

## Evolution of cacti is largely driven by genetic drift, not selection

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*Summary:* Since at least 1930, evolutionary explanations have focused on Darwin's mechanism for driving evolution, namely natural selection (and one of its side effects, adaptation), mostly neglecting other evolutionary forces. Yet, genetic drift probably plays a huge role in plant evolution, as does drift's supporting cast of gene-gene interactions and inbreeding. I argue that cactus evolution should be largely driven by drift, rather than selection, because of high rates of polyploidy, inbreeding, and endemism, which collectively result in highly reduced effective population sizes for virtually all of the Cactaceae.

*Zusammenfassung:* Seit mindestens 1930 haben sich Erklärungen der Evolution auf Darwin's Mechanismen zur Steuerung der Evolution fokussiert, d.h. auf natürliche Selektion (und besonders Adaptation als Seiteneffekt), und haben andere evolutiven Kräfte ausser Acht gelassen. Wahrscheinlich spielt aber die genetische Drift eine wichtige Rolle in der Pflanzenevolution, ebenso wie die Auswirkung der Drift auf Gen-Gen-Interaktionen und Inzucht. Ich gehe davon aus, dass die Evolution der Kakteen hauptsächlich durch genetische Drift gesteuert wird, und nicht durch Selektion. Als Gründe für diese Sicht sind die hohen Raten von Polyploidie, Inzucht und Endemismus zu nennen, welche zusammen zu stark reduzierten effektiven Populationsgrößen für fast alle Kakteen führen.

### Introduction

Charles Darwin published a truly great book, whose title contains one of the cruelest misnomers in all of science, *On The Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life* (Darwin, 1859). Darwin had the amazing insight, announced a year earlier at the Linnean

Society independently by both Darwin and Alfred Russel Wallace, that the primary force driving evolution is natural selection (Browne, 2002). The irony is that Darwin's long and marvelous book effectively never said a word about the origin of species, something we forget, especially when typically using the abridged title, *Origin of Species*, and that for sixty years scientists had dismissed Darwin's mechanism of selection.

Charles Darwin was quite familiar with cacti, which he saw throughout his travels in South America (Brazil, Argentina, Chile, Galapagos) during the voyage of the Beagle (Rowley, 2009). John Henslow, the person who arranged for Darwin's trip on the Beagle, described *Opuntia galapageia* and *Opuntia (Maihueniopsis) darwinii* from specimens that Darwin collected and sent him while on the voyage (Henslow, 1837). Although Darwin's travels only took place early in his life, his love for botany never waned. The famed botanist Joseph Hooker was Darwin's most reliable advocate and friend (Browne, 1996), who was at least partially responsible for motivating many of Darwin's later botanical books (Darwin, 1862, 1865, 1875, 1876, 1877, 1880).

To most modern biologists, the word evolution evokes notions of selection and adaptation. Even the definition of life itself is often equated with adaptation and selection (Oró, 2002). Consider adaptive explanations given for succulence (water storage in xeric environs), taproots in geophytes (for water storage and protection from grazing), winged seeds (for wind dispersal) in *Pterocactus*, long spines (for protection against herbivory by mammals) in many cacti and their possible sister taxon Didiereaceae, vibrant red upright fruits in many cacti (for seed dispersal by birds), and even the size and shape of xylem cells that supposedly reflect the ultimate size of stems. Speciation, in particular, and origins of new taxa, in general, have become inextricably

linked with selection and adaptation (Mayr, 1947; Schluter, 1998). We talk about adaptive niches into which specific species fit, but into which sister taxa fail to exist. For example, *Pediocactus sileri* clings to small gypsum cliffs, while *Pediocactus paradenei* lives in nearby high-altitude woodlands, while *Pediocactus (Toumeyia) papyracantha* is widely distributed in low desert fields of grama grass. Many cactus biologists explain speciation as a result of adaptation to new niches, which is very much in accord with Ernst Mayr's ideas of allopatric and peripatric speciation (Mayr, 1947). And adaptation is the direct result of selection for beneficial genes. Adaptive radiation is still the paradigmatic but inaccurate view, dating back not just to Darwin's great 1859 book, but at least to Jean-Baptiste Lamarck's *Philosophie Zoologique* (1809).

What did Charles Darwin contribute to our understanding of cactus evolution? Other than the huge benefit of placing biology in an evolutionary framework, with descent by modification from common ancestors (tree of life), I argue that Darwin's contributions on selection may have been misleading. From a very unfair historical retrospective, hindsight being 20/20, Darwin may have muddled our views by over-emphasizing the importance of natural selection, especially selection's largely non-existent role in speciation. Even from the most naïve of perspectives, one would expect natural selection to play a huge role in extinction. By comparison, it is not altogether obvious how selection induces speciation. While there exist tortured explanations of selection acting to reduce fitness of hybrids between previously isolated populations (e.g. breaking of hypothesized co-adapted gene complexes (Dobzhansky & Pavlovsky, 1958), the logic is not applied consistently. We tend to forget that selection and mutation are not the only evolutionary forces. Instead, as Charles Goodnight says, we have the 'four horsemen of evolution': selection, mutation, migration, and genetic drift. We also have two other huge players in cactus evolution: (1) gene-gene interactions (epistasis and linkage disequilibrium), which are implicit in discussion of co-adapted gene complexes, and (2) genome duplications, which have particularly huge effects with polyploidy (allopolyploidy and autopolyploidy) in which all of the chromosomes are duplicated throughout the individual. Here, I want to show how selection probably makes little difference in the evolution of cacti, but instead their evolution is probably driven by genetic drift by and through gene-gene

interactions and polyploidy.

I posit that drift is probably much more important than selection in the evolution of most plants and animals and this is especially true in many cacti because they are highly inbred. "[N]atural selection is but one narrative among other origin stories" (Lancaster, 2003:79) (with apologies for quoting Roger Lancaster out of context). The limited role of selection in cacti is further diminished by both gene-gene interactions and intense inbreeding. Speciation in many cacti is probably largely driven by polyploidy.

## Selection

Other than work on pollination, there is no persuasive evidence that selection and adaptation have played a major role in cactus evolution. Most discussion of cactus evolution merely describes systematics and taxonomy, i.e. patterns not process (Wallace & Gibson, 2002). There are also several reports of heterozygosity and population structure ( $F_{ST}$ ,  $G_{ST}$ ,  $F_{IS}$ , etc), but seemingly without attribution of these measures to selection (e.g. Hamrick *et al.*, 2002). These empirical measures are affected by all the evolutionary forces (selection, mutation, drift, migration), which also includes the influences of inbreeding, genetic mixing, and gene-gene interactions. The gorgeous work of the cactus morphologists Franz Buxbaum, Norman Boke, and Irving Bailey largely avoided discussion of selection. More recent cactus morphologists Jim Mauseth and Art Gibson hinted at correlations between cactus anatomy, environment, and habit, implying that selection and maybe adaptation are at work, but never explicitly trying to tease apart the various evolutionary forces (Gibson, 1973, 1977a,b, 1978; Mauseth & Plemons-Rodriguez, 1998; Mauseth, 1999, 2006). Even Art Gibson and Park Nobel's classic *Cactus Primer* (1986) said surprisingly little about evolution of cacti and even less about selection. Their very short final section titled "The evolution of cactus characters" asks if there is a selective trade-off between large leaves and either stem succulence or CAM photosynthesis. They hypothesize that shrubby or tree-like forms living in environments where the individuals need to outgrow shade may be due to selection. However, they do not test this and rightfully complicate the story by arguing that such selective pressures probably change if the cacti ever experience freezing temperatures. Finally, Gibson and Nobel state that no research had even yet been done to discern whether the selective advantage of spines (assuming such an advan-

tage exists) is due to protection against temperature or herbivory. I have not found any direct measures of selection in cacti using classical methods (Endler, 1986; Falconer & Mackay, 1996).

While selection undoubtedly plays some role in cactus evolution, it is not at all obvious that selection plays a larger role in cacti than in any other plant or animal. And, as I show below, plants and animals have much more drift than other taxa, i.e. bacteria, protists, and fungi. Therefore, I suggest that we look for an adaptive explanation for one of the most unique and exaggerated characters in the plant kingdom, namely the cephalium. After this case-study, we will return to discussion of the other evolutionary forces, especially genetic drift, and some of its causes such as inbreeding and polyploidy.

Selection and adaptation clearly cannot explain one of the most conspicuous morphological features in cacti, the cephalium. The genus *Melocactus* provides an extreme example, with no photosynthetic tissue ever growing once a cephalium is produced. Most of the cephalium provides no selective advantage insofar as it is not photosynthetic and flowers are only produced from the most recent year's growth. Older parts of the cephalium serve no obvious use. It is possible to conjecture that a tall cephalium may get the attention of more pollinating animals, but the genus *Melocactus* is largely self fertilising and their principal animal pollinators are hummingbirds (Nassar & Ramirez, 2004; Nassar *et al.*, 2007). Old specimens of *M. paucispinus* or *M. violaceus* with cephalia that are twice as tall as the green portion of the stem look comical and seem to place the individual at a selective disadvantage due to the huge amount of unused tissue. Terminal cephalia on *Discocactus* and *Backebergia* also provide no photosynthetic tissue despite new chlorenchyma growing below their cephalia. *Backebergia militaris* cephalia seem especially wasteful with their abscission after a few years (Mauseth, 2006). What selective pressures would make these terminal cephalium bearing species so canalized and apparently maladaptive? The only terminal cephalium bearing plants that do not experience huge photosynthetic losses are those with ring-like cephalia in *Arrojadoa* and *Stephanocereus leucostele*, but still there is no obvious selective advantage here.

Likewise good selective or adaptive advantages have never been proffered for lateral cephalia and pseudocephalia. While there is certainly less loss of photosynthesis with these

lateral floral structures than with terminal cephalia, verifiable benefits have not been documented. While developing flowers and fruits may need protection from the weather and herbivory, this has never been convincingly tested. Some species include individuals both with and without lateral cephalia, such as *Pilosocereus gounellei* subsp. *zehntneri* and *gounellei*, yet neither has been shown (nor even asserted) to be more fit or more highly adapted. Sunken lateral cephalia can alter the direction in which stem tips are tilted, increasing total surface area of the photosynthetic stem exposed to sunlight (Vazquez-Sanchez *et al.*, 2007). But several cacti without lateral cephalia also have tilted stems (e.g. *Ferocactus* spp.) or stem tips (e.g. *Stephanocereus (Pilosocereus) luetzelbergii*). Furthermore, tilting of stem tips due to lateral cephalia in *Cephalocereus columna-trajani* imposes a fitness cost by rendering stem branching impossible, at least without stems breaking (Zavala-Hurtado *et al.*, 1998).

A simpler explanation than selective advantage and adaptation for cephalia and pseudocephalia in cacti is pleiotropy. Many cactus ovules produce copious hairs and bristles in species that lack a cephalium, possibly as protection against thermal perturbations or herbivory (which is also untested). Why couldn't regulation of nearby stem tissue result in these hairs and bristles also being produced in tissues adjacent to where ovules are produced? Such evolutionary developmental constraints seem to exist on a cactus that virtually produces a cephalium, namely *Lophocereus (Pachycereus) schottii* (Martorell *et al.*, 2006).

### Genetic drift

Unlike selection, genetic drift is a random evolutionary force. Although the mathematics underlying drift and selection are intricate, the concepts are simple (Wright, 1931; Gillespie, 1998). Selection means that the probability of successfully living and reproducing is determined by an individual's genotype. Certain alleles and certain combinations of alleles at multiple loci produce fitter individuals than others, at least in a given environment. One can then set-up equations to predict exactly how genotype frequencies (probability of each allele at each locus) of any given population will change from one generation to the next in the face of selection. By contrast, drift is a random force associated with finite population sizes. Every genotype cannot possibly exist in a finite population,

where a population can be defined as the set of inter-breeding individuals. For simplicity, assume that each genetic locus has only two alleles. This is true for some genes such as human Rh factor, which has positive and negative alleles in many populations, but not other genes such as blood type, which has three alleles, A, B, and the null allele. Next say that we are simultaneously examining  $n$  genetic loci with two alleles per locus. Then there are  $2^n$  possible genotypes, which can be a huge number, especially with thousands of genes in an individual (there are only about  $2^{250}$  quarks in the entire universe). For small populations, it is possible that by chance alone many multi-locus genotypes are missing from the population. For small populations it is even possible that one of the two alleles at a single locus is missing from the population, by chance alone. This effect is known as drift. Drift is large for (1) small population sizes, (2) high amounts of inbreeding, (3) low migration rates between different populations, (4) high number of alleles per locus, and (5) high linkage disequilibrium.

Although Darwin never articulated anything about genetic drift, drift can still be envisioned as a Malthusian phenomenon. Organisms can potentially produce an exponentially growing number of offspring, but the environment constrains population sizes to be roughly fixed. Assume that all individuals are equally likely to be in the next generation – they all have equal fitness – but the majority of individuals are killed off in random fashion each generation. There is a finite probability that no individuals in the next generation will retain a given allele. If population sizes are small enough, even if fitnesses amongst individuals are not equal, this random effect can result in elimination of the fittest allele. That is, drift can dominate selection.

Evolutionary biologists realize that all four evolutionary forces – selection, mutation, migration, and drift – act on all populations at all times. What matters is the relative size of these four forces. The relative role of drift is inversely proportional to long-term effective population size. It is telling that the seminal empirical work on the evolutionary role of selection was done by Rich Lenski, with his students and colleagues, working with bacteria (Bennett *et al.*, 1990). While this work is remarkably good and important, we must remember that the population size of genetically-mixing bacteria are often on the order of millions or billions of individuals. By contrast, plants and animals breed in small iso-

lated populations, where we expect drift to dominate selection. For example, many eastern Brazilian cacti are endemic to isolated mountains and outcrops (Taylor & Zappi, 2004), greatly reducing effective population sizes (Moraes *et al.*, 2005). The IUCN red list (2009) provides population estimates for some cacti. For example, two of the only five known populations of *Melocactus paucispinus* have fewer than fifty individuals, while the sympatric *Micranthocereus streckeri* has only one known population with fewer than fifty individuals. There are certainly a few species of cacti with huge actual population sizes, such as *Coryphantha vivipara*, which lacks clonal reproduction and has a range of almost one-quarter of the United States plus parts of Canada (Benson, 1982). However, such large population sizes are atypical (Preston, 1948). Furthermore, effective population size – which is the quantity with real evolutionary importance – can be no larger than actual population size.

Using within-species nucleotide divergences for silent sites and estimated mutation rates, Lynch (2007) computed order-of-magnitude estimates of long-term effective population sizes multiplied by ploidy level as shown in Table 1.

While the final estimate was not computed using cacti, a twenty-year generation time is approximately true for many cacti. Furthermore, while Lynch envisioned these estimates being used for haploid or diploid organisms, polyploidy in cacti will further reduce the estimate of effective population size. Even for populations of a few thousand individuals (e.g. *Obregonia denegrii*), long-term effective population size for this species must be much smaller than the long-term effective population of perennial plants given in Table 1.

Effective population size is a function of breeding system, generation time, and history. Long-term effective population size is the harmonic mean of effective population size at each generation (Hartl & Clark, 1989). Drift is much larger if there was a population bottleneck in the past. Thus, even cacti with large population sizes, such as saguaros, probably have relatively small effective population sizes because population sizes are usually in decline, with rare and episodic large recruitment years (Pierson & Turner, 1998). Large organisms with long generation times, such as cephalium-bearing cacti, have particularly small effective population sizes and hence lots of genetic drift. There are very few if any estimates of effective population size in cacti, either long-term or short-term. The above

**Table 1.** Order-of-magnitude estimates of long-term effective population sizes multiplied by ploidy level for various groups of organisms

organisms	long-term effective population size $\times$ ploidy level
prokaryotes (eubacteria and archaea)	$10^8$
non-parasitic unicellular eukaryotes	$10^7$
invertebrates	$10^6$
vertebrates	$10^4$
annual plants	$10^6$
perennial plants (trees with 20 year generations)	$10^4$

arguments indicate there is no reason to presume that cacti have typically long-term effective population sizes, even for perennial plants. Cactus evolution is thus much more under the influence of drift than are most other organisms.

### Inbreeding

Inbreeding is a measure of how closely related (on average) interbreeding organisms are in a population. Inbreeding can be complete in self fertilising organisms. There is, however, no example of complete outbreeding. Individuals can only breed with close relatives, usually in the same genus or species. Most evolutionary biologists believe that there was a last common ancestor for all extant life, which means that there would still be inbreeding (albeit not much) even if a plant somehow hybridized with an animal.

Inbreeding increases drift. Obligately selfing organisms have an effective population size of one. Even with obligate outcrossing taxa, inbreeding reduces effective population size to a smaller value than the number of interbreeding individuals because breeding is never truly random. The greater the inbreeding coefficient, the smaller the effective population size and the greater the role of drift. While population geneticists routinely estimate these inbreeding coefficients, we will stick with a more qualitative discussion.

Ecologists inferred surprising amounts of inbreeding in plants and animals, noting that offspring invariably mated close to their parents (Shields, 1982). This independently corroborates population genetics estimates of effective population size. Cacti are especially prone to large amounts of inbreeding and genetic drift. Many cacti are facultatively self fertilising. Many cacti are clonal (e.g. *Opuntia*, *Cylindropuntia*) reducing effective population size to one (Grant & Grant, 1980; Clark-Tapia *et al.*, 2005; Ricardo *et al.*, 2006; Mandujano *et al.*, 2007). Some highly

inbred species that ordinarily are self fertilising (e.g. *Melocactus andinus*, *Stenocereus eruca*) occasionally outcross, but this only increases effective population sizes slightly (Griffith, 2001; Nassar & Ramirez, 2004; Clark-Tapia *et al.*, 2005; Ricardo *et al.*, 2006; Nassar *et al.*, 2007). Obligate outcrossing species tend to be rare in endemic species or species found only on isolated mountain-tops or valleys. Dioecious species with equal numbers of female and male individuals (*Mammillaria dioica*, *Echinocereus coccineus*) also have half the effective population of related monoecious species. Effective population size is even lower if the sex ratio is not one-to-one.

### Ployploidy

Ployploidy is doubling of all chromosomal material. Polyploidy is an umbrella term for allopolyploidy and autopolyploidy. In both, offspring inherit a full (diploid) set of chromosomes from both parents, often due to unreduced gametes, and hence the offspring have four copies of each chromosome. Allopolyploidy means that the two parents were of different species, whereas autopolyploidy means that both parents were of the same species. Since most species concepts seem rather meaningless for plants, I ignore this distinction (Rowley, 2007; Soltis *et al.*, 2007). For instance, the biological species concept was invented and is really only applicable for animals insofar as congeneric (and sometimes confamilial) plant species can often hybridize (Cracraft, 1989). There are quite a few intergeneric cactus hybrids (Anderson, 2001; Arias & Terrazas, 2008).

I, however, do not use the term polyploidy to simply mean the doubling of the *number* of chromosomes. Evolution can double the number of chromosomes without doubling any chromosomal material other than centromeres. All chromosomes can effectively be chopped into two pieces, something known as karyotypic fission or

chromosomal fission (Todd, 1970; Kolnicki 2000). From a cursory glance of karyotypes, chromosomal fission does not appear to occur in cacti (Goldblatt & Johnson, 2008), but may occur in other succulents, such as with the outrageously high chromosome numbers in the Crassulaceae (Uhl, 2007). When speaking of doubling the number of chromosomes, I will use the term polyploidy if there is twice as much DNA per cell and will use the term chromosomal fission if there is little or no increase in the amount of DNA per cell, other than due to duplicate centromeres.

Polyploidy results in instantaneous reproductive isolation (Grant, 1971). If parents have two of each chromosome (diploid), their offspring have four of each chromosome (tetraploid), and gametes are reduced (half the number of chromosomes), then any such backcross between parent and offspring will have three of each chromosome (triploid). Such triploid backcrosses cannot produce viable gametes because it is impossible to *evenly* divide three chromosomes between two daughter cells. Taxonomists have agreed that allopolyploids are new species, although there is still debate on whether to call autopolyploids new species (Rowley, 2007; Soltis et al., 2007). Regardless, polyploidy is a predominant form of speciation in flowering plants. In cacti, polyploidy is especially prevalent in the Opuntioideae, with fewer but still many occurrences in the Cactoideae (Stockwell, 1935; Katagiri, 1953; Pinkava & McLeod, 1971; Pinkava et al., 1973, 1977, 1985, 1992, 1998; Weedin & Powell, 1978; Ross, 1981; Pinkava & Parfitt, 1982; Weedin et al., 1989; Cota & Philbrick, 1994; Cota et al., 1996; Das et al., 1999; Lichtenzweig et al., 2000; Powell & Weedin, 2001; Negrón-Ortiz, 2007; Goldblatt & Johnson, 2008).

Chromosomal fission seems to occur in succulents (although probably not cacti) and does not result in reproductive isolation or speciation. If a 'normal' parent backcrosses with its offspring that has undergone chromosomal fission, the resulting backcross can still undergo normal gamete formation. At meiosis, each large parental chromosome pairs with its homologous pair of fissioned descendant chromosomes. If the American succulent Crassulaceae largely evolved via chromosomal fission, then this may explain the huge size of the comparium of interbreeding species amongst many genera (Uhl, 2007). Cacti do not appear to have chromosomal fission; they always seem to duplicate all nuclear DNA whenever they duplicate chromosome numbers. Diploid cacti typically have 3–4 pg of DNA per

nucleus (Bennett & Leitch, 2005), although *Pereskia grandifolia* has only 2 pg (De Rocher et al., 1990). By contrast, the octaploid *Weberbauerocereus winterianus* has 14.2 pg of chromosomes per nucleus, which is four times the typical diploid level of DNA (Bennett & Smith (1976) citing unpublished work of P. Barlow), indicating true polyploidy.

Polyploidy, but not chromosomal fission, diminishes the role of selection vis-à-vis increasing mutation rates. In newly formed polyploids, roughly one-quarter of the genes are highly methylated (Adams et al., 2003), resulting in a drastically higher mutation rate of cytosine nucleotides to thymine (Gorelick, 2003), gene silencing and even deletions (Pikaard, 2001). Methylation of duplicated or newly inserted genes occurs in all living organisms, probably as a defense mechanism against parasitic DNA (Yoder et al., 1997; Wendel & Doyle, 2005); methylation largely shuts down gene activity.

Polyploidy probably is detrimental to individual plants due to more than just increased nucleotide mutation rates. Polyploidy also increases generation time – it takes lots of time and energy to synthesize chromosomes. Chromosomes are enormous molecules hence polyploid nuclei and cells are physically larger (Masterson, 1994), plus can have faster cell elongation rates (Sugiyama, 2005). It has also been hypothesized that this change in cell shape disrupts production of wood fibers, including in cacti, making it impossible for stems to stand upright (Stebbins, 1938; Gibson, 1973, 1977b; Gibson & Nobel, 1986).

In summary, not only have many new cactus taxa probably originated via polyploidy, this polyploidy reduced the role of selection by severely reducing effective population size and by increasing mutation rate.

### Gene-gene interactions

There are two forms of gene-interactions, genotype effects called linkage disequilibrium and phenotype effects called epistasis. Linkage disequilibrium will be crucial to our explanation of why selection is relatively unimportant, while epistasis encapsulates our heuristic notion of co-adapted gene complexes.

Linkage disequilibrium is a probabilistic and often poorly understood quantity that is calculated for an entire population. Linkage disequilibrium is measured amongst selected alleles at two or more genetic loci, quantifying how statistically independent those alleles are (Gorelick &

Laubichler, 2004; Liu & Lin, 2005). Consider two genetic loci, one with three alleles and the other with four alleles. There are then a dozen linkage disequilibrium coefficients ( $3 \times 4$ ) (half of the linkage disequilibrium coefficients in this example will be redundant because the matrix of linkage disequilibrium coefficients must have rows and columns that sum to zero). Consider the coefficient for the first allele at one locus and the third alleles at the other locus. Given that we know that an individual has the first allele at the first genetic locus, the linkage disequilibrium coefficient tells us how well we can predict that this individual will also have the third allele at the second genetic locus. Take human Rh factors and blood types as a two-locus example. Knowing blood type, can we predict Rh factor of an individual? If these two loci are independent, the answer is no and all linkage disequilibrium coefficients will be zero. If however, say, most people with B blood type are Rh-negative, then the population has non-zero linkage disequilibrium for that pair of alleles.

How can an entity as abstruse as linkage disequilibrium have evolutionary significance? First, in order to measure epistasis and thus the magnitude of co-adapted gene complexes, we need to know linkage disequilibrium (Wagner *et al.*, 1998; Gorelick, 2004). Second, if there is a lot of non-zero linkage disequilibrium, then the population has effectively many fewer genetic loci upon which selection can act (Barton, 1995; Lynch, 2007). If selection acts to favour an allele at one genetic locus and linkage disequilibrium is large, then alleles at all other tightly linked genetic loci will go along for the ride. Selection will thus really only affect a single genetic locus and not many different ones independently. This is known as genetic hitchhiking and severely diminishes the role of selection (Barton, 1995; Lynch, 2007). As a hypothetical example, say that selection strongly affects spine length, but then selection may have little or no effect on genes that control the amount of succulence, height of plant, shape and colour of flowers, etc.

Over the course of eukaryotic evolution, genome size increased at a much higher rate than did the number of chromosomes (Lynch, 2007). Even by chance, this leads to much higher rates of linkage disequilibrium since crossing-over recombination is a rare event, with approximately one or two crossing-over events per generation per pair of homologous chromosomes. Plants and animals have much larger genomes than unicellular organisms or colonial organisms

with few specialized cell types, such as algae and fungi (Lynch, 2007). Thus, all plants and animals should have more drift and less selection than bacteria, protists, and fungi simply as a function of genome size. Cacti are fairly typical flowering plants with regards to their genome size (Bennett & Leitch, 2005).

Phenotypic gene-gene interactions, known as epistasis or epistatic interactions, are even less well understood than their genotypic counterpart of linkage disequilibrium. Without several unrealistic and constraining assumptions (Gorelick, 2004; Kouyos *et al.*, 2006), we can only define and measure epistasis for a pair of genetic loci. Nonetheless we need epistasis to rigorously define co-adapted gene complexes. A gene complex is nothing more than two or more genetic loci with non-zero linkage disequilibrium. The adjective co-adapted, however, implies that there is some fitness advantage (selection) to having a pair of linked genetic loci (Willett & Burton, 2003; Takahasi & Tajima, 2005). While nobody is measuring the magnitude of co-adapted gene complexes (i.e. the magnitude of epistasis) for more than two genetic loci (Cheverud & Routman, 1995; Kouyos *et al.*, 2006), this has not stopped the rampant use of the term co-adapted gene complexes throughout the literature. This makes many hypotheses untestable. For example, consider one of the most commonly provided hypotheses for speciation (Dobzhansky & Pavlovsky, 1958; Marshall *et al.*, 2002). A single population becomes divided into two geographically isolated populations due to some environmental exigency (e.g. climate change, isthmus or rift formation). The two isolated populations each separately form co-adapted gene complexes (positive epistasis). At a later date, if the two populations again come in contact with one another, then they cannot viably interbreed because this would break apart the two co-adapted gene complexes (negative epistasis). Without a way to measure multi-locus epistasis, this hypothesis cannot be tested. This is a far cry from the hypothesis that polyploidy, which may have no selective advantage, results in speciation. Like linkage disequilibrium, epistasis probably severely inhibits the role of selection and enhances the role of drift. However, unlike with linkage disequilibrium, we can yet empirically test these effects of epistasis.

## Discussion

Charles Darwin opened our eyes to evolutionary mechanisms by which populations of interbreeding individuals evolve. Unfortunately, he was not privy to the genetic mechanisms by which many of these evolutionary changes were generated nor faithfully passed on to subsequent generations. Darwin understood that mutations increased variation, which was the fodder upon which evolution fed. He also understood that selection decreased variation and that the interplay of mutation and selection drove evolution. Darwin, however, did not and could not have understood the mathematical nuances underlying drift, which also reduces variation, nor the genetic mechanisms underlying polyploidy and gene-gene interactions. It is also peculiar that while scientists for sixty or seventy years accepted Darwin's ideas of descent with modification, that, unlike today, they generally did *not* accept his ideas about natural selection. Instead, neo-Lamarckian and mutationist notions ruled the day until Sewall Wright, Ronald Fisher, and John Haldane each independently united selection and Mendelian genetics (Provine, 1971). Ironically, these three founders of population genetics, especially Wright, simultaneously created theory of genetic drift and gene-gene interactions, which were largely ignored by evolutionary luminaries such as Ernst Mayr.

Cacti are large perennial plants, with fairly common polyploidy and remarkably small long-term effective population sizes. Long generation times and extreme endemism means that many cacti will have even more genetic drift than typical plants and animals. As any grower of *Sicobaccatus dolichospermaticus*, *Copiapoa cinerea*, or *Browningia candelaris* can assert, these plants can have longer generation times than humans. Most cacti are fairly narrow endemics, some extremely so, as with many *Pediocactus* species. As another example, cacti in eastern Brazil are also narrow endemics, living on metamorphic and sedimentary rock outcrops in a sea of flat plains that probably never saw cacti, even before human-cultivated sugar cane and eucalyptus plantations (Taylor & Zappi, 2004). There are a few wide ranging cactus species, such as *Opuntia polyacantha* and *O. fragilis*, but these are the exception and are often polyploid, indicating that effective population size is probably a fraction of what we would naïvely suspect. In fact, many of these polyploids appear to be parthenogenetic or completely asexual or at least rarely produce viable seeds,

meaning that effective population sizes are one or very low. Polyploidy results in speciation, but the new species are not necessarily any better adapted than their parent species. In fact, polyploidy probably triggers non-adaptive radiations in these groups.

Evolution of cacti, especially long-lived ones with small ranges and polyploid taxa, should be dominated by genetic drift and mutation. Selection should play relatively small roles in these taxa, despite adaptationist just-so-stories. Even convergent evolution for short squat succulent plants could merely be an ancillary effect of high polyploidy, where these plants simply cannot grow tall woody stems. Of course, these assertions are hypotheses that beg for rigorous testing using phylogenetic comparative methods

Not only should cactus evolution be dominated by drift, so should some other succulents. Agaves come to mind because of their even higher rates of polyploidy than cacti and their long generation times due to semelparity. Succulents in which the opposite is likely true, that selection may be more important than drift, would be annual species with low ploidy levels, such as some Aizoaceae.

Because of minimal wood development, most cacti do not fossilize and hence their phylogenies are constructed exclusively using extant taxa. These phylogenies have relied on both morphological and molecular characters. It is generally believed that the most informative characters for such phylogenies are neutral. These characters provide the plant with no selective advantage or disadvantage, thereby allowing us to distinguish convergent evolution from true relatedness. With neutral characters, it has been possible to build and test phylogenetic hypothesis about cacti, including recent robust in-roads into understanding how they are related to other succulent groups, such as *Ceraria*, *Portulacaria*, and *Didiereaceae* (Nyffeler *et al.*, 2008). With an emphasis on neutral characters, it should only be natural to consider that such neutral characters may play a huge role in evolution of these plants. I have argued here that we should largely abandon adaptationist arguments about cactus evolution premised on selection (Williams, 1966; Gould & Lewontin, 1979) and instead focus on genetic drift and its supporting cast of gene-gene interactions, inbreeding and polyploidy.

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