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Asexuality and Epigenetic Variation

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Epigenetic processes are of fundamental importance for all living organisms as an individual phenotype is shaped by both its genome and its epigenome (Richards, 2006; Bossdorf et al., 2008). By *epigenetic*, we mean all molecular signals that are literally on top of DNA, such as cytosine methylation, chromatin marks, histone modification, and RNAi (Allis et al., 2007), many of which are responsible for classical developmental processes, as seen by embryologists. Collectively, we refer to all of these molecular epigenetic signals as the *epigenome* (Suzuki and Bird, 2008). Like Holliday and Pugh (1975), we consider these molecular epigenetic signals to be the nuts and bolts underlying classic epigenesis *sensu* Waddington. The developmental programs that lead to differentiated cell phenotypes are based on the interaction of genomic control mechanisms with programmed epigenetic signals that are established very precisely in space and time at the scale of an individual (Bird, 2002; Meissner et al., 2008). The integration of these intrinsic epigenetic signals by the genome enables cellular differentiation of all multicellular eukaryotic organisms (Jaenisch and Bird, 2003; Holliday, 2006; Allis et al., 2007). While this is true for any individual of any taxon, there are evidently some important differences among the epigenomes of different taxa (Suzuki and Bird, 2008); but there might even be some epigenetic variation among individuals of the same taxon that is not necessarily caused by genetic variation (Richards, 2006, 2008).

These two interacting variables, the genome and epigenome, though connected during development and differentiation can vary more or

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less independently from one another; and as a result, we need to try to disentangle genetic from epigenetic variation in the shaping of an individual phenotype (Gorelick, 2004a, 2005; Bossdorf et al., 2008; Richards, 2008). Furthermore, a growing body of literature shows that organisms can have heritable epigenetic variation despite little or no heritable variation in DNA nucleotides (Cubas et al., 1999; Rakyan et al., 2003; Chong and Whitelaw, 2004; Weaver et al., 2004; Blewitt et al., 2006; Manning et al., 2006; Richards, 2006; Whitelaw and Whitelaw, 2006; Crews et al., 2007; Vaughn et al., 2007; Kucharski et al., 2008; Jablonka and Raz, 2009). While these phenomena are particularly difficult to investigate for organisms that reproduce sexually (with constant genetic mixing during meiosis and syngamy), asexual organisms are a better model system to study epigenetic variation (Massicotte and et al., in press).

In this chapter we first demonstrate how asexual lineages allow us to measure epigenetic variation that is distinct from genetic variation in natural populations. Then, we show how epigenetic variation can be used to demarcate generations—and thus to define individuals and heritability—in asexual organisms. While epigenetic signals often vary over the course of development, the same epigenetic signals can in some instances be more or less immutable from one generation to the next. Such constancy of epigenetic signals probably also exists in asexual lineages. Epigenetic signals are thus a convenient measurement "device" that allows us to define individuals, generations, heritability, and even species in asexual taxa.

By *asexual*, here, we mean those eukaryotic taxa that never engage in *amphimixis*, i.e., genetic mixing. Asexual taxa can include lineages that rely on hybridogenesis, gynogenesis, parthenogenesis, or autogamy, including complete automixis, restitutional automixis, and even apomixes (Gorelick and Carpinone, 2009) (see Table 6.1). With *autogamy*, each individual produces both eggs and sperm, which then fuse with one another to form a zygote. With *complete automixis*, females undergo meiosis, but then two egg nuclei from the same meiosis fuse with one another to form a zygote. Complete automixis includes many forms of parthenogenesis and possibly gynogenesis. With *restitutional automixis*, including premeiotic doubling, females undergo meiosis, but diploidy is restored only by the egg cell or its mitotic progeny spontaneously duplicating all chromosomes (i.e., endomitosis or endoreduplication).

Epigenetic signals are generally more readily alterable than are DNA nucleotides (Gorelick, 2005; Angers et al., 2010). Heritable and inducible epigenetic variability may thus allow asexual taxa to succeed without genetic mixing of DNA nucleotides. The frozen niche variation (FNV) model (Vrijenhoek, 1984) suggests that for a population composed of multiple different clones, each clonal lineage uses only a fraction of the total niche, reducing competition among individuals and allowing efficient resource utilization. This model can be extended to variation among the epigenomes of individuals from the same clonal lineage, thus creating epiclonal lineages. According to the FNV model, selection should favor epiclonal lineages that have minimal overlap and, as a result, enhance the ecological success of a clonal lineage. However, epimutations typically are less heritable than changes in nucleotide sequence because of the necessity of the epigenetic reset prior to the initiation of development. Epigenetic reset can be seen in the classical case of resetting a mature multicellular adult composed of highly differentiating cells to a small (almost unicellular) zygote or preembryo that is composed of totipotent cells. Epigenetic reset could also be seen in molecular signals, such as cytosine methylation or telomere degradation, which are reset to "juvenile" levels following meiosis and syngamy (Gorelick and Carpinone, 2009). It is not clear yet whether the balance between these two factors—epimutations and epigenetic reset—will cause evolution of obligate asexual taxa to be more or less affected by epimutations than by DNA nucleotide mutations.

TABLE 6.1
Nomenclature of Asexuality

TERM	DEFINITION	EXAMPLES
Amphimixis	Outcrossing Reduction division + gametes + syngamy	Most plants and animals, any taxon with male individuals
Complete automixis	Both gametic nuclei are products of the same meiotic division Reduction division + syngamy (either with or without gametes)	Stick insect (*Bacillus atticus*), dewberry (*Rubus caesius*), *Paramecium aurelia*
Restitutional automixis	Meiosis but no gametes and no syngamy Endomitosis in lieu of syngamy Reduction division but no syngamy	Lumbricid earthworms (e.g., *Octolasion cyaneum*), garlic chives (*Allium tuberosum*), probably *Giardia intestinalis*
Parasex	No discrete reduction division or gametes Syngamy + reduction	*Aspergillus nidulans*, possibly some heliozoans
Apomixis	No meiosis or syngamy	Possibly bdelloid rotifers and orabatid mites, although these may be cryptically automictic
Autogamy	Self-fertilization	Mangrove killifish (*Kleptolebias* [*Rivulus*] *marmoratus*), many flowering plants
Endomitosis	Duplication of chromosomes without nuclear division (aka endoploidy or endoreduplication)	Probably all eukaryotes
Fertilization	Fusion of gametic cell membranes (aka plasmogamy)	All amphimictic and completely automictic taxa
Syngamy	Fusion of gametic nuclei or pronuclei and subsequent mixing (i.e., decondensation and unpairing) of homologous chromosomes (aka karyogamy)	All amphimictic and completely automictic taxa
Parthenogenesis	Reproduction without sperm	Stick insect (*Bacillus atticus*), dewberry (*Rubus caesius*), *Paramecium aurelia*, hammerhead shark (*Sphyrna tiburo*), teiid lizard (*Aspidoscelis* [*Cnemidophorus*] *tesselatus*)
Gynogenesis	Reproduction in which sperm induce development of the egg to form an embryo or preembryo, but the sperm nucleus or pronucleus does not fuse with the egg nucleus or pronucleus	Summer flounder (*Paralichthys dentatus*), Amazon molly (*Poecilia formosa*), diploid hybrid *Chrosomus* [*Phoxinus*] *eos-neogaeus* complex
Hybridogenesis	Apparent amphimixis but eggs comprised only of maternal chromosomes	Hemiclonal *Poeciliopsis monachalucida*, hybrid frog *Rana esculenta* complex

If nothing makes sense except in light of population genetics (Lynch, 2007), then we need to know how the four horsemen of evolution—selection, mutation, gene flow, and drift—are affected by lineages being sexual versus asexual. Incidentally, such a broader understanding of the roles of different evolutionary factors in sexual versus asexual lineages, including the role of epigenetic variation, will also be relevant to one of the most fundamental puzzles of all evolutionary theory, the evolution of sex (Gorelick and Heng, 2011). Selection should have roughly equal effects on obligate asexual lineages and related amphimictic taxa. Gene flow should also be roughly the same in obligately asexual and amphimictic lineages. It is difficult to generalize whether sexual and asexual taxa will have different mutation rates. The only instances when their mutations rates should clearly differ are when asexuals evolved via polyploidy and geographical parthenogenesis. Neoploids have higher proportions of cytosine methylation and, hence, higher mutation rates than their diploid ancestors (Adams et al., 2003; Rapp and Wendel, 2005; Salmon et al., 2005). This also results in higher epimutation rates, due to loss of methylation following mismatch repair of deaminated 5-methylcytosine (Gorelick, 2003). Geographical parthenogens tend to live in harsher environments and, hence, might have higher mutation rates than amphimictic sister taxa (Vandel, 1928; Lynch, 1984). Only with drift do we unequivocally expect higher rates of evolution with asexual lineages than with sister sexual lineages. First, however, we must digress.

Genetic drift is defined for any locus, including many different loci that contain epigenetic signals. A *locus* is a specific location on a eukaryotic or prokaryotic chromosome. A location on a map, including a chromosomal map, is not inherently genetic or epigenetic. What matters are the items that reside at that location. By analogy, on a map of Canada, the *location* of the province of Quebec has no linguistic attributes but can be considered francophone only when we ask who resides there. A location on a chromosome can be considered genetic if we ask which DNA nucleotides reside at that locus or epigenetic if we ask about methylation at that locus. Similarly, a locus could be considered epigenetic if we ask whether the polymorphism observed at a given histone is methylated, unmethylated, acetylated, deacetylated, phosphorylated, or not phosphorylated. As a final example, we could ask how many cytosines are methylated at a given promoter. Drift at any of these loci could be considered epigenetic drift, although we are reluctant to distinguish epigenetic from genetic (Gorelick and Laubichler, 2008). Effective population size of obligate asexual lineages is one. Therefore, evolution of obligate asexual lineages should be dominated by genetic drift of epigenetic alleles (Richards, 2008) and, as we saw in the previous paragraph, to a lesser degree by mutation and epimutation, which could also be considered synonymous.

Because population genetics was developed before the elucidation of DNA as the carrier of genetic information, within the conceptual framework of population genetics, mutation can mean DNA point mutations as well as epimutations (Gorelick and Laubichler, 2008). We have drift not only at DNA loci but also at epigenetic loci. Thus, when studying epigenetics and asexuality, we are justified in focusing on epigenetic drift and epimutations and, of course, their impacts on additive (epi)genetic variation (Gorelick, 2005).

In this context, studying epigenetic variation of asexual taxa has several advantages. First, asexuality makes the investigation of epigenetic variation not related to genetic variation much easier and allows us to quantitatively measure degrees of epigenetic variation. As epigenetic variation can potentially influence phenotypes, influence sustained phenotypic plasticity, and/or be considered as a phenotype by itself, such measurements are obviously important. Furthermore, epigenetic variation helps to demarcate clones (individuals) and epigenetic reset helps to define generations, thus allowing us to apply the traditional conceptual apparatus of evolutionary theory to these taxa.

EPIGENETIC VARIATION • *What Can Asexual Organisms Tell Us?*

A growing body of literature suggests that epigenetic mechanisms might be of particular importance in driving microevolutionary processes, including speciation, mostly because subtle variation in gene expression can strongly influence the phenotypic outcome even without having to modify the underlying DNA sequence. In that sense, heritable epigenetic variation and its effects on the regulation of gene expression can be see as a logical extension of the paradigm of regulatory evolution.

Epigenetic variation has many sources. One can classify epigenetic variation into three classes, reflecting its dependence upon underlying DNA nucleotides (Richards, 2006; Bird, 2007). Genetic information can control epigenetic marks via the interaction in *cis* and *trans*. This represents the obligate epigenetic variation, which can be modeled as epistatic interactions between DNA sequences and epigenetic markers (Gorelick, 2004b). However, epigenetic variation is not exclusively linked to DNA sequence variation. Facilitated—semi-independent, e.g., *agouti* locus (Morgan et al., 1999)—and pure—fully independent, e.g., monozygotic twins (Fraga et al., 2005)—epigenetic variation have also been observed. The independence of epigenotype and DNA sequences highlights the potential of individuals with identical DNA sequences to express different phenotypes. The amount of variation of a phenotype that is linked to facilitated or pure epimutation can therefore be a function of stochastic events and/or environmental influence (Angers et al., 2010).

Before trying to reach conclusions about the effects of epigenetic variation in the evolution of any organisms, it is important to look at the epigenetic variation in natural populations (Kalisz and Purugganan, 2004; Richards, 2008). It is particularly important to define how much epigenetic variation in natural population is not strictly related to DNA sequence variation, i.e., how much facilitated and pure epigenetic variation exists (Massicotte et al., in press). To do so, we need to be able to isolate the proportion of the phenotype that is not encoded by DNA. Some examples can be found in the literature (we know that there is some variation in natural populations), but we do not yet have much of a picture of how much facilitated and pure epigenetic variation exists, except perhaps for studies of monozygotic twins (largely human; Rakyan et al., 2004). An equally important problem is to determine how much of the existing epigenetic variation (of any kind) is heritable. This is especially relevant in medical contexts as a growing body of evidence suggests that some environmentally induced variation in the epigenome can indeed be passed on to future generations (Pembrey et al., 2006; Hitchins et al., 2007).

The question is, How can we isolate the effect of epigenetic (facilitated and pure epigenetic variation) versus DNA-induced genetic and epigenetic effects (obligatory epigenetic variation) in order to highlight the phenotypic variation that is the result of individuals having different epialleles? The easiest solution would be to control for DNA-based variation. How can we do so and still investigate the epigenetic variation in natural populations? Naturally occurring populations of clones are found in organisms that reproduce asexually via parthenogenesis, gynogenesis, and androgenesis; (Bird, 2007; Avise, 2008; Scali and Milani, 2009). Individuals that belong to the same clonal lineage are all genetically identical. Populations are composed of many individuals from a single lineage, unlike with monozygotic twin studies in which we can investigate variation in a sample size of only two, albeit across many sets of twins in a population. We can also borrow ideas from quantitative epigenetics and try to measure what proportion of additive genetic variance is due to heritability of some life-history traits that are related to an epigenetic state (Rutherford and Henikoff, 2003; Gorelick 2005).

PARTITIONING EPIGENETIC VARIATION

Because epigenetic marks are reversible, they are more susceptible to being altered than are

DNA nucleotides. The modification of the original epigenetic pattern that controls gene expression is called an *epimutation* (Jeggo and Holliday, 1986). Epimutations can become established over the course of development and/or at maturity. As an example, the removal of an epigenetic mark, such as DNA methylation, can lead to improper gene expression in space and/or in time at the scale of an individual and, consequently, modify the phenotype. Epimutations can be the result of stochastic events, i.e., the inability to establish or to maintain the programmed epigenetic pattern through cell multiplication (mitosis). Such epigenetic variation among individuals can lead to the formation of epialleles (Kalisz and Purugganan, 2004). Naturally occurring epialleles have been described, mostly in plants, e.g., *Linaria* floral symmetry (Cubas et al., 1999) and tomato ripening (Manning et al. 2006). More interestingly and in contrast to the genome, the environment can influence the epigenome and lead to epimutations (Jaenisch and Bird, 2003). For example, Pembrey et al. (2006) showed that smoking by teenage human males adversely affected their offspring and grand-offspring, even though the males quit smoking long before they fathered offspring. The consequences of prescribing 5-azacytidine for treating human ailments are particularly insidious because this chemical inhibits maintenance methylation throughout the genome, effectively removing methylation from cytosines. Furthermore, much of the 5-azacytidine is urinated into the water supply, wreaking environmental damage that is currently unregulated (Gorelick, 2005). Epimutations are far more common than DNA mutations over the course of an individual's life span. Numerous examples of the integration of extrinsic signals and the subsequent response of shaping of the epigenome have been observed: temperature (Sheldon et al., 2002), diet (Feil, 2006), and chemicals (Crews et al., 2007) being three examples. Both the stochastic events and the integration of the extrinsic signal represent ways by which the phenotype can be modified without changing the underlying DNA sequence, resulting in more phenotypic plasticity (Angers et al., 2010).

Epigenetic variation can thus occur at different levels and scales: (1) within an individual, (2) among individuals in a population, and (3) between populations.

Epigenetic variation that possibly modifies the phenotype can occur at the scale of an individual (in addition to integration of intrinsic signals related to the developmental program, which technically are also epigenetic signals). The inability to maintain the initial epigenetic state at any given locus in some cells leads to variation among cells of the same tissue. This process is called *variegation*. An example of a variegated phenotype is the *agouti* locus that controls coat color in mice. This system illustrates well the metastability concept of epialleles (Rakyan et al., 2002, 2003). The development of cancer clones is another example of how epigenetic modifications can have phenotypic consequences during the life cycle of an individual.

There will, of course, also be a certain amount of epigenetic variability among individuals of the same population (variable expressivity) (Rakyan et al., 2002). This level of variation is integrative of the stochastic events that occur at the scale of an individual and/or to the effect of the environment. Environmental influence on the shaping of the epigenome is a well-known process (Jaenisch and Bird, 2003). Individuals experiencing similar environmental pressures should have more similar epigenetic profiles, while individuals experiencing different environmental pressures (inhabiting different areas, eating different food, higher exposition to contaminants, etc.) should have more dissimilar epigenetic profiles. As a result, epigenetic variation at the scale of a population is a function of stochastic events and of the heterogeneity of the environment (both in space, allowing individuals inhabiting different parts of an environment to have different epigenetic profiles, and in time, allowing modification of the epigenetic profile of individuals over the course of development).

Epigenetic differentiation of populations will be dependent on heterogeneity of environmental conditions. Two recently separated populations under similar environmental pressures should have low epigenetic differentiation. Over time, these two populations should evolve divergent epigenetic signatures due to experiencing different environmental pressures, as well as epimutations, epigenetic drift, and selection pressure. How should we measure this epigenetic divergence? Genetic tools for measuring differentiation, such as Wright's F statistics, all have heritability of the variation as a premise. The good news is that Wright did not presume the molecular mode of inheritance—DNA versus epigenetic—insofar as population genetics was largely formulated prior to the realization that DNA was the primary carrier of the genetic code (Gorelick and Laubichler, 2008). Therefore, the conceptual apparatus of heritability measurements and the estimation of genetic variation can easily be adopted to quantify epigenetic variation and heritability.

DEFINING GENERATIONS AND INDIVIDUALS • *What Can Epigenetic Reset Tell Us?*

In order to define development or evolution in eukaryotes, one has to demarcate generations, which is ordinarily a seemingly trivial task when the alternation of haploid and diploid generations is bookmarked by meiosis and syngamy. However, how do we define development or evolution in asexual eukaryotes, such as with parthenogenesis (strictly clonal), gynogenesis (where the sperm of parental species is needed only to trigger embryogenesis but sperm DNA is not incorporated into the zygote, possibly with or possibly without egg meiosis), or restitutional automixis (endoreduplication and no syngamy), in all of which meiosis and/or syngamy are lacking? In asexual taxa, we have an intuitive notion of what constitutes generations, especially by comparison with amphimictic sister taxa. Here, we propose that the start of a generation in all multicellular eukaryotes can be demarcated by epigenetic reset, such as of cytosine methylation or chromatin marks, which are clearly needed for development (Santos and Dean, 2004; Gorelick and Carpinone, 2009). Although this might make quantitative geneticists cringe, epigenetic resets can provide a sufficient demarcation of generations to rigorously define heritability in all eukaryotes, including asexual ones. The term *genetic* should be synonymous with *heritable* (a synonymy that we have reluctantly not invoked in this chapter) (Gorelick and Laubichler, 2008); hence, the fidelity of epigenetic reset provides a measure of heritability and, consequently, implicitly provides a definition of generations. Epigenetic reset also defines individuals in such so-called asexual lineages, demarcating the end of one individual and the start of the next. Also, due to the relative fluidity of epigenetic signals, at least when compared with DNA nucleotides, asexual individuals may well possess different epigenetic profiles (variable expressivity).

THE PROBLEM OF DEFINING GENERATIONS AND INDIVIDUALS IN ASEXUAL TAXA

Is epigenetic reset needed to define generations and individuals in asexual taxa? This depends upon how one defines *epigenetic*. Contrary to popular misconceptions (pardon the pun), meiotic females of most taxa do not produce single-celled gametes with only a single haploid nucleus. Furthermore, most (all?) zygotes are not single-celled with a single diploid nucleus and alternation (see next paragraph for details). Moreover, ploidy is fuzzier than usually believed. Thus, we need something other than return to a single-celled stage to demarcate generations in multicellular organisms. Regardless, in multicellular eukaryotes, reset of development from a large multicellular stage to a small unicellular or oligocellular stage would provide a demarcation between generations and individuals; but this is epigenetic *sensu* Waddington and all biologists before the molecular era. Our contribution here is to make this definition of generations and individuals more molecular and, in many ways, more quantifiable.

Instead of demarcating generations by meiosis and syngamy, we could try defining generation by reversion to a single-celled state with either a single haploid or diploid nucleus, as apparently occurs during meiosis and syngamy. However, this single-celled state is often illusory. The product of meiosis in many females is a single cell with either two or four haploid nuclei. See, for example, bisporic and tetrasporic megagametogenesis in angiosperms (Klekowski, 1988). The so-called polar bodies are often not jettisoned or digested until after the sperm fertilizes the egg. In many organisms, such as humans (Austin, 1965), most stages of female meiosis are not even initiated until the egg, which is still really diploid, is fertilized by the sperm. Thus, in human females there is no true haploid phase. Things get even crazier in some plants, such as *Gnetum*, where the egg nucleus is embedded in a single huge cell with thousands of haploid nuclei (Friedman and Carmichael, 1996). However, it cannot be universally true that sperm are needed for female meiosis. Female coral and sea urchins complete meiosis before fertilization (Austin, 1965; Longo, 1973). Moreover, automixis occurs in many parthenogens without sperm. With these parthenogens, does a second egg pronucleus trigger meiosis? While male gametes almost always go through a single-celled stage with a single haploid nucleus, females seldom do. Females are the more fundamental sex, at least if we accept the paradigm that anisogamy evolved from isogamy (Bell, 1978). Thus, for organisms in which males do not exist, we need something other than gametes with single nuclei to demarcate generations.

Contrary to what is in most textbooks, most outcrossing sexual taxa also do not go through a single-celled stage with only a single diploid nucleus. In most organisms, including humans, gametic pronuclei duplicate all haploid chromosomes prior to the two pronuclear envelopes dissolving to form a zygote (Austin, 1965; Gwatkin, 1977; Veeck, 1999; Gorelick and Carpinone, 2009). Thus, in diploid taxa, zygotes have four copies of each homologous chromosome (4C). The first stage in which cells each contain a single diploid nucleus is usually the two-cell stage. For sexual taxa, we could demarcate the start of a generation as the production of a 4C zygote or the subsequent two-celled 2C (diploid) stage.

For the foregoing reasons, we cannot rely on reversion to a single-celled haploid or diploid state to demarcate generations in most organisms, regardless of whether they are sexual or asexual. For sexual (outcrossing) lineages, we can simply demarcate generations by when genetic mixing occurs. How, though, can we demarcate generations for asexual taxa? The answer here depends on the form of asexuality, automixis versus apomixis, i.e., with or without meiosis.

Can we demarcate generations by changes in ploidy? This might provide a surrogate for single-celled stages (Kondrashov, 1994), but, as we discuss in the next three paragraphs, this approach has a few problems. First, it cannot account for endoploidy. Second, it cannot account for lineages that alternate between more than two ploidy levels, i.e., more than just haploid and diploid. Third, and perhaps most fundamentally, ploidy level has no obvious connection with development and epigenesis. We therefore, in the next section, propose an epigenetic demarcation of generations.

Should we define individuals of new ploidy as new individuals and new generations? Does it make sense to define each sperm as a separate haploid individual? What about each egg cell, especially if it never undergoes an unambiguous haploid state? For unicellular taxa, alternation of ploidy may be the best we can do for defining individuals and generations, but we can do better for multicellular taxa. Alternation of haploid and diploid generations seems like a contrived definition of generations, especially with endoploidy rampant in many eukaryotes (Cavalier-Smith, 1995). Human heart and liver cells are highly polyploid (Anatskaya and Vinogradov, 2004), but this should not raise them to the level of another individual (possibly parasitic) in your body. Endoploidy also exists in

invertebrates (Johnston et al., 2004; Mello, 2005). There is some evidence that endoploidy is common in cells with high metabolic demand in animals (Vinogradov et al., 2001; Anatskaya and Vinogradov, 2002), such as cardiac or flight muscles. Endoploidy is also common in plants, although it is not obvious whether ploidy levels are correlated with metabolic demand in plants (De Rocher et al., 1990; Palomino et al., 1999). Nonetheless, changes in ploidy, without other changes, do not seem to warrant the demarcation between two generations or between two individuals.

Endoploidy probably has interesting epigenetic effects in its own right. From a developmental perspective, endoploid cells appear to be terminal, the zenith of ontogeny. At least in animals, endoploid cells are usually (always?) highly specialized and probably incapable of further mitotic or meiotic divisions. From a molecular perspective, we suspect that endoploid cells have a disproportionate number of their regulatory loci methylated and are highly heterochromatic.

How do we define generations and individuals in taxa that, instead of alternating only between haploid and diploid stages (for the moment, ignoring how fuzzy these stages are, especially for female gametes), cycle between haploid, diploid, tetraploid, and perhaps higher ploidies and then undergo the decreasing cavalcade of ploidies: tetraploid, diploid, haploid? This occurs in some members of the genera *Polysiphonia* (red alga), *Ectocarpus* (brown alga), *Pyrsonympha* (Excavata, oxymonad), and *Giardia* (Excavata, diplomonad), some of which even have higher ploidy levels in this cycle (Müller, 1967; Hollande and Carruette-Valentin, 1970; Goff and Coleman, 1986). Each change in ploidy should constitute a new generation if and only if there is an associated epigenetic reset. We do not have a good answer here if there appears to be one extended epigenetic rest spanning all increases in ploidy and a second extended epigenetic reset spanning all decreases in ploidy (cf. Davis et al., 2000; Farthing et al., 2008).

EPIGENETIC DEMARCATION OF GENERATIONS

We propose that the crux of what constitutes a new generation and a new individual is epigenetic reset. Heuristically, we want to demarcate a generation by the *abrupt shift* from (1) a huge, complex, multicellular organism with many specialized cell types, many of which are incapable of further cell divisions, to (2) a small, unicellular, or oligocellular organism, with one type of totipotent cell. The one or two totipotent cells then divide mitotically, *gradually differentiating* into the complex multicellular individual. This is classic epigenesis. Notice that this heuristic definition does not mention ploidy levels. While some taxa, such as bryophytes, pteridophytes, and many algae, have complex multicellular haploid and diploid stages; others, such as most animals, do not. Thus, we are left with the uncomfortable situation that gametes and gametophytes may or may not be considered separate haploid individuals. This seems peculiar insofar as many of these haploid entities undergo extensive development. Even animal sperm undergo extensive development, from a prototypical spherical cell to an elongated cell virtually devoid of cytoplasm. We thus need a more precise—less heuristic—definition of epigenetic reset to demarcate individuals and generations.

We thus propose that epigenetic reset of various molecular epigenetic signals provides a definitive demarcation of individuals and generations in all eukaryotes, including autogamic, automictic, and apomictic taxa. We simply have to plot a time series of some molecular epigenetic signal and then use time series methods to detect discrete jumps in the signal. Epigenetic signals, such as cytosine methylation and chromatin modification, are known to take discrete jumps during or immediately following meiosis and syngamy in outcrossing sexual taxa (El-Maarri et al., 2001; Santos and Dean, 2004; Ruiz-García et al., 2005). Why not use discrete jumps in these same molecular epigenetic signals to define individuals and generations in self-sexual and asexual taxa? Why not use these signals to determine whether endoploid tissues

constitute a separate (parasitic) individual, albeit one that is an evolutionary dead end? Why not use these signals to determine whether the 1N-2N-4N-8N-16N-8N-4N-2N-1N ploidy cycle is composed of eight generations? Or is it fewer? For unicellular eukaryotes, do molecular epigenetic signals allow us to define individuals and generations despite a lack of development in either haploid or diploid stage? Why not use these signals to determine whether there are interesting asymmetries between female and male gametes, possibly providing clues as to causes of differential genomic imprinting? While we do not believe that such inquiries should influence existing abortion and contraception debates, it would be fascinating if these signals helped to inform us whether egg or sperm cells are ever really individuals.

Epigenetic reset provides virtually the same demarcation of generations as existed for outcrossing sexual organisms that used genetic mixing as the demarcation. The epigenetic reset demarcation provides the same number of generations, although the timing of the start of generations may be slightly different from when genetic mixing occurs. In outcrossing sexual taxa, molecular epigenetic signals are usually thought to have a sawtooth pattern over multiple generations, with the period of the signal being one generation (Gorelick and Carpinone, 2009). Over the course of diploid development, the frequency of a molecular epigenetic signal over all or a portion of the genome (epigenome) changes gradually and monotonically. For example, telomeres gradually degrade, while cytosine methylation at regulatory loci gradually increases. There is a rapid (albeit not instantaneous) shift in these epigenetic signals during gametogenesis (Farthing et al., 2008). This epigenetic reset is a return to levels that existed at the start of the previous haploid generation but not necessarily to levels at the start of the diploid generation. Molecular epigenetic changes probably then change gradually and monotonically over the course of haploid development, although data are not available to corroborate this. However, during or immediately following syngamy, there is a second rapid shift in these epigenetic signals to those levels that existed at the start of the previous diploid generation. Outcrossing sexual taxa have two distinct types of generation, haploid and diploid. Over the course of a generation, the time history of molecular epigenetic signals in outcrossing sexual eukaryotes undergoes two gradual monotonic periods, interspersed with two discrete jumps, with the pattern repeating in subsequent generations.

In the hypothetical situation in which all epigenetic variation (marks associated with the developmental program and epimutations) is heritable, the offspring epigenome would be identical to the parental epigenome and strict sense heritability would be equal to one. This situation is highly improbable because the epigenetic reset is needed to initiate proper development of individuals of the next generation, at least in mammals. As a result, most of the epigenetic marks are cleared each generation. However, the epigenetic marks associated with developmental programs are reestablished each generation with a high fidelity to enable proper development of organisms (epigenetic signals associated with developmental programs are highly heritable because they are genetically determined, i.e., obligate epigenetic variation). Furthermore, the epigenetic resets of meiosis and syngamy might not be perfect, allowing transgenerational epigenetic inheritance of some epimutations, thus leading to a certain level of additive epigenetic variation. If both epigenetic resets were perfect, we would expect to see no facilitated or pure epigenetic component of additive genetic variance because all changes in epigenetic signatures would be reset (assuming the low probability of the appearance of the same epimutation in the next generation). Although the epigenetic mark itself might not be present in the germ line, some particular molecular mechanisms have been proposed to lead to faithful reestablishment of the marks by passing on small interfering RNA via the cytoplasm of gametes (Chandler, 2007). There is thus a tension or trade-off between the two

parts of this chapter: Epigenetic resets make transgenerational inheritance of epimutations more difficult, but epigenetic reset allows demarcation of generations and individuals. We can have our cake and eat it too because epigenetic reset can sometimes be imperfect, although exactly how imperfect is an outstanding empirical question that may vary across taxa, as we discuss in the next section. Among individuals, epigenetic variation also helps to define individuals, as we discuss at the end of this chapter.

Notice that epigenetic reset demarcates generations even in obligately self-fertilizing lineages, for which there is never genetic mixing. This includes lineages with autogamy, complete automixis, and restitutional automixis. The question, however, remains whether an epigenetic reset demarcation of generations works for forms of ploidy cycling lacking any evidence of meiosis, such as the putatively apomictic gynogenetic fishes like *Poecilia* and *Chromosomus* [*Phoxinus*] *eos-neogaeus*. For apomictic gynogens, sperm somehow seem to provide the epigenetic signal that resets development and presumably also resets cytosine methylation signatures.

Because epigenetic resets are imperfect, time series analysis may be needed to detect these resets. Time series may also be needed because epigenetic resets are not instantaneous. Continuous periodic time histories are often analyzed with Fourier series. Sawtooth waves, however, are a mix of discrete and continuous variables. Therefore, the orthogonal basis used to estimate the molecular epigenetic signals should ideally contain discrete and continuous functions, e.g., sinusoids and step functions. We therefore suggest using Walsh-Hadamard series to estimate where the discrete jumps occur, which demarcate generations (Elliott and Rao, 1982).

Asexual or self-sexual individuals may not undergo meiosis and/or syngamy but should still undergo molecular epigenetic resets once or twice each generation. Otherwise, they will have no way of resetting diploid and/or haploid development each generation. Taxa with autogamy or complete automixis should undergo two molecular epigenetic resets each generation because they still undergo both meiosis and syngamy. With many instances of parthenogenesis, standard meiosis almost certainly occurs after premeiotic endomitosis; there is no syngamy and, thus, only one epigenetic reset (Dawley, 1989; Gorelick & Carpinone 2009). Apomictic gynogenesis is unusual insofar as the epigenetic reset is initiated by something other than meiosis and syngamy. Only one reset is important, the one before the initiation of the developmental program. The sperm that triggers development may serve as the signal for the epigenetic reset, although the sperm genome (i.e., sperm nuclear DNA) is not incorporated. Taxa with restitutional automixis, such as lumbricid earthworms (e.g., *Octolasion cyaneum*) and garlic chives (*Allium tuberosum*), may undergo only one molecular epigenetic reset each generation because they have meiosis but no syngamy. Their endomitotic duplication resembles that of endoploidy in outcrossing sexual taxa and, therefore, may not count as a generation. Using the demarcation of epigenetic reset, we may be unable to distinguish haploid and diploid generations in taxa with restitutional automixis.

We should also point out that there has been contemporary debate about when generations start in amphimictic (outcrossing) lineages, especially in humans, largely due to political tensions between in vitro fertilization researchers and antiabortion activists (Spallone, 1996). The terms *conceptus* and *preembryo* describe the state between fertilization and blastocyst (aka embryo) stage, with the subtle message that generations and individuals are not clearly demarcated in humans. Preembryos are composed of many cells that will not form the next generation but instead contribute to the placenta. Preembryos can be split into monozygotic twins and, hence, represent an indeterminate number of individuals. Just as epigenetic signals are not reset instantaneously, epigenesis from a fertilized egg to a blastocyst with a primitive streak also does not happen instantaneously.

Parasex refers to life cycles with syngamy but without a traditional reduction division. Instead, these organisms go from a diploid to a haploid state by jettisoning one homologous chromosome at a time, going through a succession of aneuploid states until only one copy of each homologue remains (Pontecorvo, 1956). Recent work shows that this process is a highly modified form of meiosis (Forche et al., 2008). However, it is not obvious whether epigenetic reset occurs during or following these successive aneuploid events. Thus, the epigenetic time history of parasexual lineages may be like restitutional automicts (one epigenetic reset per generation) or like amphimicts and complete automicts (two epigenetic resets per generation). Nevertheless, we should still be able to use epigenetic resets to demarcate generations in the few lineages of fungi that are parasexual.

Other than with apomictic gynogenesis, in which sperm pronuclei (products of meiosis!) trigger epigenetic reset, we hypothesize that *obligate* apomictic reproduction does not exist in multicellular eukaryotes because there is nothing akin to meiosis or syngamy to induce the needed epigenetic resets. This needs to be tested in two parallel ways. First, we need to look for cryptic meiosis in putatively obligately apomictic lineages (Solari, 2002). Second, we need to see whether epigenetic reset occurs each generation in these lineages. By epigenetic reset, we refer to the molecular signals, such as cytosine methylation, because it is already obvious that developmental reset *sensu* Waddington (1940, 1957) occurs.

Having defined generations for asexual organisms, conventional definitions of heritability apply. We can apply parent–offspring regression or full-sib (but not half-sib) analysis.

DEFINING INDIVIDUALS AND HERITABILITY

Once we define generations for asexual taxa, it becomes easier to define individuals. If two organisms are separated by an epigenetic reset, i.e., a generation, then they must be different individuals. The only question remaining is whether two individuals in the same generation are the same individual (Smith et al., 1992; Scrosati, 2002). Here, we need to rely again on epigenetic variation, as we did in defining asexual species. Epigenetic reset is not perfect and is, in fact, less heritable than DNA nucleotides. There are small stochastic differences in epigenetic reset from generation to generation as well as between individuals within a generation. In meiotic taxa, each gamete should have slightly different molecular epigenetic resets, allowing us to distinguish individuals even if these are not outcrossing taxa, as with automixis or autogamy. If eukaryotes still exist without meiosis, there will probably still be molecular epigenetic reset occurring during ploidy cycling. Stochastic differences in this epigenetic reset should allow us to distinguish individuals following ploidy cycling. If two organisms differ in their epigenetic marks at more than a certain number of loci, then call the two organisms different individuals. If the two differ in fewer than that predetermined number of epigenetic loci, then say that they are clones, i.e., the same individual. The only thing arbitrary about this definition is setting the threshold number of epigenetic loci that differ. The same tack has been taken to define prokaryotic species (Moreno, 1997; Stackebrandt et al., 2002).

Having rigorously defined generations and individuals for obligately asexual lineages, defining heritabilities of any signals is now trivial, whether the signals or traits are ultimately caused by DNA nucleotides, epigenetic marks, or both. Simply apply Lush's classic definition of heritability: additive genetic variance divided by phenotypic variance (Lush, 1937). Being able to demarcate individuals and generations allows us to apply conventional quantitative genetic techniques, such as parent–offspring and full-sib analysis, as well as more sophisticated methods, such as restricted maximum likelihood (Knott et al., 1995). The measured phenotype can be anything from color of a maize kernel or *agouti* mouse to amount of cytosine methylation present on a stretch of DNA.

CONCLUDING REMARKS

Asexual lineages can illuminate the relative importance of epigenetic signals in evolution. Conversely, epigenetic signals can be used to define generations, individuals, and even species in asexual lineages. How well we can accomplish both of these goals depends on the extent of epigenetic variation in natural populations, and we still have much work to do in order to evaluate this, including how imperfect epigenetic reset is during meiosis, syngamy, or more generally ploidy cycling.

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