaced it with other forms of epigenetic regulation (Gorelick, 2003a). For better or worse, several classical model organisms lack cytosine methylation or have very little cytosine methylation that does not appear to be functional, such as the nematode *Caenorhabditis elegans*, the fission yeast *Schizosaccharomyces pombe*, the budding yeast *Saccharomyces cerevisiae*, and most (all?) Diptera species, especially the Drosophilidae (Jollos, 1934; Proffitt, Davie, Swinton & Hattman, 1984; Wolffe & Matzke, 1999; Lyko, Ramsahoye & Jaenisch, 2000; Allis *et al.*, 2007). We predict that only lineages completely lacking 5-methylcytosine in their nuclear DNA will be able to survive without some form of meiosis. Budding yeast, fission yeast, and some nematodes have lost all cytosine methylation, but are sexual. Some dinoflagellates and kinetoplastids (asexual, unicellular) only have modified forms of cytosine methylation, namely 5-hydroxymethyluracil and β-D-glucosol-hydroxymethyluracil (Rae & Steele, 1978; Gommers-Ampt, Teixeira, van de Werken, van Dijk & Borst, 1993). These organisms undergo some development, and hence must somehow reset epigenetic signatures, but possibly without meiosis (however, see the discussion below concerning evidence that kinetoplastids may be automictic). Thus, we predict that only eukaryotes that completely lack cytosine methylation will completely lack meiosis.

Evolutionary origins

We predict that obligate asexuality cannot occur in lineages that have complex developmental pathways mediated by cytosine methylation. Thus we make the bold prediction that all multicellular eukaryotes will have some form of meiosis or syngamy. This is in stark contrast to conventional wisdom that holds that there are many diverse obligate asexual eukaryotes (Schön & Martens, 2002; Loewe & Lamatsch, 2008). The epigenetic reset associated with meiosis (including syngamy) is the proverbial elixir of youth (Sonneborn, 1954; Bell, 1988). Although definitive evidence for meiosis and epigenetic resetting does not yet exist in the basal eukaryote *Giardia* (diplomonad), meiotic machinery (Ramesh *et al.*, 2005) and recombination (Cooper *et al.*, 2007) have been uncovered in *Giardia*, which undergoes alternation of generations between free-swimming and encysted forms (Bernander *et al.*, 2001). Unisexual salamanders in the genus *Ambystoma* have existed without outcrossing sex for 2.5–5.0 million years, with some form of meiosis followed by syngamy between

gametes of one or more species (automictic gynogenesis or hybridogenesis; Spolsky, Phillips & Uzzell, 1992; Bogart, Bi, Fu, Noble & Niedzwiecki, 2007). Admittedly, there appears to be some 'leakage' of sperm nuclear DNA into these unisexual female salamanders as a result of hybridogenesis (Bogart *et al.*, 2007). Even the three most famous ancient asexual animals (bdelloid rotifers, parthenogenetic brine shrimp, and parthenogenetic oribatid mites) each show some signs of automixis or epigenetic resetting (Ricci, Santo, Radaelli & Bolzern, 1999; Gorelick, 2003b; Domes, Norton, Maraun & Scheu, 2007). The other famed ancient asexual lineage, darwinulid ostracods, now appears to be facultatively amphimictic (Smith, Kamiya & Horne, 2006). The phylum Gastrotricha may be typical for protists insofar as until recently all species were believed to be obligately asexual, whereas all species are now believed to be facultatively sexual (Weiss, 2001). Flowering plants with the *Taraxacum*-scheme of putative apomixes undergo restitutional meiosis and probably also crossing-over recombination (van Baarlen, van Dijk, Hoekstra & de Jong, 2000). We predict that putatively apomictic flowering plants, such as guinea grass (*Panicum maximum*) with *Panicum*-like 'megagametophytes' composed of four diploid cells, are actually products of complete automixis, and hence are sporophytes (Kaushal, Malaviya, Roy, Pathak, Agrawal, Khare & Siddiqui, 2008). We predict that their megaspore mother cell undergoes meiosis, two products of which immediately fuse to form a single-celled diploid analogue to the gametophyte, which then undergoes three mitotic duplications. One of the four resulting diploid cells becomes the zygotic embryo, whereas a second diploid cell is fertilized to form triploid endosperm. Sexual cycles are slowly being found in supposedly 'imperfect' fungi (O'Gorman, Fuller & Dyer, 2009). 'Furthermore, it is exceedingly difficult to find 'genuine' asexual eukaryotes [insofar as] vestiges of ancient sexual mechanisms have been found' in many putatively asexual protists (Solari, 2002: 1). Only with strained nomenclature should a modified meiosis constitute an instance of obligate asexuality. How many facets of meiosis have to exist before we deem an organism to be sexual? Might theory carry us further if we simply equate sex with meiosis, including complete automixis and restitutional automixis (Boyden, 1950; Cavalier-Smith, 1995) and probably syngamy?

Because mitosis evolutionarily preceded meiosis and we claim that reduction division preceded the epigenetic reset, the earliest eukaryotes must have been obligately apomictic (asexual). However, because of the conservative nature of meiosis in virtually all extant eukaryotes (Kondrashov, 1997), we can predict that the last common ancestor of extant eukaryotes was sexual, at the same time admitting that this is a fairly weak prediction. Unicellular eukaryotes may evolve back to a seemingly atavistic (derived) asexual state. It is also not obvious which unicellular eukaryotes will require an epigenetic reset, other than to predict that those with more development will be in more need of reset. Clearly single cells can undergo extensive development, as is evident from human sperm cells, but we have no way of quantifying degree of such developmental changes.

Senescence

Assuming that the Hayflick limit is real, the more cells and the more cell types in a eukaryote, the fewer consecutive generations of asexual (apomictic; mitotic) reproduction can occur before meiosis is required (i.e. the less often the eukaryote can use facultative apomixis). The maximum number of consecutive mitotic division without an intervening meiotic division may vary between lineages and environments, although this should not affect the qualitative nature of the following prediction. We predict that the number of successive generations of reproduction via apomixis will be inversely proportional to number of cells and cell types per individual. Here we invoke a naïve concept of individual, not conceptualizing asexual progeny as part of some superorganism that includes their parent and all other members of the lineage (e.g. Weismann, 1882; Lustig, 2000). Consequently, protists should be able to undergo many generations of asexual reproduction before eventually requiring rejuvenescence via amphimixis, complete automixis, or restitutional automixis, consistent with work on ciliates (Woodruff, 1917; Sonneborn, 1954; Bell, 1988). We predict that the Hayflick limit should be greater for plants than for animals simply because metazoans have far more cell types than plants. Indeed, many crop plants have survived vegetatively for centuries without any form of meiosis or syngamy (e.g. apples, bananas, pineapples, potatoes).

We predict that stress will induce sexual reproduction. Although not a novel prediction, this is consistent with other theories and with empirical results. Physiological and even mental stress disables maintenance methylation enzymes (Kovarik, Koukalová, Bezdek & Opatrny, 1997; Demeulemeester, Van Stallen & De Proft, 1999; Weaver, Szyf & Meaney, 2002). This can have large epigenetic effects both within a generation (ontogeny) and between generations (phylogeny) (Gorelick, 2004; Gorelick, 2005; Richards, 2006; Osborne & Gorelick, 2007). The more stress, the more environmentally-induced epimutations and hence the greater need for an epigenetic reset. Because meiosis and its putative derivative

syngamy are the only ways to induce large-scale reset of cytosine methylation, sex will be triggered by stress.

HISTORY

We briefly compare the theory herein with our predecessors' ideas about rejuvenescence, sex as a means of reducing heritable variation, whether self sex counts as sex, and definitions of sex.

The notion that sex induces rejuvenescence dates back to Bütschli (1876), and Maupas (1886; 1889; 1890), and possibly back to Braun (1853 [1851]), but was largely and erroneously discredited by Weismann (1889–1891; Lustig, 2000). Margulis & Sagan (1986b, 1988) followed Maupas with regards to rejuvenescence. They believed that meiosis provides a developmental (epigenetic) reset for multicellular organisms, something that they implicitly hint may not be needed in unicellular protists (this is odd insofar as Margulis made a brilliant career out of understanding protists). On the other hand, Calkins (1915), Sonneborn (1954), and Bell (1988) believed that rejuvenescence is primarily (exclusively?) relevant for unicellular automictic eukaryotes. We believe that rejuvenescence holds the key to sex in all eukaryotes. What we add to insights of these earlier authors is that: (1) sex reduces heritable variation in epigenetic signals and ploidy; (2) syngamy is a modified form of meiosis; and (3) starvation and desiccation inhibit maintenance methylation, thereby providing extra incentive for implementing a cytosine methylation reset via meiosis. Otherwise we are rejuvenating the erstwhile idea of a 19th Century archivist (Maupas) that sex induces rejuvenescence.

Although the current long-lived paradigm is that sex functions by increasing heritable variation in a population, undercurrents of the contrary notion that sex functions to decrease heritable variation existed in the 1800s. 'After reading Malthus, Darwin concluded that the "final cause of sex" is that it keeps the amount of variation within certain limits' (Ghiselin, 1988: 10). Darwin proffered a *reductio ad absurdum* argument: if sex did not reduce variation, then there would be as many species as there are individuals (Darwin, 1838–1839), an argument that may have been motivated by reigning notions of blending inheritance. Indeed, Darwin's argument has a modern Darwinian flavour insofar as diminution of heritable variation is exactly what modern evolutionary biologists expect in the face of selection. However, presently, only a very few biologists believe that sex reduces heritable variation, with that minority being largely comprised of those who deal with self fertilizing or otherwise strongly inbred taxa. Shields (1988: 268) went so far as to state that, 'inbreeding sex is the only reproductive strategy available to long-lived organisms with large genomes'.

In referring to any event where meiosis occurs as sex, we are following the lead of Balfour (1885), Montgomery (1906), Brachet (1911), Boyden (1950, 1954), Mahendra & Sharma (1955), Williams (1975), and Margulis & Sagan (1986b). Self fertilization via fusion of female and male gametes invariably counts as sex [e.g. the simultaneous hermaphrodite mangrove killifish, *Kryptolebias* (*Rivulus*) *marmoratus*] (Avise, 2008). However, parthenogenesis in which two female gametes fuse is often referred to as asexual. 'As Wilson (1925) quotes Brachet with approval, there is "a veritable bridge set up by Nature between fertilization and natural parthenogenesis." . . . To continue to describe parthenogenesis as asexual can only lead to confusion as we attempt to understand evolution of specialized sexual reproductive processes . . .' (Boyden, 1950: 820). 'Balfour (1885) was the first to show that parthenogenesis, development by an unfertilized egg cell, is sexual and not asexual reproduction, and all more recent analyses confirms his opinion' (Montgomery, 1906: 83). An even more extreme circumstance is restitutional automixis, for which Klekowski (1973) coined the pejorative 'subsexual'. Inconsistent use of the word sex seems wrong, especially if it comes from biases against self sex. Part of this bias undoubtedly comes from our mammalcentric view. Mammals are probably the only broad taxonomic group in which parthenogenesis has never been documented in nature. Part of the inconsistency in defining sex also may arise because obligate self fertilization does not result in genetic mixing.

The distinction between fertilization (fusion of cells; plasmogamy) and syngamy (fusion of pronuclei; karyogamy) was recognized by Maupas (1890) in his debates with Weismann. Maupas persuasively, but historically unsuccessfully, used this distinction to justify that rejuvenescence was the primary function of sex (Lustig, 2000).

Conservation of ploidy, euploidy, is a long-recognized function of sex. Mahendra & Sharma (1955) assert that the *sine qua non* of sex is restoration of chromosome numbers via reduction division. This is an idea that dates back at least to Wilson (1900) citing Weismann (1885, 1887) and Hertwig (1890) (see also Zacharias, 2001), and was firmly supported by Cleveland (1947). It is hard to understand why euploidy has taken short shrift in theories of the origin and maintenance of sex, despite being variation-reducing.

Cleveland (1947) believed that meiosis originated to reduce ploidy that was a product of endomitosis. His proposed chronological sequence of evolutionary events was: (1) evolution of diploidy; (2) restitutional automixis spurred by endomitosis; (3) complete auto-

mixis, which he referred to as autogamy; and finally (4) amphimixis. We completely concur with this sequence. Maynard Smith & Szathmáry (1995) tentatively suggest a similar sequence, although without the third step of complete automixis. Kondrashov's (1994) ploidy cycling also hints at restitutional automixis being the origin of sex, although he envisioned endomitosis alternating with one-step (not two-step) meiosis. Furthermore, Cleveland's paper is one of the few to make an explicit distinction between reduction division during gamete formation and reduction division following syngamy, so-called gametic meiosis versus zygotic meiosis, both of which we believe occur each generation with amphimixis, autogamy, and complete automixis.

Balbiani (1861), Bütschli (1876), Maupas (1889, 1890), Montgomery (1906), and Alexander & Borgia (1979) considered micronuclei of *Paramecium* to be functional equivalents of gametes. Maupas (1890) and Wilson (1900) even called the two conjugating cells gametes. With such a perspective, complete automixis in these ciliates is equivalent to pedestrian self fertilization in plants and metazoans. Ciliate automixis would therefore be considered sexual.

For many years, biologists have suspected that there is a link between environmental stress and rejuvenescence. Calkins (1915) showed that cyst formation serves two related purposes in protists: protection from stresses and correction of stress-induced chromosomal errors.

We follow in the footsteps of Jablonka & Lamb (1995: 215–216) in asserting that, 'Meiosis may have been essential for the efficient restoration of the epigenetic marks required to return cells to the ground state. We do not want to argue that the *origin* of meiosis was associated with its role as an epigenetic renovation system, but we do believe that this role has been important in the evolution of multicellular organisms and in the shaping of the sexual process seen today'.

In light of this historical accounting, no single aspect of the present study appears to be particularly radical, even though the overall perspective may seem strikingly different from the paradigmatic views of the origin, functions, and maintenance of eukaryotic sex.

DISCUSSION

In attempting to answer what the origin and functions of sex are, we had to revisit the question of exactly what sex is. One reason for this is the conventional and seemingly misleading notion that obligate self sex, such as automictic parthenogenesis (complete automixis) and restitutional automixis, do not constitute forms of sexuality. By contrast, we consider self sex a bona fide form of sexuality. Almost 75 years ago, Gustafsson (1935) realized that parthenogenesis was a form of meiosis, and not an atavistic reversion to mitosis. Another reason for clarifying what exactly is sex is that the epigenetic aspects of sex have largely been disregarded. By defining sex to be ploidy maintenance plus epigenetic resetting, either with or without outcrossing, we hope to have captured the evolutionary essence of sex, at the same time retaining the gist of sexuality encapsulated by those who equate sex with the dynamic duo of meiosis and syngamy.

Many others have defined sex to be merging of different genomes (Margulis & Sagan, 1986a). This appears to box its advocates into a needless corner. On one hand, Margulis & Sagan (1986a: 156) assert that meiosis (not fusion of different genomes) provides the adaptive advantage of sex via rejuvenescence, whereas, on the other hand they claim that autogamic taxa repudiate the claim that sex (for them, fusion of different genomes) rejuvenates genomes. Even more striking, had they defined sex to be fusion of any genomes, including identical genomes (mitotic copies), then their definition would be very close to ours, with syngamy being nothing more than a modified form of meiosis.

Cytosine methylation is the most fundamental epigenetic signature (Allis *et al.*, 2007); hence, it should be no surprise that it may be involved in evolution of sex. Not only does cytosine methylation exist in all but a few highly derived eukaryotes, but it also exists in prokaryotes (Russo *et al.*, 1996) and has been implicated in the transition from an RNA-world to a DNA-world (Poole, Penny & Sjöberg, 2000), gene regulation (Russo *et al.*, 1996), and genomic defence (Bestor, 1996). Cytosine methylation has probably played a role in evolution of sex determination, dioecy/gonochorism, and sex chromosomes (Gorelick, 2003a); thus, it is no great leap to imagine that it also played a large role in the evolution of meiosis and syngamy.

Although we have largely focused on cytosine methylation at regulatory loci, the arguments herein apply to many other inter-related molecular epigenetic signals that control ontogeny, such as (1) cytosine methylation on telomeres, centromeres and structural genes; (2) histone modification via acetylation, phosphorylation, and cytosine methylation; (3) other forms of chromatin marks, including X-inactivation and genomic imprinting; and (4) interfering RNA (Gorelick & Laubichler, 2008).

We derive deep insights about evolution of sex from lineages in which individuals only have sex with themselves. Obligate complete automixis, obligate restitutional automixis, and especially obligate autogamy result in extreme homozygosity, implying that

neither independent segregation nor crossing-over recombination can create genetic variation during sex in these obligately self sexual lineages. The existence of evolutionarily long-lived lineages with obligate self sex therefore indicates that increased heritable genetic variation is probably not the *sine qua non* of meiosis. A simple *gedankenexperiment* adds credence to the evolutionary primacy of automixis: who did the first sexual organism have sex with? If the driving force behind sex were independent segregation causing increased genetic variation, then we would expect more lineages to have many pairs of smaller chromosomes, holding chromosomal content (*C*-values) constant (a process known as chromosomal or karyotypic fission) as appears to occur in plants amongst pteridophytes (Haufler & Soltis, 1986; Soltis & Soltis, 1987) and in mammals amongst carnivores and deer (Todd, 1970; Kolnicki, 2000). The many taxa with only a few large chromosomes repudiates the notion that segregation drove the evolution of sex. It appears that, ontogenetically, all meiotic organisms undergo restitutional automixis (i.e. meiosis followed by endomitosis). Taxa with amphimixis, autogamy, and complete automixis then undergo a second modified meiotic cycle known as syngamy. Syngamy is merely a modified form of meiosis, with both a division restoring previous ploidy levels and the epigenetic reset (Fig. 1). Cumulatively, the evidence indicates that the origin and maintenance of sex may have nothing to do with genetic mixing. Thus, we propose that euploidy and the epigenetic reset associated with meiosis and syngamy were the original and still primary functions of sex.

Obligate self sex is not an evolutionary dead-end, devoid of opportunities for adaptation or speciation, as many have claimed (Stebbins, 1957; Avise, 2008). With such extreme inbreeding, virtually any genetic change results in a new lineage of so-called microspecies. Although DNA sequences in such taxa may have much less variation than with amphimictic sister taxa, both obligate selfing and obligate outcrossing taxa should have similar epigenetic variation. The epigenetic reset associated with meiosis and syngamy is not perfect. Therefore, epimutations, many of which are environmentally induced, can result in substantial epigenetic variation upon which selection can act (Jablonka & Lamb, 1989; Rutherford & Henikoff, 2003; Gorelick, 2004, 2005; Lushai & Loxdale, 2007). From an evolutionary perspective, such heritable epigenetic variation is equivalent to any other form of additive genetic variance (Jablonka, Matzke, Thieffry & Van Speybroeck, 2002; Gorelick & Laubichler, 2008).

Unlike with purely mitotic reproduction (apomixis), self sex in the form of autogamy, complete automixis or restitutional automixis provides a lineage with sufficient crossing-over recombination to thwart the operation of Muller's ratchet. Self sex not only precludes accumulation of DNA nucleotide mutations, but also the accumulation of epimutations. Furthermore, self sex is a form of ploidy cycling, which thereby relieves mutational load experienced by obligate apomicts (Kondrashov, 1994).

A misconception is that sexual organisms always go through single-celled haploid and diploid stages. As was evident with the discussion of fertilization versus syngamy, evolutionarily what matters are not the numbers of cells, but rather the number of nuclei. Although epigenetic resets associated with meiosis and syngamy can reset complex multicellular diploid stages into much simpler haploid individuals, these need not be stages with a single haploid nucleus. Flowering plants with bisporic and tetrasporic female haploid stages have a minimum of two or four haploid nuclei (Klekowski, 1988). Even if, contrary to our theory, syngamy results in a 2C zygote, double fertilization in Gnetales and basal angiosperms with diploid endosperm shows that the initial diploid stage need not contain a single diploid nucleus or even a single diploid cell (Friedman, 1990; Williams & Friedman, 2002). In double fertilization, the two sperm are products of a single mitotic division, and the two egg cells that are fertilized are also mitotic copies of one another. Thus, although the theory here suggests that haploid and diploid stages should start out with a small number of nuclei, the floor is certainly not one such nucleus.

Genetic mixing is icing on the cake; possibly advantageous, but probably of at best secondary importance (Margulis & Sagan, 1986b). Both increases and decreases of additive genetic variance are possible consequences of sex. There can be three functions of syngamy: restoring ploidy via a cell division, reset of development, and genetic mixing (Jennings, 1912).

Existing evolutionary theories of sex fall into two camps: increasing genetic variation and rejuvenescence (Mooney, 1993), with the former being the current paradigm. Although both theories may contribute to maintenance of sex, only rejuvenescence can explain origin of sex because no genetic mixing (neither segregation nor crossing-over recombination) occurs in obligately automictic or autogamic lineages, with their high homozygosity.

Meiosis and reduction division are remarkably consistent across all extant eukaryotes: animals, plants, fungi, and protists (with the possible exception of parasex). Even the putatively asexual diplomonads and kinetoplastids contain homologues of many genes that are believed to be only used in meiosis and mismatch repair (Bell, Harvey, Sims & McCulloch, 2004; Ramesh *et al.*, 2005). They also show signs of reduction division (Hope, MacLeod, Leech, Melville, Sasse, Tait & Turner, 1999; Bernander *et al.*, 2001).

Such conservatism across all eukaryotes is indicative of strong selective forces to quell variation. Two billion years of selection should remove virtually all additive genetic variance in a trait. Conservatism across all eukaryotes in a trait such as meiosis is consistent with sex decreasing heritable epigenetic signals, and not necessarily sex increasing variance in heritable genetic signals.

Typically, evolutionary questions about the maintenance of any form of genetic variation are best answered using population and quantitative genetics. By contrast, population and quantitative genetics are acknowledged to be woefully inadequate for explaining the origins of variation (Müller & Newmann, 2003). Origins of evolutionary variation are largely the bailiwick of evolutionary developmental biologists (evo-devo), falling under the rubric of epigenetics. Thus, it would be apropos if epigenetic resetting provided the primary function of sex.

Epigenetic resets that are associated with meiosis (including syngamy) reduce epigenetic variance. They virtually eliminate large within-generation (within-individual) epigenetic variance that choreographs development and possibly drives the Hayflick limit of successive mitotic divisions (Sonneborn, 1954; Hayflick & Moorhead, 1961; Howard, 1996; Tollefsbol & Andrews, 2001; Ruiz-García *et al.*, 2005). Epigenetic resets also eliminate the variance-creating effects crossing-over recombination has on nuclear epigenomes. Selection reduces heritable variance in any trait, and this appears to be exactly the case with the wholesale epigenetic resets associated with meiosis and syngamy. Diminution of epigenetic variance provides for classical Darwinian individual-based selection, as noted by Maupas (1890), without any need to invoke multi-level selection or kin selection. Each offspring benefits by inheriting an epigenetic reset that weathered development in previous generations from gametophyte to gamete to zygote to mature diploid organism. Only later did outcrossing sex (amphimixis) arise from self sex (probably from restitutional automixis to complete automixis to autogamy to amphimixis; Fig. 4). Epigenetic resetting therefore provides a plausible Darwinian mechanism underlying the evolutionary origin, maintenance, and ubiquity of sex.

Returning to the epigraph from Hamilton (1988), we too would like to see sex used for its oldest purpose, which we assert is self sex as a conservative variation-reducing form of rejuvenescence. Too often, we forget that self sex can be for much more than recreation.

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