

SEX REDUCES GENETIC VARIATION: A MULTIDISCIPLINARY REVIEW

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For over a century, the paradigm has been that sex invariably increases genetic variation, despite many renowned biologists asserting that sex decreases most genetic variation. Sex is usually perceived as the source of additive genetic variance that drives eukaryotic evolution vis-à-vis adaptation and Fisher's fundamental theorem. However, evidence for sex decreasing genetic variation appears in ecology, paleontology, population genetics, and cancer biology. The common thread among many of these disciplines is that sex acts like a coarse filter, weeding out major changes, such as chromosomal rearrangements (that are almost always deleterious), but letting minor variation, such as changes at the nucleotide or gene level (that are often neutral), flow through the sexual sieve. Sex acts as a constraint on genomic and epigenetic variation, thereby limiting adaptive evolution. The diverse reasons for sex reducing genetic variation (especially at the genome level) and slowing down evolution may provide a sufficient benefit to offset the famed costs of sex.

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Despite any pretense of machismo, eukaryotic sex is pretty boring and conservative. Although many of us delight in the traditional study of sexual variation, here we acknowledge that sex largely reduces variation. We thus hope to partially obviate the paradox of sex, thereby dethroning the queen of problems in evolutionary biology. There has been a coalescing of ideas from multiple independent perspectives that sex acts to decrease genetic variation, especially at genomic and chromosomal levels (admitting that sex largely increases genetic variation at the levels of individual genes). Although such notions exist in the early writings of Darwin and Weismann, in more recent times the variation-reducing qualities of sex have been brought to light by ecologists, cancer biologists, population geneticists, paleontologists, molecular biologists, and epigeneticists. In addition, genome theory is a departure from the traditional gene theory where individual

genes serve as the information unit and genetic variation occurs mainly through mixed gene mutations. Genome theory, where whole genomes serve as the informational unit, maintains that sex should reduce variation at the genome level. We hope to refocus debates about sex, which have been mired in looking for how increasing additive genetic variation might be beneficial and thereby impose what we believe to be fictitious costs of sex. By resynthesizing the panoply of naysayers, we show that sex is a conservative force that acts primarily as a coarse filter to remove variation, consistent with a mechanism (meiosis) that has been unchanged for 1–2 billion years.

One of the great quandaries in evolutionary biology is the existence of extensive diversity in the face of unrelenting selection and drift. Sexual populations do not adapt or do so at a much slower rate than expected (Futuyma 2010). For asexual (actually,

apomictic) animals and plants, mutation rates high enough to balance selection and drift, with no other evolutionary forces, would be lethal. Darwin realized this problem and consequently posited that sex increases variation, although based on naïve notions of genetics.

The single biggest “breakthrough” at resolving the origin of variation came from Weismann’s brilliant opus (1886). Building upon his colleagues’ discovery of the chromosomal basis of meiosis and outcrossing, Weismann proposed that sex increases variation in populations. Weismann rightly understood in 1886 that meiosis can increase variation at the level of individual genes, but was utterly wrong in 1891 that meiosis can increase variation at the level of chromosomes. His error was in not realizing that one copy of each homologous chromosome was passed on to each product of meiosis. Most work on evolution of sex has since been predicated on an uncritical acceptance of Weismann’s (1891) synthesis.

Since the 1930s, there has been too great a focus on individual genes and not enough focus on whole genomes with plenty of linkage disequilibrium, epistasis, and epigenetic effects. We are not asserting that sex entirely reduces additive genetic variance nor that genetic mixing via meiotic recombination does not provide some advantages. Instead, we assert that these are two distinctive functions of sex and that the main one is to ensure the existence of a given species by maintaining genome system identity. In contrast, the increased diversity at the gene level by meiosis is secondary, as the combination of genes contributes to new features of existing systems rather than altering the system in a fundamental way. Clearly, if it was merely just for increasing genetic diversity, sex would not have evolved in the first place insofar as asexual systems display much higher levels of genetic diversity. Although meiotic crossing-over recombination provides new allelic combinations, it does not alter loci or even the physical order of loci. We are only advocating a change in emphasis and perspective. Consider the analogy of alleles as colors of pixels on a computer screen, loci as physical location on the computer screen, and the genome as the computer screen. Even genome duplication can be handled with this analogy as a higher resolution screen. Meiotic crossing-over recombination may change the colors on the screen and may slightly alter the perceived image. But, we believe that the primary function of sex is to ensure that the computer screen itself, and not the image thereon, maintains its integrity.

By invoking many independent and unsurprising pieces of evidence about evolution of sex, we arrive at the surprising conclusion that sex does not generate the diversity that drives evolution per Fisher’s fundamental theorem of natural selection. Instead sex acts as a fundamental constraint on eukaryotic evolution, resulting in sluggish evolution and a paucity of adaptive evolution *sensu* Futuyma (2010).

Definitions of Sex

Before delving into arguments for sex reducing genetic variation, we first need to define sex. Definitions are arbitrary; the best definition (if such a thing exists) depends on its utility. As Günther Wagner (2010:1359) stated in discussing definitions of fitness, “[T]he meaning of a theoretical concept does not derive from its ‘definition’ (explanation) but from its status and use in the context of a theory and the associated experimental practice.” We have made a modicum of progress at understanding evolution of sex by defining sex to be meiosis (Gorelick and Carpinone 2009; also see Blute 2009). However, we first discuss other common definitions of sex and briefly describe their problems. Circumscribing a definition of sex is essential if we want to ascertain how sex affects genetic variation.

Agrawal (2009), who works extensively on evolution of sex, assumes that sex equals independent segregation of chromosomes plus crossing-over recombination, a definition that appears in many textbooks. Yet, many organisms when they undergo meiosis to produce gametes suppress crossing-over recombination, a phenomenon especially well-known in male *Drosophila* (Morgan 1914). Yet nobody would accuse sexually voracious *Drosophila* of being nonsexual or asexual for want of recombination. In many, if not most eukaryotes, crossing-over recombination can also occur during mitosis (Pontecorvo and Käfer 1958), further confounding the definition of sex as segregation and recombination.

Biologists dealing with both prokaryotes (bacteria, archaea) and eukaryotes often define sex to be any process that mixes genes (Margulis and Sagan 1986; Normark 2009). This could mean fertilization, but also horizontal gene transfers as the result of infection. Just because an individual is infected by a virus that transmits some of its DNA should not mean that we call the infected individual sexual. We do not understand how the definition of sex as genetic mixing gained so much traction, especially in light of transposable elements and mitotic recombination.

Many biologists equate sex with outcrossing in eukaryotes, that is, where eggs and sperm (or two eggs in isogamous taxa) come from two genetically different individuals. Such a definition means that self-fertilization does not count as sex. Likewise, when egg and sperm arise from genetically identical clones, this also would not count as sex. Yet, self-fertilizing organisms undergo the exact same processes—meiosis, including independent segregation and crossing-over recombination, and syngamy (fertilization is fusion of an egg and sperm cell; syngamy is fusion of an egg nucleus and sperm nucleus)—as their outcrossing relatives. Even with outcrossing sex, the two sexual partners usually are very similar genetically, that is, are of the same species (Shields 1982). Because self-sex is fundamentally no different from outcrossing sex (Gorelick and Carpinone 2009), we reject this definition of sex as outcrossing. Self-sex and sex between closely related

individuals, however, have played a huge role in understanding the variation-reducing role of sex.

Several biologists (e.g., Martens et al. 2009) state that sex is the combination of meiosis and syngamy. Although nicely circumscribing sex, this still implies that some forms of parthenogenesis, gynogenesis, and androgenesis do not count as sex, even though meiosis must occur each generation. If all chromosomes duplicate without a nuclear division either at the start or end of meiosis (aka premeiotic doubling or restitutional automixis), an organism can have alternation of haploid and diploid generations with segregation and crossing-over recombination, despite no syngamy. Likewise, in gynogenesis, fertilization of an egg by a sperm is required to resume arrested egg meiosis, but the sperm nucleus is discarded, hence no syngamy occurs despite the necessity for both egg and sperm meiosis. A few researchers prefer to define sex as simply meiosis (Boyden 1950; Williams 1975), especially because syngamy is probably nothing more than a modified form of meiosis (Gorelick and Carpinone 2009). Nonetheless, we consider sex to be meiosis plus syngamy because this is the most palatable of the traditional definitions of sex, although readily admit that meiosis plus endomitosis provides an equally valid form of sex.

Darwin and Weismann

We review the various reasons proposed for sex-diminishing genetic variation, starting with the 19th century arguments of Darwin and Weismann. It is crucial to examine Darwin and especially Weismann because many of the modern problems understanding evolution of sex stem from propagation of their errors.

Darwin's argument for sex reducing genetic variation predates the *Origin of Species* (Darwin 1859) and Mendelian genetics (Mendel 1866). Darwin (1838–1839) argued that if sex did not reduce variation, then there would be as many species as there are individuals (see Ghiselin 1988; Gorelick and Carpinone 2009). Darwin believed in blending inheritance, which also led him to believe that sex reduced variation, as his cousin Francis Galton pointed out a few years after Darwin's death in "Regression towards mediocrity in hereditary stature" (Galton 1886). Averaging diminishes variation.

Prior to the rediscovery of Mendel's work, Weismann believed in blending inheritance, but remarkably proffered an argument that sex decreases some genetic variance, yet increases some other genetic variance (Weismann 1886). He argued that sex indeed decreases variation in large genetic deviations, as it must if species concepts are to remain. His argument is predicated on huge population sizes. With blending inheritance, most individuals will have identical genetic constituency. If a large mutation arises, however, this can cause a speciation event, *à la* Goldschmidt

(1940). However, Weismann argued that the situation was different for small genetic deviations, which varied stochastically across all members of the large population. In modern terms, small genetic deviations were random and effectively neutral, thereby allowing variation to be maintained or slightly increase in the face of sex. Small genetic deviations, in modern terms, are nucleotide differences such as point mutations or indels that are only readily observable via sequencing (and via the phenotypic changes they cause). In terms that were just becoming known to Weismann, large genetic deviations mean chromosomal rearrangements that are visible with a light microscope. But with these large rare genetic deviations, Weismann agreed with Darwin that sex reduces variation.

The above argument was abandoned by Weismann (1891; also see Churchill 2010) once the reduction division of meiosis was explicated by Hertwig (1890). Weismann retained his old belief that sex increases variation in small genetic deviations, but now believed that the initial meiotic chromosomal doubling caused increases in variation of large genetic deviations (Weismann 1891; Meirmans 2009). Weismann (1891) learned that meiosis begins with a chromosomal doubling followed by a pair of reduction divisions (citing Platner 1888, 1889; Hertwig 1890), except for possibly in what we would now call automictic parthenogens, which Weismann used to support his 1891 theory. Weismann then thought that the number of possible genotypes due to meiotic segregation for an organism with n pairs of homologous chromosomes was $\binom{2n}{n} = \frac{(2n)!}{(n!)^2}$, that is, the number of combinations of sampling n chromosomes from a cell containing $2n$ chromosomes. We now know this is a severe overestimate, with there being only $2n$ possible haploid genotypes because each haploid product of meiosis receives one copy of each homologue. But in 1891, Weismann further over-inflated this estimate of the number of possible haploid genotypes to a value of greater than $\binom{2n}{n}$ by believing that the initial meiotic chromosomal doubling meant that a gamete could have two identical copies of any given homologous chromosome, for example, two copies of the maternal copy of chromosome 8. Weismann thus championed the notion that meiosis created genetic variation of large genetic deviations ("idants," "rods," "chromatosomes") in what we would now call the chromosomal or genome level, in addition to increased variation of small genetic deviations (ids), in what we would now call genes. Weismann (1891) asserted that fertilization and amphimixis increase variation in small genetic deviations (genes), whereas meiosis even with parthenogenesis could increase variation in large genetic deviations (chromosomes)—although, from a modern perspective, it seems that such a process will quickly drift to fixation. Thus, the typical modern view of Weismann in which he asserted that amphimixis functions to increase genetic variation, both of small and large deviations (genes and

chromosomes), is overly simplistic. By not understanding that one copy of each homologous chromosome was passed to meiotic daughter cells, Weismann (1891) inadvertently helped confound evolutionary theory for over a century, although the real problem resides with evolutionary biologists that have failed to wade through the subtleties of Weismann's 1891 arguments. The curious thing is that Weismann's 1886 ideas—not his more famed 1891 ideas—much more closely reflect the work of several modern researchers (e.g., Heng 2007; Gorelick and Carpinone 2009; Wilkins and Holliday 2009). It is remarkable that Weismann (1886) seems to have gotten the relationship between sex and genetic/genomic variation correct given the state of pre-Mendelian genetics. But why have so many modern researchers instead so readily accepted Weismann's (1891) incorrect notion that sex unfailingly increases genetic variance?

Paradigm

Since Weismann (1891), the paradigm has been that sex increases additive genetic variance, providing the fodder by which natural selection can increase fitness of populations. Fisher's (1930) fundamental theorem states that rates of change of fitness are proportional to additive genetic variance. Although mutation is the ultimate source of genetic variation, Fisher believed that sexual mixing of genes was the only way to adequately maintain this variation in all lineages except those of the simplest organisms. Neo-Darwinism rests on the pillars of selection, mutation, and genetic mixing. However, until Williams (1966), this selection when applied to evolution of sex was essentially group selection. Williams (1975; also see Williams and Mitton 1973) later provided possible (although tenuous) individual-based selective advantages for sex. Since 1975, the paradox of sex has been largely predicated on finding selective advantages of genetic mixing and explanations for the cost of males, although still with a tinge of group selection (Williams 1992). Thus, many biologists still define sex as genetic mixing (Margulis and Sagan 1986; Kirkpatrick and Jenkins 1989; Agrawal 2009), not meiosis. Even when the "cost of meiosis" is discussed, it is still in the vein of costs of segregation and crossing-over recombination (Treisman and Dawkins 1976; Uyenoyama 1984), despite existence of other functions of meiosis (e.g., maintenance of euploidy, repair of double-strand DNA damage, epigenetic reset). There have been challenges to the idea that sexual reproduction maintains diversity and that diversity explains the origin and maintenance of sex (Felsenstein 1985). However, at the fundamental level, most explanations of the function of sex are linked to genetic diversity without regard for the level at which genetic diversity is measured, such as population level or individual level. For example, sex has been considered beneficial for populations in two major ways. According to John Maynard

Smith (1978), sexual populations can evolve more rapidly to fit a changing environment by combining different mutations together in an individual; and sex can reduce the load of deleterious gene mutations in a population by aggregating bad mutations into an individual, resulting in that individual not surviving and the elimination of the mutations. At the individual level, the benefit of sex is mainly linked to offspring with gene variations with increased potential for fitness. Clearly, the function of "genetic mixing" is important here, whether they are "good genes" or "bad genes."

Most evolutionary discussions regarding the advantages of genetic mixing have been based on a faulty assumption that asexual species reproduce identical genomes. Williams (1975) argued for his lottery principle that asexual reproduction produces little or no genetic variety in offspring (like buying a large number of lottery tickets that all have the same number), limiting the chance of "winning," whereas sexual reproduction produces diverse genomes (lottery tickets with a greater variety of numbers and therefore a greater chance of "winning"). By reanalyzing genome sequencing data, this generally accepted assumption has been debunked (Heng 2007). Empirical comparison of asexual bdelloid versus sexual monogonont rotifers demonstrates that asexual lineages can have similar or greater genetic variation than their sexual relatives (Mark Welch and Meselson 2001; Fontaneto et al. 2009), with the caveat that it is nigh impossible to objectively define populations and species for obligate asexual lineages. Even Weismann (1891) performed experiments showing substantial genetic variation in parthenogenetic ostracods. Asexual lineages probably also possess the same levels of epigenetic variation as sexual lineages (Gorelick et al. 2011), and epigenetic variation is fundamentally no different from genetic variation (Gorelick and Laubichler 2008). Unfortunately, however, it will take time to change the long-held and probably erroneous belief that sexual lineages have more genetic variation than asexual sister taxa.

Not only are old arguments still pervasive, but they help drive the direction of more modern empirical research. For example, parthenogenetic taxa are often considered to be evolutionary dead ends because there is something aberrant about not having sex with others (Ah-King 2009). Comparison of related sexual and asexual lineages supposedly helps bolster the notion that sex increases genetic variation. There are apparently no evolutionarily long-lived asexual plant lineages and only a few long-lived asexual animal lineages (bdelloid rotifers, oribatid mites, and darwinulid ostracods). These data are taken to imply that obligate asexual lineages are less fit than their sexual counterparts, due to lack of genetic mixing, which is a classic Weismannian and Fisherian argument. However, it is virtually impossible to define asexual species, especially for those who prefer the biological species concept. Biologists cannot agree on a species concept for extant outcrossing organisms, and struggle even more to define asexual species (Birky and Barraclough 2009). Circumscription

of a species or population is a large factor in determining amount of genetic variation in that species or population. Even more problematic for making inferences about longevity of sexual versus asexual taxa is how asexuality is defined (Scali 2009). For example, ancient asexual oribatid mites are believed to all be completely automictic (Heethoff et al. 2009). Self-fertilization occurs between two products of the same meiotic division. Therefore the standard comparative approach (e.g., Schön et al. 2009) only tells us about evolution of outcrossing (amphimixis), and nothing about evolution of meiosis or syngamy.

Despite a paradigm that seems to have persisted since 1891, there has been a strong undercurrent from many different sub-disciplines indicating that sex largely decreases additive genetic variance. There are other benefits of meiosis, such as maintenance of ploidy, synapsis of homologous chromosomes, elimination of chromosomal rearrangements, and epigenetic reset. Next, we review many of these disparate and robust arguments, which each indicate that the costs of sex may be balanced by the variation-reducing benefits of meiosis.

Population Genetics and Punctuated Equilibrium: Sex Slows Evolution

Conventional wisdom holds that asexual taxa will have less genetic variation than their sexual relatives. Asexual lineages will thus become specialists with a narrow ecological niche (frozen niche variation hypothesis; Williams 1975; Vrijenhoek 1984). Asexuals sprout many independent (reproductively isolated) specialist lineages, whereas sexuals evolve as one panmictic generalist lineage. When looking at selection at the species level or any level greater than the individual, this means that asexuals have faster evolution than sexuals. With asexuals, there are many more subpopulations to select among. This means that sex slows down evolution, even if generalist, sexuals are therefore much less subject to extinction than asexuals (Williams 1975; Bell 1982).

The counterpart to the frozen niche variation hypothesis for asexual lineages is the general purpose genotype hypothesis, which states that asexuals should occupy wide niches compared with sexuals. Although sexual species occupy narrow niches, they are also hypothesized to episodically undergo cladogenesis (Stanley 1975). Thus, in the short run, sex severely slows down evolution. Only in the long-run, after relatively rare origination events, do sexual taxa have rates of evolution as large as that of asexuals.

The above argument has been accepted by many paleontologists under the guise of punctuated equilibrium, where for sexual taxa, evolution is usually extremely slow, that is, lack of

phyletic gradualism (i.e., short-term stasis), punctuated by rare instances of rapid evolutionary origin of new taxa. This view has also been espoused by proponents of Wright's shifting balance theory (Provine 1986; Goodnight 1995). Sex usually results in extreme slowing of evolution, and it is not altogether clear whether the few periods of rapid evolution are attributable to outcrossing, meiosis, or syngamy.

The above arguments are predicated on the empirical assumption that asexual populations have much less additive genetic variance than related sexual populations. What do the data say about this, especially since gel electrophoresis (Hubby and Lewontin 1966; Lewontin and Hubby 1966) and DNA sequencing (Kreitman 1983)? Although comprehensive comparative reviews have not yet been done especially for taxa that we suspect are apomictic, there is growing evidence that asexuals have just as much additive genetic variance as related sexuals. Bdelloid rotifers have incredible amounts of heterozygosity, although this could be due to premeiotic doubling (restitutional automixis) (Mark Welch et al. 2009). Apomictic plants, such as the mustard *Bochera holboellii*, have roughly as much allelic sequence variation as sexual relatives (Corral et al. 2009). We thus turn to more robust arguments that have been proposed for how sex slows evolution.

Meiosis Maintains Ploidy and Corrects DNA Damage That Causes Mutations

Bill Shields advanced "the apple does not fall far from the tree" ecological argument for sex reducing genetic variation (Shields 1982, 1988). If parents possess some adaptive advantage and random deleterious mutations, then their offspring are best off by inheriting their parent's original (premutation) genotype. Inbreeding and possibly mitotic recombination provide a template by which mutations can be corrected, while co-adapted gene complexes are maintained. Shields provides a theoretical argument and extensive ecological evidence that offspring usually take up residence adjacent to their parents and that there is extensive inbreeding in most plants and animals investigated. For Shields, sex fosters inbreeding, which thereby fosters homogeneity among offspring. Shields' contribution was to note that, even at the level of genes and gene combinations, sexual reproduction combined with inbreeding constrained the generation of hitherto unselected genotypic diversity.

Shields arguments are echoed in definitions of species, especially the biological species concept (as though other species concepts are nonbiological). Individuals can only interbreed with those who are genetically similar to themselves, that is, having largely identical genomes. Genetic similarity can even be used as a

proxy for defining species, as is done when chromosomal banding is used to differentiate species (King 1990). Even crosses between closely related taxa are impossible, such as humans and great apes. There is no extreme outcrossing, such as between plants and animals or between different animal phyla (although see Williamson 2006 for radical notions). Reproductive isolation ensures that genetic variation within populations will be constrained, allowing for local adaptation.

Harris Bernstein (1977) and colleagues (Bernstein et al. 1981, 1988) echo Shields arguments, but on a molecular genetic level. They proffer that the function of sex is for DNA repair. Synapsis brings homologous chromosomes into association, allowing double-stranded DNA damage to be detected and corrected. Bernstein et al. (1988) note the commonness of selfing hermaphrodites and even the taxonomically widespread occurrence of premeiotic doubling (restitutional automixis), which are beneficial in eliminating new genetic variation due to point mutations and which cause no new genetic variation. Bernstein's idea of decreasing genetic diversity by repairing DNA damage that would otherwise cause mutations was appreciated by Forsdyke (2007), who considered the reason for sex to be decreased diversity at the gene level and increased genetic diversity among gametes, coupled with chromosomal pairing that ensures equal partitioning of chromosomes among gametes.

Page and Hawley (2003) extended Bernstein et al.'s (1981) argument by positing that the function of synapsis during the first metaphase of meiosis is not only to conserve individual genes, but to conserve ploidy. Without the molecular machinery for meiotic crossing-over recombination, reduction division would not occur properly. Aneuploidy—gain or loss of one or a few chromosomes—is usually fatal, at least in animals. Cavalier-Smith (2002) also states that facilitating ploidy cycling is a main function of sexual reproduction. Even the world's leading expert on meiosis, Robin Holliday admits that, “the initial function of chromosome pairing was to *limit*, not enhance, recombination” (Wilkins and Holliday 2009: 3, italics in original). Prior to this “surprising” conclusion, most researchers felt the function of reducing genetic diversity was a byproduct of chromosomal pairing. If diploidy first evolved to correct double-strand DNA errors, then maybe the quintessence of meiosis, including synapsis, is reduction division (Otto and Goldstein 1992; Orr 1995). This is also the essence of Kondrashov's (1994) ploidy cycling arguments, in which most asexual organisms undergo alternation of haploid and diploid generations (also see Kondrashov 2001), arguing that ploidy cycling eliminates mutational load. Although ploidy cycling may often contain cryptic meiosis (Solari 2002; Ramesh et al. 2005; Smith et al. 2006; Cooper et al. 2007; Gorelick and Carpinone 2009; Gorelick et al. 2011), we should not dismiss the importance of euploidy. With diploidy, but without reduction division, runaway polyploidy would ensue (Mahendra and Sharma

1955). Once the number of chromosomes becomes sufficiently high, aneuploidy becomes common, as seen in taxa with over 500 chromosomes, for example, the pteridophyte *Ophioglossum* (Löve et al. 1977), the monocot *Voanioala* (Johnson et al. 1989), and the eudicot *Echeveria* (Uhl 2007). Aneuploidy also seems common in the ant *Dinoponera lucida*, with $2n = 106 - 120$ chromosomes (Mariano et al. 2008).

The above arguments regarding inbreeding, repairing double-strand DNA damage, (e.g., double-strand breaks, interstrand crosslinks) and maintenance of ploidy have at least one thing in common: offspring will look just like their parents. An epigenetic perspective yields something similar: development works best when offspring precisely mimic what was successful for their parents and grandparents.

Sex Reduces Epigenetic Variation

Many population genetic models have been used to explain the maintenance of sex, assuming that sex increases genetic variation vis-à-vis genetic mixing. This makes sense insofar as population genetic models are remarkably good at explaining static phenomenon, but are notoriously poor evolutionary models for explaining origins of novelty (Müller and Newmann 2003). The evolution of sex debate has therefore been separated into two camps: “maintenance” and “origin” of sex. As ideas about the origins of sex are difficult to test, most available hypotheses have focused on the maintenance of sex (Wilkins and Holliday 2009). Gorelick and Carpinone (2009) took the evolutionary tack most suited to explaining evolutionary origins, namely epigenetics, with the corollary of also applying to its maintenance.

During meiosis, not only does synapsis of homologous chromosomes and reduction division occur, but there is an epigenetic reset. Cytosine methylation and chromatin signatures are erased in primordial germ cells (PGCs) and reestablished on gametes during meiosis (Davis et al. 2000; Farthing et al. 2008; Zechner et al. 2009). Although epigenetic erasure occurs prior to meiosis, cell specification has already occurred—PGCs are predestined to undergo meiosis after at most a few mitotic divisions (Ewen and Koopman 2010). Reestablishment of epigenetic marks occurs during gametogenesis. (Gamete formation is not a discrete event, localized in time, as can be seen from PGC development and arrest of egg meiosis, sometimes for decades.) Epigenetic signatures are not reset to levels of the adult tissues that underwent meiosis, but rather to levels that gametes had in previous generations. Likewise, following syngamy, cytosine methylation and chromatin signatures are erased and reestablished to the same levels that they were at during previous embryonic stages. For plants and animals, an individual gradually develops from a zygote to a mature adult under aegis of these epigenetic signals. The

zygote is single-celled and totipotent; the adult contains billions of specialized cells (McShea and Brandon 2010). The process of meiosis returns complex diploid adult cells to a simple haploid state, necessitating epigenetic reset. Syngamy turns a haploid (sometimes multicellular) state to a simple diploid state, again necessitating epigenetic reset. Epigenetic reset reduces variation. Epimutations accumulate over the course of diploid and haploid development, while epigenetic reset removes much of this epigenetic variation by restoring epigenetic signatures to levels that worked in the previous generation(s).

Cancer Evolution Surprisingly Reveals the Main Function of Sex

Cancer is a typical evolutionary process (Nowell 1976; Crespi and Summers 2005; Heng et al. 2006b, 2010; Merlo et al. 2006; Ye et al. 2007; Heng 2009; Nicholson and Duesberg 2009; Vincent 2010). Recent studies of cancer genome evolution demonstrated the importance of genome level alterations during in vitro immortalization process (Heng et al. 2006a,b,c, 2009, 2010; Heng 2009; Ye et al. 2009). In this time-course experiment, fibroblast cells with p53 mutations and normal karyotypes (46 chromosomes) were cultured and followed over a period of two years of continuous culture. When the cells grew to 80–90% confluence in a culture dish, these cells were divided into two new dishes for further growth. Each instance of this subdividing is called a cell passage. A proportion of cells from each passage can be harvested to determine whether new karyotypes have formed, and how many of these newly formed karyotypes are clonal (detectable from multiple cells) or nonclonal (only detectable in one cell). Here we define the concept of cloning as sharing the same karyotypes, indicating that parent cells produce identical or similar offspring (Heng et al. 2006a,b,c). The domination of clonal karyotypes indicates the stability of a cell population, and the length of time to detect dominant karyotypes indicates the evolutionary time window for such domination. Dynamics between clonal and nonclonal chromosome aberration can be used to study patterns of evolution. Interestingly, two distinctive phases of karyotypic evolution have been documented. One is the discontinuous phase where karyotypes are highly variable, coupled with high levels of nonclonal chromosome aberrations (NCCAs). Due to drastically altered karyotypes between cell passages and within the same passages, evolutionary relationships are impossible to trace. In contrast, in the stepwise phase where gradual changes of karyotypes are observed that last for hundreds of passages, a majority of cells share common clonal chromosome aberrations (CCAs) coupled with low levels of nonclonal chromosome aberrations. In this stable phase, evolutionary relationship between different passages and among cells within the same passage can

be easily traced based on the similarity and gradual alteration of the karyotypes. This relationship not only contributes to our understanding of stochastic cancer progression, but reveals different patterns of evolution. In particular, the pattern of evolution in the discontinuous phase is similar to asexual evolution that displays punctuated evolution patterns, whereas the stepwise phase is similar to many sexual species where karyotypes can be traced. Because the punctuated phase is very unstable as indicated by the high level of NCCAs, it follows that asexual species should also have a diverse genome, which is contrary to the traditional thinking that asexual species have identical genomes (despite sequence divergence within asexual lineages). This realization has led to the question of the basic assumption that sexual species are more diverse than asexual species. To make sense of the association of genetic diversity with asexual species and of genetic homogeneity with sexual species, we proposed that sexual reproduction serves as a “filter” to eliminate altered genomes (Heng 2007). Sex as an evolutionary filter solved key differences between cancer evolution and organismal evolution in sexual species, insofar as a mechanism to stabilize somatic cell genomes is lacking in cancer cells.

Despite the seemingly huge difference between evolutionary biologists (who care about heritability of traits of individuals across generations that are defined by meiosis and syngamy) and cancer biologists (who are interested in the heritability of traits within somatic cell populations mediated by mitosis), these two very different subjects share key similarities: they both study evolving biological lineages. If we adopt a system viewpoint and consider each individual in organismal evolution and each cell in somatic cell evolution as an individual system, then common evolutionary patterns emerge. Both organismal and somatic cell evolution generate stable genomes when the environment is benign and unstable genomes when systems are under stress. Organismal evolution can purify and conserve the genome with a sexual filter, whereas somatic evolution cannot purify itself due to the limitations of mitosis. This is why somatic evolution can occur over such a relatively short period of time compared with organismal evolution that may take millions of years. In fact, any chromosomal pairing that eliminates altered genomes and any epigenetic reset could be thought of, in abstract, as means of maintaining system stability. The discovery we made studying somatic cell evolution is just such a link between system instability and the patterns of evolution.

Clearly, there is no sex involved for somatic cells. The significance of the somatic cell system is the conceptual realization that gene-level alteration and genome-level alteration represent fundamentally different aspects of evolution. To maintain the genome-defined biosystem, karyotypes must be maintained (by system stability in somatic cells and sexual reproduction in most eukaryotic organisms), whereas the gene-level alterations only

mainly contribute to microevolution. Thus, the main function of sex is to maintain the genome, while a secondary effect is to promote gene-level diversity. Gene-level diversity may arise from sex as an unselected side effect of a system that functions to reduce genomic changes.

Genome Theory: Resynthesis of the Function of Sex

Cancer models are ideal for watching evolution in action: each case can be considered a single run of successful somatic cell evolution. As soon as we treated each cell within a population as an individual, the dynamics of genome evolution could be studied. Based on data/concepts from organismal and cancer evolution, the genome theory was recently introduced (Heng 2009). Fundamentally different from gene theory, genome theory maintains that each individual gene is not the information unit subject to selection because the meaning of gene is defined by genome context, including topology within the nucleus, environment, genotype-by-environment interactions, indirect genetic effects, and eco-evo-devo. The eukaryotic genome represents a major evolutionary transition, with the genome serving as a main platform for evolution (Durand and Michod 2010). Because only the entire genome, and not the gene, can define a species (not to be dismissive of barcoding), the significance of genetic mixing at the gene level is drastically reduced both for maintaining and/or creating new systems or species. In contrast, the function of maintaining the population or species by preserving the genome context is of great importance for species (assuming species selection is real; Jablonski 2008; cf McShea and Brandon 2010). Based on genome theory, genome-level alterations are mainly linked to macroevolution, whereas gene-level alterations are linked to microevolution. Epigenetic alterations are also involved more at the genome level due to their global regulation.

In terms of genetic mixing, the genome and genes function differently. Selection of adaptive genome stability results in species identity, whereas altered genes can provide changed features within the species, possibly facilitating short-term adaptation. Because the environment is constantly changing, gene mutations and the combination of them are in constant flux, only the genomes are preserved over a long period of time. The system must be able to exist first, and this is of primary importance to the perpetuation of the genome. Although genetic recombination contributes to genetic diversity, it does so secondarily and within the framework of the chromosomally defined genome. Resynthesis of the function of sex based on the genome theory has drastically changed the way we study sex and evolution, with sexual reproduction as the key that distinguishes between drastic genome alteration mediated macroevolution and gene muta-

tion mediated microevolution (Heng 2009). In addition, the main function of sex as a filter explains the difference between sexual and asexual species, insofar as the asexual process generates more diverse genomes because of the less-controlled reproduction systems, whereas sexual reproduction generates more stable genomes.

Many of Darwin's and Weismann's arguments were based on studying phenotypes without the influence of gene theory. With acceptance of gene mixing as the key factor for evolutionary diversity, there has been a century-long struggle to demonstrate that the main function of sex is to increase genetic diversity. Results have been highly controversial, but the concept survives. The variation-reducing function of sex can now be fully appreciated by adopting genome theory.

Conclusion

If sex—by which we mean meiosis alternating with either syngamy or endomitosis—reduces genetic variation, then this benefit may substantially balance the costs of sex and meiosis (e.g., costs of males and mating, sexually transmitted diseases, recombinational load). Sex is beneficial to all offspring and the population because sex removes deleterious changes to genes and genomes. Ploidy is restored. DNA damage (e.g., oxidatively altered bases) is repaired and mutations are thereby avoided. Cytosine methylation and chromatin marks are reset to levels that worked for both parents. Large chromosomal arrangements are either purged (very likely) or the resulting offspring form new species (very rarely). For facultatively sexual lineages, asexual/apomictic reproduction—as we see with cancer tissues—results in extreme variation in all of the above phenomena. Only sex, *vis-à-vis* meiosis and/or syngamy, eliminates variation, especially large variation, to preserve a genome-defined system. As with any biological system, variations of small effect are tolerated. This is the argument of evolution as a tinkerer (Jacob 1977; Poole et al. 2001), which was largely Weismann's 1886 reasoning. Although sex may be thought of as Ponce de León's fountain of youth, it really is as boring as a metronome, restoring genetic and especially genomic and epigenetic signals to states that were successful in previous generations. Sex thereby slows (constrains and restrains) evolution, enabling lineages to conservatively defer extinction.

Although many hints that sex reduces genetic variation have existed for over a century, the paradigm has persisted that sex increases genetic variation. Although variation-inducing effects of sex exist on a very local scale, for example, of (neutral) synonymous substitutions, for genome-scale variations there has never been decent evidence that sex increases variation. The primary reason evolutionary biologists believed that sex induces genetic variation is that Darwin's (1859) and Fisher's (1930) theories

needed a recurring source for genetic variation. Given that most lineages are sexual, sex was a convenient answer. . . although, we believe, a wrong answer.

We do not quibble with Fisher's (1930) notion—which really dates back to Darwin (1859) and Weismann (1891)—that (additive) genetic variance is the engine driving evolutionary change. We only contest that sex helps generate that heritable variance, especially at the genome level. Weismann's (1891) *deus ex machina* of sex generating genomic variation was a seemingly necessary step in bolstering Darwin's theory of natural selection, which had not yet found general acceptance, and was predicated on an excusably erroneous notion of pangenesis prior to the rediscovery of Mendelian genetics. It is possible that Darwin and neo-Darwinian thinking overemphasized the relative role of selection compared with the other evolutionary forces of mutation and drift or stochastic genome alteration (e.g., Lynch 2007; Gorelick 2009; Heng 2009). Overemphasis on selection was broached by Sewall Wright's (1931) shifting balance model and Motoo Kimura's (1983) neutral theory. As Futuyma (2010) insightfully noted, the two biggest advances in evolutionary theory in the past half century were Kimura's neutral theory and constraints on evolution.

Our contribution is to incite the paradigm shift that eukaryotic sex is a brake, not an engine, of evolution. Sex is ancient, conservative, and virtually ubiquitous in eukaryotes. This is the trademark of a trait that has been under intense selection for millions (even billions) of years. Intense and persistent selection is consistent with a trait that limits or reduces variation.

Alternatively, as one reviewer suggested, maybe sex is a clutch, neither an engine nor a brake. Like a clutch, most of the time sex causes almost no change and, in fact, limits the speed of evolution to some narrow band. Indeed, drivers on steep hills often use a clutch as a brake. However, rarely the clutch is engaged and there is a shift to a new gear vis-à-vis a change in genomic architecture leading to a new genome context (Ye et al. 2007; Heng 2009).

We reviewed arguments from a diverse assemblage of biologists—ecologists, cancer biologists, population geneticists, paleontologists, molecular biologists, genome theorists, epigeneticists—who implore that sex reduces genetic variation. Despite the fact that sex reduces genetic variation in multiple biological systems and from multiple disciplines, mainstream researchers have chosen to believe otherwise. They have largely relied on gene theory, believing that mixing of genes is fundamental. Knowing what we know today, the general assumption of sexual species displaying diverse genomes is wrong. Contrary to the current paradigm, almost all evidence suggests that the genome, rather than genes, defines the system and that the initial function of chromosome pairing was to enhance repair of DNA damage while limiting aberrant chromosome segregation (not enhance recombination), and that sexual reproduction main-

tains the genome identity at multiple levels. With this paradigm shift, there is much less of a problem explaining why sex persists. There is much less need to invent an endless set of population genetic models justifying the genetic mixing aspect of sex. Instead meiotic crossing-over recombination becomes a relatively minor epi-phenomenon that hitchhikes along with the variation-reducing aspects of sex. We argue that the queen of problems in evolutionary biology—the existence, persistence, and ubiquity of sex—was never a problem, but rather exactly what evolutionary biologists should expect from a variation-reducing phenomenon.

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