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Mitosis circumscribes individuals; sex creates new individuals

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Abstract Many aspects of biology, such as population genetics and senescence, are predicated on identifying individuals and generations. Conventional demarcations of individuals and generations, such as physiological autonomy, unicellular bottlenecks, and alternation of generation, are rife with problems. Do physically separated cuttings or plant ramets constitute separate individuals or generations? Are chimaeras one or more individuals? To resolve these problems, Clarke (Biol Philos 27(3): 321–361, 2012) proposed that individuals are circumscribed by mechanisms that constrain heritable variance in fitness. Simultaneously, Gorelick and Heng (Evolution 65(4): 1088–1098 2011) showed that sex constrains heritable variance Therefore, for eukaryotes, meiosis and karyogamy provide a consistent way to demarcate individuals and generations. Epigenetic reset associated with meiosis and karyogamy rejuvenates the next generation, but not the parent(s) that engaged in the sex act. Wholesale epigenetic resets that probably only occur with meiosis and karyogamy imply that monozygotic twins are two different individuals, but apomictic progeny are diffuse parts of one disaggregated individual. Mitotic heritability circumscribes an individual, whereas meiotic heritability demarcates new individuals and generations.

Keywords Meiosis, meiotic · Karyogamy · Epigenetic · Monozygotic twin · Endoploid, endomitosis · Chimaera, chimera, chimerism, chimaerism

Introduction

In many areas of organismal biology, such as population genetics and population biology, it is crucial to discern what constitutes an individual or generation (Clarke

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2010). If we cannot demarcate individuals and generations in a systematic fashion, then our science can be questioned (Santelices 1999).

Do clones constitute a single or multiple individuals? While we have some conception of what constitutes distinct individuals for humans, what about cuttings from a wine grape, chopped up pieces of a starfish, asexual progeny of aphids in summer, or thousands of trunks of a square kilometer clone of quaking aspen? Is there a difference between number of individuals in a litter of nine-banded armadillos (monozygotic quadruplets), a litter of Wied's marmosets (dizygotic chimaeras), and a clutch of parthenogenetic whiptail lizards (complete automicts)? When are individuals considered to be members of the same generation? Are two seedlings from the same generation if they are from the same two parents of western bristlecone pine, but the seeds were produced five thousand years apart? If I am a clone of my mother, then am I my mother's sister or her child? Are all sperm in an ejaculate separate haploid individuals or part of the diploid individual that produced them? My goal is to answer these questions solely in the context of evolution and ecology, using sex—by which I mean meiosis and karyogamy, along with the associated wholesale epigenetic reset—to demarcate separate individuals and generations.

The following broad questions motivate my definition of individuality. (1) What are evolutionary trajectories in population genetics and what generates heritable variation? To measure heritability, we must know whether two individuals are the same or different and whether are they are in the same generation (e.g. siblings) or instead are parent and offspring. To understand genetic drift, we must know effective population size. (2) Can we model and predict senescence? Senescence occurs in an individual, so it is crucial to know who the individual is. (3) How can we quantify biodiversity using abundance data? Should a clonal plant count as one or more individuals in demographic studies?

Unlike many previous works on circumscribing individuals, I am not asking how physiological autonomy evolved, but instead provide a useful new definition of individuals. Quality of a definition is gauged by its utility and consistency of meaning and connotation across many contexts (Wagner 2010; Gorelick 2011). The definition of individuality herein is consistent with modern views on levels of selection and the resulting theory consistent with modern views on senescence and population genetics. A few of the implications are also counter-intuitive.

I propose that meiosis and karyogamy, along with the associated wholesale epigenetic reset, provide a demarcation of individuals and generations. First, I present traditional demarcations of individuals and generations, highlighting their foibles and the value-added in replacing these with a definition based on meiosis, karyogamy, and epigenetic reset. Second, I borrow from philosophy of biology and levels of selection to define individuals by "identifying mechanisms which constrain the extent to which populations of biological units exhibit heritable variance in fitness" (Clarke 2012: 338–339). I then show how this implies that we should demarcate individuals with sex, which may mean self sex. Third, I show how this definition implies that monozygotic twins are separate individuals, but that chimaeric and dikaryotic autonomous entities constitute multiple individuals in one body. However, this requires examination of wholesale epigenetic reset, not just

meiosis and karyogamy. Fourth, I discuss implications of circumscribing individuals by mitotic heritability and demarcating new individuals and generations by meiosis and karyogamy, such as population genetics of apomictic taxa, individuals not needing to be spatially or temporally localized (Janzen 1977), and measuring beta-diversity.

Problems with traditional demarcations of individuals and generations

Traditional methods for demarcating individuals and generations include (1) genetic uniqueness and homogeneity (Santelices 1999), (2) physiological autonomy and unity (Buss 1987; Pradeu 2010), as well as the related notion of levels of selection (Maynard Smith and Szathmáry 1995), (3) alternation of generations, and (4) return to a single-celled state ('unicellular bottlenecks'). In this section, I describe problems with these demarcations, but reserve discussion of levels of selection because I want to build upon it, as well as build upon epigenetic similarity. See Clarke (2010) for additional demarcations of individuals and critiques thereof.

Genetic similarity

Demarcating individuals by genetic similarity is problematic due to clonality. Is a clonal patch of quaking aspen (*Populus tremuloides*) one individual? Monozygotic twins are 'genetically identical' except for mutations, so would constitute one individual. There are also difficulties if we use genetic homogeneity, rather than genetic uniqueness. Relying on genetic homogeneity, the progeny of obligately self-fertilising organisms are much more likely to constitute a single (disaggregated) individual than offspring of obligately outcrossing sister taxa. Carrying this argument further, offspring of highly outbred parents are more likely to be considered separate individuals than offspring of highly inbred parents. There are however some appealing aspects of using epigenetic homogeneity to demarcate individuals, which I consider below.

Physiological autonomy

Physiological autonomy refers to any subset of an organism that can survive and reproduce on its own, which is an arbitrary construct because no organism can survive without its biotic environment. Physiological autonomy and unity do not make for decent demarcations of individuals because of eusocial insects (Hymenoptera; Isoptera) and naked mole rats (*Heterocephalus glaber*), *inter alia*. Eusociality indicates that the physiologically autonomous unit is the colony, not reproductive or non-reproductive colony members. Even if a eusocial colony contains multiple mating females and males, the population size, not just effective population size, would be one. Taking this argument to an extreme, species have been considered a single disaggregated individual (Hull 1976; Shcherbakov 2010). In the opposite direction, plant cuttings are counter-examples to physiological autonomy circumscribing individuals. Plant stems may seem physiologically dependent on roots and

leaves. But, because of copious ground tissue and latent meristems, detached stems can grow roots and leaves and thereby become physiologically independent.

Not only is physiological autonomy a poor demarcation for individuals because of disaggregation of putative individuals, but also because of the reverse, aggregation of putative individuals. Genetically unique individuals often graft with other genetically unique conspecifics, forming chimaeras. Conspecific root grafts are the norm in angiosperm and conifer trees (Basnet et al. 1993; Suzuki and Grady 2004; Lanner 2007). More confounding, the highly clonal *Populus tremuloides* has extensive root grafting between different clones (Jelinkova et al. 2009). Heterospecific grafting of trees with phosphorus-providing mycorrhizal fungi and nitrogen-fixing eubacteria can be advantageous, but does this affect individuality? Humans form conspecific chimaeras via placental exchange of maternal and fetal cells (Evans et al. 1999) and exchange of cells between dizygotic twins (Ross and Thomas 1978; Rubocki et al. 2001). Furthermore humans are comprised of about 10 % of their own cells and 90 % of microbial cells. An extreme form of conspecific (actually, sibling) chimaerism occurs in Wied's marmoset, *Callithrix kuhllii*, whose somatic and germ-line tissues are composed of cells derived from mitotic divisions of multiple zygotes (Ross et al. 2007). Chimaerism, especially if advantageous, means that physiological autonomy and genetic homogeneity yield conflicting demaractions of an individual.

Chimaeric portions of algal and angiosperm populations can have higher fitness than genetically pure putative individuals (Basnet et al. 1993; Santelices et al. 1996), partly debunking Grosberg and Strathmann's (2007) notion that defectors amongst the chiaemeric constituents provide fitness disadvantages and thereby render the chimaera something other than an individual. Grafted chimaeric gymnosperm roots and conspecific microchimaerism in humans may also confer fitness advantages (Suzuki and Grady 2004; Boyon et al. 2011).

Conjoined twins, especially when dizygotic, provide a vexing case for using physiologically autonomy to demarcate individuals. Separating conjoined human twins is often fatal, rendering twins a single physiological unit. Yet, they are really no different than grafted roots of conspecific plants, which can usually be separated without fatality. In both instances, separation decreases fitness, but with conjoined humans the fitness decrease is more extreme.

Symbioses provide problems with using immunological responses to define individuals (Pradeu 2010, 2011). If a host does not immunologically reject cells of different origin that are needed for survival of the host, then Pradeu considered those cells to be part of the host individual. These could be photoluminescent bacteria in light organs of cephalopods, gut bacteria in animals, nitrogen-fixing bacteria or phosphorus-absorbing fungi in plants. Conversely, if the host has immunological reactions to these other cells, then Pradeu argued that they should not be considered part of the individual. But, if a human has an auto-immunological disease, are they then multiple individuals, whereas a healthy human is only a single individual? With vertical transmission of symbionts from a parental host to its offspring, do offspring constitute the same individual as their parent? I therefore consider microbial symbionts to be equivalent to any other chimaeric cell, regardless of whether the foreign cell is conspecific with the host.

Chiamaeras and symbioses show that individuals depend on each other, including across species. Humans depend on many other species for food, photosynthetic organisms to generate oxygen, and microorganisms to recycle our wastes. Physiological autonomy cannot exist.

Unicellular bottlenecks

For many taxa, neither haploid nor diploid states go through a single-celled haploid or diploid stage, hence there is no unicellular bottleneck each generation sensu Grosberg and Strathmann (2007). Consequently return to unicellularity cannot be used to demarcate generations. Females usually do not have a single-celled haploid state. In almost all animals, egg meiosis is arrested and only completed after plasmogamy by one or more sperm (Austin 1965; Asch et al. 1995). Furthermore, polar bodies are sometimes not ejected. A mature metazoan egg cell almost always contains one mature haploid egg nucleus, one or more sperm cells, and sometimes one or mature polar bodies. In animals, usually only males go through a single-celled haploid stage (Austin 1965; Gorelick 2012). Likewise, there is no single-celled diploid stage because both egg and sperm nuclei/pronuclei undergo an endomitotic division immediately prior to karyogamy (Schatten 1994; Veeck 1999; Gorelick and Carpinone 2009). The zygote has 4C, not 2C, chromosomal content, and therefore the first division is a cleavage (not mitotic) division to form a pair of diploid cells. Plants are even more extreme in lacking a single-celled stage. Angiosperm pollen tubes retain both sperm cells until the moment of double fertilisation (Williams 2008). Angiosperm female haploid stages (gametophytes) can be monosporic, bisporic, or tetrasporic (Klekowski 1988; Friedman and Ryerson 2009), i.e. angiosperm female gametophytes can be formed from one, two or all four of the products of meiosis. The gymnosperm *Gnetum gnemon* has a female gametophyte with a haploid egg nucleus embedded in a cell containing thousands of other haploid nuclei that arose from all four products of meiosis (Friedman and Carmichael 1996). Maybe we could demarcate generations by return to a haploid or diploid state with only a few cells or nuclei (how many is a few?), but this is essentially demarcating generations by epigenetic reset, a reset to a simpler developmental state with fewer cell types and less complexity.

Alternation of generations

Alternation of generations of sexual eukaryotes is predicated on one generation being defined by diploidy, the subsequent generation by haploidy via meiotic reduction division, and finally a return to diploidy (Bower 1890, 1935). Return to diploidy is usually via karyogamy, but could instead be via endoploidy, including premeiotic doubling (Kondrashov 1994; Gorelick and Carpinone 2009; Stenberg and Saura 2009). By demarcating generations, we are also demarcating individuals—a member of the next generation is a different individual from members of the previous generation. The biggest problem with using alternation of generations to demarcate individuals is endoploidy, also known as endomitosis, endoreduplication, and somatic polyploidy, and sometimes included under the umbrella of genetic

homogeneity. Even excluding gonadal tissues, most putative individuals are composed of cells with differing ploidy levels, almost always multiples of the base diploid number of chromosomes. Mature human red blood cells and plant phloem sieve cells have no chromosomes. Many 'diploid' animal and plant tissues contain cells that have duplicated their chromosomes (S phase), sometimes repeatedly, without completing a mitotic nuclear division. In animals, cells with high metabolic demand, such as muscle and liver cells are highly endoploid (Cifuentes-Diaz et al. 1991; Anatskaya and Vinogradov 2004; Johnston et al. 2004). Plants have lots of endoploidy, especially succulent tissues (Lukaszewska and Sliwinska 2007; Breuer et al. 2010). If changes in ploidy demarcate a new generation, then our muscle and liver cells are a different generation and a different individual than the rest of us, which seems like nonsense. By contrast, I have no problem saying that my gametes are a part of the next generation.

We could excuse endoploidy in 'diploid' tissues if such cells and their mitotic daughter cells do not undergo meiosis. Endoploid cells seem like terminal development stages and, in animals, are almost always somatic cells. More problematic are plants if their endoploid cells can undergo meiosis, possibly following several intervening mitotic divisions. Most troubling are eukaryotes in which virtually all cells cycle between multiple ploidies, not just between haploid and diploid. Instead of the standard 1N → 2N → 1N haploid-diploid ploidy cycle, species could routinely go through the following ploidy cycle: 1N → 2N → 4N → 8N → 16N → 8N → 4N → 2N → 1N (Hollande and Carruette-Valentin 1970; Goff and Coleman 1986). In such instances, it is still unknown whether increases in ploidy are due to endoploidy or karyogamy, although I expect endoploidy. It is often unknown whether the decreases in ploidy are due to meiosis. However, in the 1N → 2N → 4N → 2N → 1N ploidy cycle of the brown alga *Ectocarpus siliculosus*, the two reduction divisions are meiotic (Müller 1967), hence different ploidy levels (haploid, diploid, tetraploid) represent different generations with different individuals. Given that only meiosis and karyogamy have associated reset of development and epigenetic signals (Gorelick and Carpinone 2009), changes in ploidy due to endoploidy or non-meiotic parasex (gradual expulsion of one of each pair of homologous chromosomes, without meiosis, if such a process exists) should not be demarcated as new generations and individuals.

Core argument: meiosis demarcates individuals; mitosis circumscribes individuals

Many advances in understanding individuality from an evolutionary perspective arose from studying levels of selection (Ghiselin 1974; Hull 1976, 1992; Maynard Smith and Szathmáry 1995; Okasha 2006). Dawkins' (1982) *Extended Phenotype* took that tack, even if you do not believe that genes are the primary unit of selection. Buss' (1987) *The Evolution of Individuality* is almost exclusively about levels of selection and is especially important due to his work on placozoa, possibly the most basal extant animal. Similarly, Michod has examined incipient individuality and keeps refining notions of "evolutionary transitions in individuality" (e.g. Shelton

and Michod 2010). It seems prudent to continue with this successful line of inquiry, that individuality has something to do with transitions from one level of selection to another.

Ellen Clarke (2012: 338–339), a philosopher of biology, stated that individuals can be circumscribed by "identifying mechanisms which constrain the extent to which populations of biological units exhibit heritable variance in fitness". Clarke's circumscription, which I adopt herein, is compatible with work on units of selection insofar as selection severely minimizes heritable variation by eliminating all individuals except those whose phenotypes confer high fitness.

At what level does the majority of heritable variation exist in eukaryotes? Conventional wisdom dating back to Weismann (1891 [1892]) is that sex is the primary source of heritable variation, a conclusion Weismann reached to rescue Darwin's mechanism of selection, which had no variation upon which to act. Per Weismann (1891 [1892]) and Clarke (2012), sex demarcates new individuals because supposedly mitosis results in almost no heritable variance, but there is a step-wise transition to increased heritable variance with sex.

However, we recently demonstrated that Weismann was wrong and, instead, meiosis *decreases* heritable variance (Gorelick and Heng 2011), whereas repeated mitotic divisions *increase heritable variance* (Heng et al. 2011). I therefore proffer that an individual is comprised of all cells that are products of repeated mitotic division from a single cell that was itself the product of a meiotic division or karyogamic fusion. Meiosis reduces heritable variance, hence delineates the start of a new individual. Meiosis provides the evolutionary transition in individuality, but due to a decrease rather than an increase in heritable variance. Clarke (2012) and I both rely on transitions to different hierarchal levels to identify individuals, but we differ on which constrains heritable variance—mitosis or meiosis—thereby leading us to different circumscriptions of individuals.

Karyogamy is probably a modified form of meiosis. Just like meiosis, karyogamy starts with a chromosomal duplication, continues with an epigenetic reset, and finishes with a reduction division (Gorelick and Carpinone 2009). Therefore, if meiosis demarcates new individuals and generations, so will karyogamy. Karyogamy hardly increases heritable variation because genetic mixing results in decreased variance in a population (Galton 1886). Averaging decreases variance. Furthermore, mating parents are of the same species and are usually from one highly inbred population (Shields 1982).

To see how mitosis results in increased variation, examine development any multicellular eukaryote. Early embryos are comprised of undifferentiated cells that look alike. As embryos develop via mitotic divisions, cells differentiate into specialised types, such as muscle, nerve, and endocrine cells that not only have different epigenetic signatures, but often have large amounts of chromosomal variation, such as endoploidy. Offspring are less different from their parents than nerve cells are different from liver cells within a single individual. Cancer cells are the quintessential place to see mitotic variation (Heng et al. 2011). Cancer biologists often consider each cell to be an individual and thereby discuss 'mitotic heritability' (Heng et al. 2010). High mitotic variability means low mitotic heritability. By contrast, for meiosis and karyogamy, offspring closely resemble their parents.

Diploid offspring resemble their diploid parents; haploid offspring resemble their haploid parents. This resemblance is especially evident for organisms with complete automixes, i.e. organisms in which females undergo meiosis to produce four haploid egg nuclei, two of which immediately fuse with one another (karyogamy) to restore diploidy. Low meiotic variation between generations translates into high (meiotic) heritability. Thus, mitosis is the unit at whose level the majority of genetic variance occurs.

Subsidiary argument: sex creates rejuvenated individuals and 'cures' senescence

Sex originally referred to fertilisation because that was all we knew about prior to the late 1800s. Details of meiosis, vis-à-vis reduction division and associated rejuvenescence, were first elucidated in 1890 (Hertwig 1890; Maupas 1890). I use the word 'sex' to refer to both meiosis and karyogamy (not plasmogamy) because karyogamy is probably a modified form of meiosis (Gorelick and Carpinone 2009). The basic argument for sex demarcating individuals and generations goes back centuries, although the first modern rendition was Émile Maupas's (1886, 1889, 1890) notion of *réjeunissement* [rejuvenescence], which is a reversal of senescence. Sex creates new individuals that are rejuvenated via epigenetic reset compared with the parent or parents that just created them. To study the evolutionary basis for senescence, we must be able to define individuals and generations.

Sex does not rejuvenate the diploid individuals who engaged in the sex act, but instead usually weakens individuals—*la petit mort*—or brings on death of semelparous organisms (Gorelick and Carpinone 2009). Sex between diploid organisms is a dangerous act: consider sexually transmitted infections or desiccation of desert plants when in flower. The act of meiosis obliterates genetic identity. "Gametogenesis with chromosome-reductions, accompanied by reformations and, as it were, partial rejuvenescence of cell-structures, must in some way act as if especially organized for *obliterating* the individual's personally 'acquired characters,' which as a rule totally disappear in sexual reproduction" (Johannsen 1923: 133; italics in original). Or, as G. C. Williams (1966: 22) more succinctly stated, "meiosis and recombination destroy genotypes as surely as death." Or, even more simply, sex equals death. The fate of haploid organisms engaging in amphimictic karyogamy is even more definitive because they lose their genetic identity. Regardless of whether we focus on sex between diploid or haploid individuals, sex only rejuvenates the next generation, not the current one. Meiosis is death (end of individual and generation) of diploid organisms. Karyogamy is death (end of individual and generation) of haploid organisms. Because obligately apomictic lineages (if they exist) do not undergo meiosis or karyogamy, individuals do not die, but there is only a single possibly large disaggregated individual that may live for one very long generation.

The *sine qua non* of sex is resetting development, taking a complex, specialised and possibly multicellular organism and transforming it into a simple, totipotent organism comprised of one or at most a few cells (Robert 2004; Gilbert 2006;

Gorelick and Carpinone 2009). Meiosis takes a trillion-celled diploid human and ostensibly distills them down to a single-celled product of meiosis (Griesemer 2002). Karyogamy also takes complex organisms to create a simple new generation: mature eggs and sperm are complex and highly specialized cells compared with the resulting totipotent zygote. The immediate products of metazoan male meiosis is almost always four spherical haploid cells that only later develop their highly asymmetrical morphology with a head and flagellated tail. It should not be surprising that karyogamy converts complex entities to simpler ones because karyogamy is probably a modified form of meiosis (Gorelick and Carpinone 2009).

All sexual eukaryotes undergo an epigenetic reset associated with meiosis and another associated with karyogamy (Farthing et al. 2008; Ünal et al. 2011). Epigenetic refers to both classic development and to molecular signals, such as cytosine methylation, histone modification, heterochromatisation, and rebuilding of telomeres. In terms of development, both meiosis and karyogamy convert complex, possibly multicellular organisms with specialization, to simple unicellular or oligocellular organism without specialization (Robert 2004). In terms of molecular epigenetic signals, the patterns that existed at the start of haploid or diploid stages of the previous generation are largely re-established (Farthing et al. 2008; Gorelick and Carpinone 2009). At or around the time of meiosis and karyogamy are the only times that wholesale (global) changes are made to molecular epigenetic signals. During haploid or diploid development—i.e. during mitotic divisions—epigenetic signatures change much more gradually, via a combination of unidirectional programmed changes and stochastic events mediated by environmental exigencies (Gorelick 2004, 2005; Angers et al. 2010).

For the many eukaryotic organisms that alternate meiosis and endomitosis, rather than karyogamy, I make no distinction between haploid and diploid individuals. Because there does not appear to be any reduction in heritable variance during endoploid events, restoration of diploidy does not demarcate a new individual. There does not appear to be a wholesale epigenetic reset associated with endoploidy/endomitosis in premeiotic doubling. Organisms that alternate meiosis with endoploidy include lumbricid earthworms (Lumbricidae) and garlic chives (*Allium tuberosum*).

It is impossible to identify senescence if we cannot identify individuals or generations. Senescence is the flip side of rejuvenescence. Why do some plants like western bristlecone pine (*Pinus longaeva*) seem to senesce much slower than related species (Lanner and Connor 2001; Flanary and Kletetschka 2005)? Do clonal organisms senesce, such as fairy-rings of creosote (*Larrea tridentata*) that supposedly can get to 10,000 years old (Vasek 1980), the recently discovered and purportedly 13,000 year old clone of Palmer's oak (*Quercus palmeri*) (May et al. 2009), the single disaggregated clone of king lomatia (*Lomatia tasmanica*) that is supposedly 44,000 years old (Lynch et al. 1998), or clones of quaking aspen (*Populus tremuloides*) that can purportedly be up to 80,000 years old (Grant and Mitton 2010)? Which ages: ramets, genets, or something else? Does the Hayflick limit (finite number of mitotic divisions without an intervening meiotic division; Hayflick and Moorhead 1961) apply equally to spatially intact and disaggregated individuals (see next section), such as siphonophores and duckweeds (Lemnoideae;

Araceae) (Haeckel 1862–1868; Vuorisalo and Tuomi 1986; Hull 1992; Dunn 2005; Dunn and Wagner 2006)? There is limited reset of cytosine methylation not associated with either meiosis or karyogamy in differentiation of human red blood cells (Shearstone et al. 2011), but this may be a very special case, especially because human red blood cells are not mitotic, losing all their DNA when mature. Many aspects of gerontology focus on telomere degradation (e.g. Bacchetti 1996; Harley 1997; Blackburn 2000; Tollefsbol and Andrews 2001; Flanary and Kletetschka 2005). Meiosis and karygamy are the only ways to reset telomeres, especially the cytosine methylation that provides for their protective role (Howard 1996; Lushai and Loxdale 2007; Zechner et al. 2009). It will be impossible to understand ageing until we have a cogent definition/demarcation of individuals.

Which demarcation of individuals is easier to use, meiosis/karyogamy or epigenetic reset?

Given that wholesale epigenetic reset probably only occurs with meiosis and karyogamy (Gorelick and Carpinone 2009), epigenetic reset and meiosis/karyogamy seem to be equivalent demarcations. However, sometimes one is easier to operationalize than the other. Meiosis is often difficult to detect, especially for automictic taxa (Solari 2002; Gandolfi et al. 2003; Ramesh et al. 2005; Signorovitch et al. 2005; Cooper et al. 2007; Gorelick and Carpinone 2009). Cryptic meiosis makes the demarcation of meiosis/karyogamy difficult to operationalise, whereas the demarcation of wholesale epigenetic reset can be detected using time series of epigenetic signals, e.g. via methylation sensitive amplified fragment length polymorphism (MSAP, MS-AFLP) or teleomere length. Epigenetic reset can also be useful for demarcating individuals and generations in female metazoans with arrested meiosis, in which meiosis can take decades to complete. Below I show how epigenetic rest can also be used to discern whether twins constitute one versus multiple individuals. But there can be disadvantages to using wholesale epigenetic reset to demarcate individuals because sometimes the epigenetic reset takes much longer than meiosis or karyogamy. For instance, vertebrate karyogamy can be rapid, whereas the associated epigenetic reset continues from karyogamy until the blastocyst stage. While the border between one generation and the next may be fuzzy when using epigenetic signals, we can at least definitively count the number of generations to the nearest whole number. Such fuzziness of demarcating generations may be problematic for studying development, but should be fine for population genetic models of multiple generations.

Difficult cases: disaggregated and chimaeric individuals

Using sex to demarcate individuals and generations has interesting implications when applied to some unusual organisms. I show how using sex to demarcate individuals implies that the individuals need not be spatially localized nor may spatially localized entities necessarily be single individuals.

Are individuals localized?

Janzen (1977) proposed that apomictic aphids and dandelions should be considered a single disaggregated individual (also see Spencer 1863 [1890]). Aphids reproduce via amphimixis in autumn, but via apomixes in summer. Per my demarcation of individuals, all summer apomictic progeny constitute a single individual. Autumnal amphimictic aphid offspring constitute new individuals in a new generation. Dandelions (*Taraxacum officinale*) reproduce apomictically and automictically, both dispersing via parachute-like fruits (Mogie and Ford 1988). Apomictic forms constitute a large diffuse disaggregated individual, whereas automictic forms constitute separate individuals per my definition. This is in contrast to those who insist that individuals be spatiotemporally localized (e.g. Hull 1980). But how spatially and temporally close do entities have to be to be considered a single individual? For an extreme view, Hull (1992) considers species to be sufficiently spatiotemporally localised to constitute individuals. Using meiosis and karyogamy, with their attendant reduction in heritable variance to demarcate individuals and generations accords with Janzen (1977). In a critique of Janzen (1977), Loxdale (2008) noted that mitotic division in disaggregated apomictic aphid clones results in large amounts of genetic variance within the clonal population, consistent with my notion that meiosis decreases genetic variance, while mitosis allows for increased genetic variance.

Confusion over the definition of individuals affects the definition of heritability. Heritability is the ratio of (additive) genetic variance to phenotypic variance and is measured by comparing offspring with parents or comparing siblings with one another. But who are the parents and offspring with apomixis? Apomictic dandelions supposedly show high heritability of environmentally-induced cytosine methylation changes (Verhoeven et al. 2010). But this is mitotic heritability, not meiotic heritability (sensu Lush 1940; Falconer and Mackay 1996). If mitosis circumscribes an individual, while meiosis and karyogamy demarcate new individuals and generations, then the only construct that makes sense for defining individuals is meiotic heritability, not mitotic heritability.

Per my demarcation of individuals, cabernet franc grape (*Vitis vinifera*) cuttings constitute a single individual despite being distributed world-wide. Similarly, Dolly the cloned sheep (*Ovis aries*) was the same individual as her genetic mother. Lack of epigenetic reset makes cloned organisms prematurely old; only epigenetic resets associated with meiosis and karyogamy rejuvenate organisms (Ünal et al. 2011). Animals cannot survive for as long as plants without an epigenetic reset, possibly because of the greater number of cell types in animals (Gorelick and Carpinone 2009).

I add the caveat that meiosis and karyogamy are not as temporally localised as usually believed. Wholesale epigenetic reset begins in primordial germ cells and ends shortly after completion of meiosis II (Allegrucci et al. 2005; Popp et al. 2010). With vertebrates, epigenetic reset starts immediately after karyogamy and ends at the blastocyst stage (Santos and Dean 2004; Loi et al. 2009). This temporal broadening of the terms ‘meiosis’ and ‘karygamy’ should not be surprising because cell fate is specified in primordial germ cells, whereas cell fate of yolk and placental

tissue may be specified long after the first diploid cleavage division (Marikawa and Alarcón 2009; cf. Gorelick et al. 2012). Wholesale epigenetic reset can be a lengthy process in female metazoans with meiotic arrest. The long duration of epigenetic reset makes it harder to pinpoint when a generation ends, which may be more precisely demarcated by a given stage of meiosis (e.g. prophase I or anaphase II) and karyogamy. However, as I next show, we need epigenetic reset, rather than meiosis or karyogamy, to decide whether monozygotic or dizygotic twins constitute one versus multiple individuals.

Monozygotic twins are separate individuals

Because the epigenetic reset associated with karyogamy ends after one cleavage and several diploid mitotic divisions, I am a different individual from my monozygotic twin because our epigenetic resets were completed after our common embryo divided. It is still not understood why monozygotic twins have different epigenetic resets nor how heritable these epigenetic resets are (Fraga et al. 2005), but here are three possible explanations. First, monozygotic twins may experience different environments from one another, i.e. stochastic variation in epigenetic marks (Gorelick 2004, 2005). Second, interactions between the twins might provide a dynamic system in lieu of an otherwise predictable environment of single births. Third, there exist intrinsic asymmetries in early diploid development (Gorelick et al. 2012). Asymmetry causes some cells to be destined for embryo and others for yolk or placenta. If monozygotic twins divide after these asymmetries are established, then the twins should have different epigenetic signatures, maybe such as different brain lateralization.

Chimaeras are a mixture of multiple individuals

Demarcating individuals by sex and its associated epigenetic reset is counter-intuitive for highly chimaeric dizygotic twins, such as Wied's marmoset (*Callithrix kuhlii*) or bovine freemartins. Do chimaeric dizygotic twins swap cells before or after the completion of the epigenetic reset associated with karyogamy? If chimaeric cells migrate between (pre-)embryos before the blastocyst stage, then dizygotic twins should be considered separate individuals because they received separate epigenetic resets. If chimaeric cells migrate between embryos after epigenetic reset, then each dizygotic twin is an epigenetic chimaera comprised of multiple disaggregated individuals. In eutherian mammals, chimaeric fetal and maternal cells that crossed the placenta count as being from two separate individuals because epigenetic reset ends at the blastocyst stage, before the placenta develops. Chimaersim makes it difficult to identify individuals, albeit hardly more difficult than identifying an individual as something separate from all of its hitchhiking microbes.

The fungal sub-kingdom Dikarya, named for its long-lived dikaryotic stage, has extreme chimaerism. Haploid mycelia undergo plasmogamy to form a dikaryon, a pair of nuclei within a cell. When cells divide, both nuclei in the dikaryon simultaneously undergo mitosis. After many mitotic divisions, a dikaryon may

undergo karyogamy to form a diploid zygote, which immediately undergoes meiosis to form a tetrad of haploid spores.

How many individuals are there in Dikarya mycelia? A dikaryotic cell contains two nuclei each with its own epigenetic reset. Therefore, the dikaryotic stage is a pair of individuals in the same body. The number of individuals does not change with mitosis, but only changes with meiosis or karyogamy. A mass of mycelia may contain some cells with single haploid nuclei, some cells with two haploid nuclei from a mating, and other cells with two haploid nuclei from a mating of different parents. Therefore, Dikarya cells contain either one or two individuals and the identity of these individuals may vary between cells. While counter-intuitive, this is the price we pay for letting levels of heritable genetic variance drive the level of selection at which we circumscribe individuals.

Implications of using sex to demarcate individuals

Most analyses in evolution and ecology require identification of individuals and many also require identification of generations. Yet, as many organismal biologists have noted, it is non-trivial defining or demarcating individuals (Hull 1976; Janzen 1977; Santelices 1999; Clarke 2010, 2012). I discuss a few implications of my demarcation of individuals, some of which are counter-intuitive.

Lack of sex does not diminish heritable variance (Vasseur et al. 1993; Crawford and Landolt 1995; Infante et al. 2003; Rottenberg and Parker 2004; Myles et al. 2011; Yuan et al. 2011). But if sex demarcates individuals, then this constitutes variation within a disaggregated individual, not variation between individuals. Thus we end up where we started, that asexual taxa lack heritable variance, but by redefining individuals and asserting that meiotic (not mitotic) heritability matters.

Demarcating individuals and generations by epigenetic resets associated with meiosis and karyogamy alters population genetic models of facultatively apomictic taxa. Effective population size and number of generations shrink to one. Mutation rate is often measured as number of substitutions per individual per generation. For apomicts, mutation rate will increase because the individual and generation are both bigger. Evolution of apomictic lineages will be dominated by genetic drift and mutation compared with automictic or amphimictic lineages. Selection will play a much larger role in evolution of automictic and amphimictic lineages because they have larger population sizes (less drift) and smaller mutation rates. These are quantitatively different evolutionary trajectories than predicted by models in which physiological autonomy and spatial contiguity are used to circumscribe individuals, which make no distinction between apomicts and automicts/amphimicts. This provides an empirical way to gauge which definition of individuals is more sensible.

Population genetic metrics must be recalculated for apomictic lineages with this new demarcation of individuals. Linkage disequilibrium is the probability of predicting which allele is at a locus if you know which allele is at another locus. Linkage disequilibrium is much lower in apomictic lineages because there is a greater chance for allelic variation within a long-lived spatially large disaggregated individual. Recasting the definition of individuals affects many other population

genetic metrics, such as F_{ST}, G×E, mutation rate, and even fitness. Fitness is defined for an individual, and average fitness is defined for a population. We could define fitness otherwise, such as fitness of a cell or mitotic cell lineage, as do cancer biologists and bacteriologists, but then would need to redefine heritability, such as to mitotic heritability.

Demarcating eukaryotic individuals and generations by meiosis implies that each product of meiosis is a separate haploid individual, i.e. for animals, each haploid egg and sperm is a separate individual. Usually eggs and sperm are thought of as part of our diploid selves. Instead, here an ejaculate consists of millions of separate single-celled haploid individuals. This, however, cannot inform ethical questions about contraception because each gamete's life effectively ends either via death or via restoration of diploidy following karyogamy. Plus, biological implications need not reflect ethical implications.

Biodiversity is often estimated using beta-diversity (Gorelick and Bertram 2010), which requires counting numbers of individuals of each taxon in each geographic area. Abundances decrease if what we believed were thousands of individuals (e.g. aspen ramets) are only one individual. Estimates of beta-diversity will change dramatically, especially if some taxa are apomictic and others not. Plant demography is a nightmare without a cogent definition of individuals (Clarke 2012).

Long-lived individuals, especially if large and spatially disaggregated, have the potential to enhance ecological stability and resilience (de Witte and Stocklin 2010).

Sociobiologists refer to a colony of eusocial ants, bees, wasps, or termites as a superorganism (Spencer 1876; Wilson 1975; Okasha 2006; Hunt 2008). Workers are all sisters, being diploid progeny of a single queen, so are separate individuals because they are products of karyogamy. Haploid hymenopteran drones are all genetically identical to their mothers, apart from recombination and mutation, and are usually not considered part of the superorganism because they do not work. However, drones are each distinct individuals because they arise from different meiotic products. The meiotic division contained an epigenetic reset, making each male a separate individual. A more fitting use of the term 'superorganism' would be for apomictic progeny, as in clonal offspring of aphids in summer, apomictic dandelions, or plant cuttings.

Concluding remarks

Clarke (2012) posited that individuals are circumscribed by mechanisms that constrain heritable variance in fitness. Clarke thinks that mitosis is the constraint, whereas I think meiosis and karyogamy are the constraint. But Clarke's definition works in either case. This definition is consistent with other works on evolutionary transitions to individuality that rely on levels of selection. Demarcating individuals by meiosis/karyogamy and circumscribing individuals by mitosis is consistent with most notions of development (epigenesis) and senescence, in which variance is evident both temporally and spatially amongst products of mitosis. However my definition of individuals provides population genetic predictions that are qualitatively different from circumscriptions of individuals based on physiological

autonomy, unicellular bottlenecks, and alternation of generations, especially for apomicts.

Sex and death make us individuals. While sex creates new individuals and generations—and often kills existing individuals—mitosis provides the glue that holds together an individual. Because it does not filter out variation, mitosis of nuclei circumscribes an individual. It does not matter whether the mitotic products are dispersed, as with a disaggregated clonal individual, or amalgamated, as with chiameric dikaryotic fungi.

Coherence of an individual is no longer measured genetically, but instead epigenetically. Given that epigenetic signals are heritable, we should consider epigenetic signals to be genetic (Gorelick and Laubichler 2008). Thus epigenetic uniqueness/homogeneity is synonymous with genetic uniqueness/homogeneity.

Using mitosis to circumscribe individuals and meiosis/karyogamy to demarcate new individuals and generations applies to all eukaryotes. However, it is not obvious how to extend this definition to eubacteria, archaebacteria, or viruses, none of which have any vestiges of mitosis, meiosis, or karyogamy. I have no idea what constitutes an individual or senescence in eubacteria, archaebacteria or viruses. Haploid population genetics seems to work well enough for eubacteria, archeabacteria, and viruses, although this could be because of their different genetic architectures and greater influence of selection in lieu of drift and mutation (Bennett et al. 1990; Lynch 2007).

Using the epigenetic reset associated with sex, including self sex, to demarcate individuals eliminates many quandaries. Are monozygotic twins separate individuals? Yes because they each undergo a different epigenetic reset (at least if not chimaeric). When does an aggregation of cells constitute a single individual versus a colony, as with volvocine algae or slime moulds? A eukaryotic individual is comprised of cells that are all products of mitosis of a single parental cell. If a slime mould slug is comprised of cells from separate meiotic divisions, then the slug is an amalgamation (chimaera) of separate individuals. With humans, only a minority of cells are the products of human mitosis. The majority of cells are hitchhikers, such as mutualistic or parasitic microbes. These microbes are not part of the individual that is us because they are not a product of human mitosis. Amongst mitotic products of a single cell, do cells with different ploidies constitute different individuals or generations? No because epigenetic reset is not associated with endoploidy or aneuploidy. Sex vis-à-vis wholesale epigenetic reset, meiosis, and karyogamy demarcates the end of one generation and the start of another. A generation could be an hour or a millennium long. Borrowing terminology from cancer biology (Omura and Goggins 2009), mitotic heritability circumscribes an individual, whereas meiotic heritability demarcates new individuals and generations, providing a conceptual simplification for eukaryotes.

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Glossary

Amphimixis (amphimictic)	Outcrossing sex, i.e. fusion of gametes from two different parents
Apomixis (apomictic)	Reproduction without any vestiges of meiosis
Automixis (automictic)	Self sex where offspring have only one parent, but gametic nuclei are formed by meiosis. Automixis can either be (1) fusion of two haploid nuclei from the same meiotic division (i.e. complete automixis, terminal fusion, central fusion) or (2) diploid gametes are formed by meiosis that have two (instead of one) chromosomal duplication (i.e. premeiotic doubling)
Chimaera (chimaeric)	An assemblage of nuclei or cells that are not all the products of a single meiotic division or single karyogamic fusion
Endomitosis (endomitotic)	Duplication of all chromosomes without a mitotic division. Endomitosis turns diploid nuclei into tetraploid nuclei
Endoploidy	Nuclei have undergone endomitosis
Karyogamy	Fusion of two haploid nuclei (actually the two nuclear membranes usually dissolve and then a new membrane is formed around the two sets of chromosomes). Karyogamy combines two haploid nuclei to form one diploid nucleus
Meiosis	Chromosomal duplication followed by two reduction divisions (meiosis I and II). Meiosis turns diploid nuclei into haploid nuclei
Plasmogamy	Fusion of two haploid cell membranes, but not their nuclear membranes

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