

REVIEW

The standard of neutrality: still flapping in the breeze?

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Abstract

Neutrality plays an important role as a null model in evolutionary biology. Recent theoretical advances suggest that neutrality is not a unitary concept, and we identify three distinct forms of neutrality. Eu-neutrality means that types do not differ in any measurable way and is thus the idealized form of neutrality. However, individuals or species that do differ in important ways can behave neutrally under some circumstances, both broadening and complicating the applicability of the concept of neutrality. Our second two types of neutrality address two quite different forms of context-dependent neutrality. Circum-neutrality means that two character states have the same direct effect on fitness but do not evolve neutrally because of differences in their circumstances. Iso-neutrality means that two types are equivalent in some population or ecological contexts but not in others, producing an isocline. Confounding of these different definitions has created significant confusion about which models are truly neutral, why some models behave neutrally even when there are large differences in reproductive outputs, and what these different views of neutrality mean to practicing biologists. These complications call into question the acceptance of neutral models as null models and suggest that a better approach is to compare the predictions of models that differ in sources of stochasticity and degree of selection.

Introduction

Two monks were watching a flag flapping in the wind. One said to the other, 'The flag is moving.' The other replied, 'The wind is moving.' Huineng overheard this. He said, 'Not the flag, not the wind; mind is moving' – The Gateless Gate (Yamada, 1979).

In every generation, barring freakish coincidence, the frequency of every segregating allele changes. This, we are taught in introductory classes, is evolution. But this near ubiquity of evolution is hardly a cause for celebration for evolutionary biologists, because it buries the wheat of 'interesting' change in the chaff of 'stochasticity'. Many of those alleles which had the temerity to change in frequency are, we suspect, charlatans that in

fact do nothing of importance to the phenotype and have only an undistinguished future as genotypes.

As evolutionary biologists, our interests often lie in those alleles that show consistent average directional changes in the short run or anomalously high probabilities of fixation over the long run. The abstractions encoded in 'average' and 'probability' mean that the simple accounting of allele frequencies for a single generation is not enough, but that some additional information, whether phenotypic or statistical, is needed to identify loci of interest. With that additional information, evolutionary theorists can hope to predict, at least on average, the directional dynamics of allele frequency and the resulting effects on phenotype. The charlatans are then revealed by their stochastic pattern of gene frequency change that does not relate to organismal adaptation. This distinction between directional and merely stochastic change has been central to the debate over the causes of observed variation in DNA sequences (Kreitman, 1996), and has reemerged in the debate over the nature of biodiversity (Chave, 2004). A central

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concern for evolutionary biologists is to understand the relationship between genotype and phenotype, and the relationship between phenotype and fitness. As we will discuss, a conceptualization of both the genotype–phenotype map and the relationship between phenotype and evolutionary dynamics is critical to developing a full understanding of the nature of neutrality.

Evolutionary biologists have long been interested in the causal forces responsible for observed variation. Darwin himself recognized that some changes in traits might be neutral (Duret, 2008). The early twentieth century featured substantial debate over whether obvious polymorphisms were neutral, indeed questioning the efficacy of natural selection (Robson & Richards, 1936; Hooper, 2002). For example, the demonstration that patterns on the shells of the snail *Cepaea nemoralis* has adaptive value in protection from predation by song thrushes (Cain & Sheppard, 1950), showed that apparent neutrality depends on the ecological context (the pattern against which the snail is viewed by predators). This opened the door to the programme of finding selective value for all traits, eventually maligned as naively pan-selectionist (Gould and Lewontin, 1979). If selection means no more than a consistent change in gene frequency as opposed to random fluctuations we will need to understand the distinct mechanisms that can cause such consistent changes. Of particular interest, here are the many ways that traits that do little in and of themselves have been found to be associated with selection.

Long before the advent of Kimura's neutral theory of molecular evolution, population geneticists were referring to alleles with no effect, or no consistent directional effect, as neutral or neutral on average (Wright, 1929; Fisher, 1930; Kimura, 1954). Organismal biologists occasionally adopt this language and describe changes in phenotype that are not associated with changes in fitness as neutral, as is the case for sex-allocation in large populations (Williams, 1979; Frank, 1990), and for life-history trade-offs (Fox, 1993; Parsons & Quince, 2007a,b). However, they more often speak of strategies that achieve equal fitness (e.g. Maynard Smith, 1982) or genotypes that map to the same phenotype (e.g. Rice, 1998).

Within population genetics, absence of selection on a trait is often equated with its having the fixation probability of a neutral allele (Kimura, 1954, 1962; Gillespie, 1974b; Frank & Slatkin, 1990; Gabriel & Bürger, 2000; Lieberman *et al.*, 2005; Lynch, 2007; Parsons & Quince, 2007b) or having an invasion exponent (deterministic or stochastic) equal to one (Orzack & Tuljapurkar, 1989; Tuljapurkar, 1990; Ratnieks *et al.*, 2007). Here, we focus on theoretical concept of neutrality as distinct from the neutral theory of molecular evolution. Our goal is not to determine whether or not an observed pattern has been produced by some type of neutral process, but rather to classify the ways that evolutionary biologists understand directional and nondirectional change in allele frequencies.

Our interest here is in differentiating between neutrality and directional forces in evolution. In the classic sense, neutrality implies that a pair of genotypes are identical in all aspects of their function and reproductive output and further that all changes in frequency are due strictly to random sampling. This is both a statement about causality of changes in frequency and a specific model for how the random effects accumulate. Unfortunately, these are not equivalent statements. There is mounting evidence that many processes do not impart directionality to the frequency dynamics in some contexts but nevertheless create a pattern distinct from the classic models of neutrality.

Distinguishing neutral and nonneutral patterns of genotypic change necessitates statistical testing. This review explores the situations in which nonneutral processes can generate patterns similar those produced by sets of neutral alleles and vice versa. If nonneutral processes can generate patterns identical to those of neutral processes, then no amount of statistical testing can distinguish these scenarios. Our goal is to engender a better understanding of the variety of processes that can produce seemingly neutral patterns and clarify the alternative meanings of neutrality currently in use in the evolutionary literature (Box 1). By classifying types of neutrality, we hope to find similarities between disparate phenomena while recognizing that a classification scheme is a tool to improve understanding.

Eu-neutrality

Eu-neutrality is the simplest, classical, form of neutrality. Two types (alleles, species, or traits) are considered to be eu-neutral if they are indistinguishable in terms of their effect on measurable, fitness-related traits, and this is true regardless of context. While eu-neutrality is trivial to define, it is not necessarily simple to model because even in this simplified scenario the dynamics depend on the details of demography and spatial structure.

Under eu-neutrality, two types are completely interchangeable regardless of their frequency, the environmental conditions, population size, etc (Chave, 2004). Eu-neutrality simply implies that no directional change in frequency is expected. This has been most often suggested for changes in DNA sequences that either have no effect because they occur in regions of the genome without coding or regulatory function or do not alter protein sequence. Such changes cannot be observed at any organismal level other than by directly sequencing the genotype.

A key feature of eu-neutrality is the context-independence of the equivalence of the types. Because they behave similarly in all contexts, the only possible causes of changes in frequency or spatial distribution are stochastic. Rice points out that in any observation of an evolving population there will be correlations between reproductive fitness and genotypes, but that these

Box 1: Categories of neutrality**Eu-neutrality**

Changes in genotype have no correlation with any fitness measure or fitness-related trait. This form of neutrality does not depend on context.

Circum-neutrality

Two sequences or traits are considered circum-neutral when all differences in their population genetic dynamics can be attributed to their genetic context. This means that the trait/sequence substitution has no direct effect on fitness or reproductive value. The trait/sequence substitution may be

correlated with some fitness measure but still have zero partial correlation with the fitness measure when other traits are controlled for. Circum-neutrality depends on the genetic context of traits/sequences and not on the direct effects of substitutions on function.

Iso-neutrality

Two types (trait values, sequences or even species) are equivalent in terms of some fitness measure in some environments or population contexts but nevertheless differ in their fitness-related phenotypes. The two types can only be considered to be neutral along some isocline that depends on the ecological or population genetic context.

changes might not be caused by differences in phenotype (Rice, 2008). However, the theoretical prediction is that repeated observations of a population containing only eu-neutral genetic variation would show no statistical association between genotype and fitness. Likewise, permuting individual genotypes (say by shuffling individuals between replicates) would not alter the statistical properties of the observations. The fundamental identifier of eu-neutrality is that the labels of individuals (their genotypes or species identity) can be shuffled without altering the expected dynamics (Chave, 2004).

Circum-neutrality

We now consider pairs of traits or sequences that have the same direct functional effects but still differ in their evolutionary dynamics. For example, multivariate genetic correlations as measured by the G matrix may cause traits that are not directly under selection to evolve as a correlated response (Lande & Arnold, 1983; Lynch & Walsh, 1998; Stepan *et al.*, 2002). In this context, a trait may have no direct causal effect on fitness and yet still behave in a way that reflects a directional evolutionary trend. We consider two distinct ways that circum-neutrality can occur. The first is when the genetic architecture leads to correlated evolution because multiple traits share a common genetic basis (i.e. pleiotropy) or are physically linked to the loci that code for the focal trait (Lauder, 1996). Both pleiotropy and linkage depend, to differing degrees, on the evolutionary history of populations, but linkage is a particularly volatile population level property, and we therefore focus primarily on the pleiotropic properties of genetic architecture. The second is through the mutational architecture and occurs when genotypes that are identical in terms of their function will differ in their position within the network of genotypes linked by mutation. Because such pairs of genotypes differ in their mutational context they will have different expected long-term fates even though all single generation measurements would suggest that they

are neutral substitutions (i.e. the coefficient s would be observed to be 0). In each of these cases, it is not functional differences between traits that cause differences from neutrality but rather the context of a trait that causes a deviation from neutrality. Circum-neutrality means that two traits or sequences have evolutionary dynamics that only depend on what happens around them, not what they do themselves.

In effect, circum-neutral trait substitutions appear neutral in terms of their effects on function but do not behave neutrally in terms of their population level dynamics. One important factor to keep in mind is that many trait substitutions may appear to have no direct fitness effect on first glance, but have significant fitness effects on closer examination. For example, a substitution that increases the recombination rate would have no effect on adult phenotypes or the number of offspring produced, but would have an effect on the mean reproductive value that the allele conferring an increased recombination rate finds itself in. Thus, recombination modifiers have a direct effect on the RV of their offspring alleles, and therefore spread (when they do spread) because they are not neutral. Even more subtle examples include meiotic drive such as biased gene conversion (Galtier & Duret, 2007). In this case, some alleles spread because they replicate at greater rates within organisms and should therefore be considered nonneutral substitutions.

Genetic architecture

Lynch (2007) has recently collected an impressive array of processes that can cause genome evolution to depend on population size. The crux of these arguments is that many traits related to genome structure might, in and of themselves, be selectively neutral, but because they are correlated with a weak deleterious effect they are effectively removed by selection in large, but not small, populations. One of the most successful applications of this reasoning applies to the proliferation of introns (Lynch, 2002).

Many arguments had been previously proposed to explain how the number of introns in eukaryotic genomes has increased over evolutionary time, including an increase in evolvability via exon shuffling, a benefit of alternative splicing, and an increase in recombination (Roy & Gilbert, 2006). In each of these hypotheses, it is the presence of the intron/exon itself that causes a change in function and fitness and leads to the expectation that the trait 'intron number' is expected to increase.

In contrast, Lynch considers the hypothesis that a change in the number of introns per gene does not have a significant effect on modularity or evolvability and therefore does not have a direct effect on organismal fitness. Instead, the effect of intron number on evolution comes from a pleiotropic effect on the deleterious mutation rate. Each intron includes a tract of DNA that can mutate, and mutations in the sites involved in intron excision may eliminate gene function. An allele with a larger number of introns is more likely to produce offspring that have deleterious mutations, and this is functionally similar to a negative selection coefficient. We can consider separately the trait 'intron number' and the trait 'gene-specific deleterious mutation rate'. On average, an allele with a greater number of introns (and the same exon sequence) will have a higher deleterious mutation rate, so the two traits are correlated. In this example, the pleiotropic effect that mutations increasing intron number tend to increase the deleterious mutation rate can be considered a property of the genetic architecture. Selection acts to limit the increase in the deleterious mutation rate and, as a by-product, reduces the spread of introns in species with large population sizes.

In principle, one can break the correlation by engineering a two-intron allele that has a lower deleterious mutation rate than another engineered one-intron allele, for instance by altering the likelihood of forming pyrimidine dimers. It is therefore the correlation between the traits 'intron number' and 'rate of deleterious mutation' that potentially leads to a pattern where reduced population size opens the door for an evolutionary increase in intron number (Lynch, 2002).

Mutational architecture

Detailed analyses of the genotype–phenotype map have advanced the idea that sets of genotypes linked by mutation can produce the same phenotype (Taverna & Goldstein, 2002; Cowperthwaite & Meyers, 2007). This means that alleles with the same phenotype differ in the probability that they will produce, through mutation, offspring with alleles that differ in phenotype and have low fitness. Even alleles that have the same phenotypic mutational spectrum can vary in their long-term likelihood of producing lineages with mutant phenotypes. In this case, there is no correlation between allele value and fitness for a broad set of alleles, and yet the future complement of alleles in the population depends on the

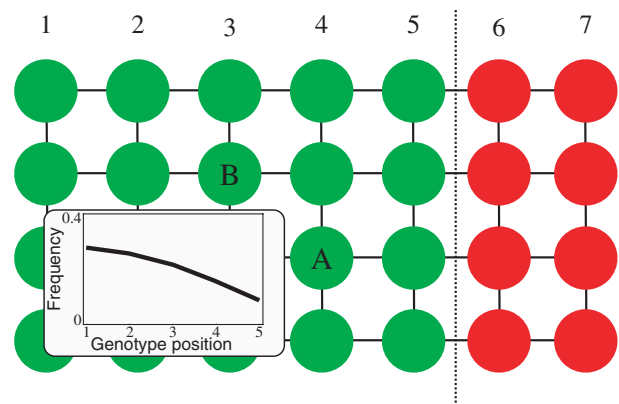


Fig. 1 A mutation network. The circles represent genotypes and the solid lines link mutational neighbours. All of the green genotypes produce the same, selectively favoured trait. The red genotypes do not. Types A and B are identical in their phenotypes, and in their offspring phenotypes. If all mutations have the same probability, genotype B will make a greater contribution to future generations than genotype A. They are therefore not eu-neutral because they are not interchangeable. The inset graph shows the frequency of the green alleles at equilibrium as a function of their column (numbered 1–5).

mutational network (Fig. 1) (van Nimwegen *et al.*, 1999). In particular, all else being equal, the population is expected to evolve farther away from the edge of the fitness-cliff. This principle, that the density of genotypes in the population tends to move away from the cliff, can be translated into a statement about neutrality in that two alleles in the neutral network can be considered to be circum-neutral.

This approach has been extended to consider competition between quasi-species, where each quasi-species represents a cloud of mutationally linked genotypes (Eigen, 1971; Wilke *et al.*, 2001). In this context, the mutational network that has a wider, flatter fitness plateau can be considered more robust to mutations and will achieve a lower fitness load and come to dominate the population (Wilke & Adami, 2003). However, this effect is dependent on the population structure: under population subdivision quasi-species that produce mutations with more deleterious effects eventually dominate the metapopulation (O'Fallon *et al.*, 2007). Alleles in the different mutational networks can be circum-neutral, but the quasi-species dynamics are nonneutral and depend on population structure.

Iso-neutrality

Frequency dependence is a central concept in population biology (Heino *et al.*, 1998). At its core, frequency dependence simply means that the relative fitness of an allele depends on the genetic context of the entire population, often because individuals have direct

behavioural interactions or have interactions that are mediated by the environment. Thus, two alleles coding for differences in behaviour may have equal fitness for some frequencies but not for others. In particular, if a polymorphism is maintained at a stable equilibrium then changes in behaviour are neutral because both behaviours experience identical returns (Charnov, 1982; Maynard Smith, 1982; Frank, 1990; Hedrick, 2006). Deterministic frequency dependence is relatively straightforward to interpret because the causes of frequency dependence come from known interactions. Many theorists have defined models without a predefined mechanism of frequency dependence (Clark, 2009), but we now know that the emergence of frequency dependence is ubiquitous. Thus, under any theoretical scenario where alternative strategies are defined by trade-offs, neutrality can only be achieved in a context-dependent way. We term this form of neutrality iso-neutrality because there will generally exist an isocline in parameter and allele frequency space along which the strategies are neutral with respect to some choice of fitness measure.

We have developed the concept of eu-neutrality to refer to allelic or species identity substitutions that are strictly 'label-changing operations' and do not alter fitness in any context. However, individuals may differ in fitness-related phenotypes but, because of trade-offs, still perform similarly in terms of reproductive output and population dynamics. To make this concept more concrete, we must first describe precisely what is meant by neutral in terms of population dynamics.

To many researchers, neutral should mean that gene frequencies change according to either a binomial sampling process (in the context of discrete, nonoverlapping generations, i.e. the Wright–Fisher model) or follow a Moran process (in the context of continuously reproducing populations), producing the same sampling properties if time is re-scaled appropriately (Leigh, 2007). When this is the case, the expected frequency of each allele at any future time is simply its current frequency, and gene frequencies follow a random walk with no directional component (Lande, 2007; Lenormand *et al.*, 2009). Because fitness measures depend on context (intergenerational effects Mangel *et al.*, 1994; migration effects Ronce, 2007; maternal effects Wolf, 2000), we must be precise about how we can identify long-term neutrality.

We will first discuss some general issues regarding the measure of neutrality in nondeterministic models. We then discuss four scenarios in which iso-neutrality has been independently discovered. Based on this analysis, we suggest that iso-neutrality is likely to be the most common form of neutrality observed in nature.

Fixation probabilities are an (almost) universal measure of neutrality

Consider a population where the frequency of two competing types changes over time. If we can calculate

the mean change in frequency as a function of current frequency, $E[\Delta p]$, then we can draw a few conclusions about the evolutionary process and about the long-term expectation as measured by the fixation probability, $U(p)$. First, in general, if $E[\Delta p]=0$ for all p , then $U(p)=p$. This simply says that whenever there is no short-term directionality then fixation probabilities are the same as for a neutral substitution (Frank & Slatkin, 1990; Proulx & Day, 2002). In effect, short-term measures and long-term measures of the direction of evolutionary change are concordant (Lande, 2007). In addition, for fixation probabilities to be identical to the neutral expectation for all initial frequencies, short-term change must also be free of directional tendencies for every current frequency. Thus, we can consider $E[\Delta p]=0$ to be a statement of short-term neutrality and $U(p)=p$ as a measure of long-term neutrality with the comforting conclusion that a process which is entirely neutral in the short-term is also entirely neutral in the long term.

We can further classify systems where the short-term change is consistently nonneutral, i.e. when $E[\Delta p]\neq 0$. In particular, if the mean change in allele frequency is always positive, then $U(p)>p$, regardless of the initial frequency, p . Again, short-term and long-term measures are consistent. Finally, if the sign of $E[\Delta p]$ changes with p , then we know that both long-term and short-term measures of evolutionary change are only neutral for some initial frequencies. The initial frequencies that show neutrality in terms of $E[\Delta p]$ are generally not the same as the initial frequencies that show neutrality in terms of $U(p)$. It is this scenario that we refer to as iso-neutrality.

Thus, when the fixation probability can be directly calculated, it is probably the best measure of neutrality or lack of neutrality (see the recent review on calculating fixation probabilities by Patwa & Wahl, 2008b). However, fixation probabilities can be difficult to calculate (and are even more difficult to measure), and because the mean change in allele frequency can reveal neutrality in the evolutionary process, we will often rely directly on the mean change in allele frequency to infer neutrality.

Iso-neutrality is inevitable (except for eu-neutrality)

In the abstract, two genotypes could differ along multiple phenotypic axes and yet still be exactly equal in terms of their mean fitness. However, on closer inspection two alternative ways of producing the same mean fitness are likely to differ in their variance or in even higher moments. Such differences in stochasticity necessarily emerge from models that include life-history trade-offs, because shifting mortality risks and birth rates necessarily has an effect on both the mean of offspring production and the variance in offspring production (Gillespie, 1974a; Bulmer, 1985; Demetrius & Ziehe, 2007; Shpak, 2007). We expect that most situations where two distinct alleles initially appear to be neutral will be revealed to be iso-neutral on closer inspection.

Classical theory for evolution under stochasticity was derived by considering demographic stochasticity and year-to-year environmental stochasticity separately (Dempster, 1955; Cohen, 1966; Gillespie, 1973, 1974b). This has often meant that models of environmental variation implicitly assumed infinite population size, whereas models of demographic stochasticity took population size to be finite. More recent approaches have advocated including both sorts of effect (Frank & Slatkin, 1990; Lande, 2007, 2008), even to the extent of defining models that track statistical features of phenotype distributions exactly (Rice, 2004, 2008).

Four routes to iso-neutrality

Variance between years

It has been long believed that when selection coefficients vary between years in an infinite (but unchanging in size) population then strategies with a larger geometric mean selection coefficients are favoured (Dempster, 1955; Cohen, 1966; Gillespie, 1973; Felsenstein, 1976). This conclusion would suggest that if the geometric mean is the appropriate measure of fitness, then two strategies that achieve the same geometric mean selection coefficient, but do so by different mechanisms, should be eu-neutral. The classical argument, however, is based on following infinite populations over an infinite amount of time, and therefore does not consider either fixation via drift or the short-term evolutionary dynamics that lead to the long-term pattern. An illustrative case is to consider two haploid genotypes in a population of fixed size where each allele has the same geometric mean offspring production in response to year-to-year fluctuations in the environment. Even though each type has the same geometric mean fitness, the short-term dynamics point towards an increase in frequency of the rare type, similar to balancing selection (Fig. 2). As suggested by the short-term dynamics, fixation of the rare allele is more likely than in the eu-neutral case (Fig. 2). In a situation where two strategies achieve exactly the same geometric mean, neutrality is violated at both short and long timescales, and the two strategies are iso-neutral when present at an intermediate frequency.

Of course, the two alleles need not have the same geometric mean, but there will still be a point of iso-neutrality in both the short-term and long-term measures, as long as the difference in geometric mean between the alleles is not too great (Lande, 2007). Lande has recently shown, in a continuous time model, that the long-term growth rate of the population is maximized at the iso-neutral point where the mean change in allele frequency is 0. His conclusion is that the population evolves towards this fitness peak, but cannot rest there because stochastic forces always overwhelm the stabilizing forces (Lande, 2007). The presence of an internal iso-neutral point is similar to frequency-dependent selection and has a similar overall

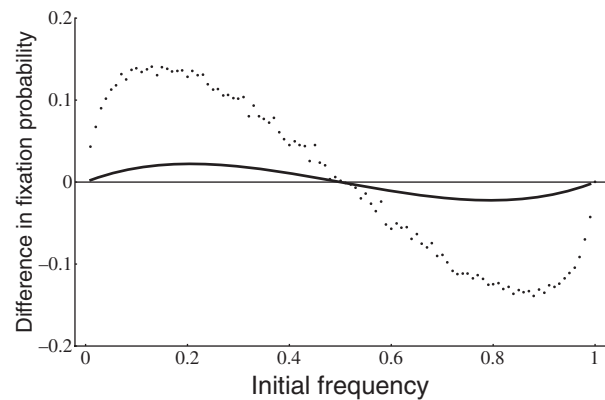


Fig. 2 Expected dynamics under simple year-to-year variability. Two genotypes are present in this simulation and the relative fitness of allele 1 in a given year is $\frac{1}{2}$ or 2 with equal probability. There is no temporal autocorrelation. The solid curve shows the expected change in allele frequency, whereas the points show the difference between the observed fixation probability and the neutral fixation probability. Population size was set to 100. Fixation probabilities were obtained by performing 50 000 simulations.

effect in that coexistence is promoted, even if it is not stably maintained over long evolutionary time scales. This can also be expressed as a kind of built-in propensity for genetic change created by variability in selection coefficients (Frank & Slatkin, 1990; see also Mustonen & Lässig, 2009).

Variance in offspring number

Reproductive success of individuals is highly unpredictable, but genotypes may also differ in the degree of buffering against environmental contingencies, leading to different levels of variance. Gillespie (1974b) approximated this process with a diffusion by making specific assumptions that rescale time with population size. Gillespie's approach showed that a trade-off exists between mean offspring production and the variance in offspring production, and that this trade-off depends on the context of population size. Using Gillespie's diffusion approximation, Taylor (2009) has shown that coalescence times of even unlinked loci are affected by this process. While this general prediction has stood the test of time, this trade-off is now recognized as involving frequency dependence. Proulx (2000) demonstrated that the mean change in allele frequency is approximated by

$$E[\Delta p] \approx \frac{p(1-p)}{\mu_1 p + \mu_2 (1-p)} \left((\mu_1 - \mu_2) + \left(\frac{\mu_1}{\sigma_1^2} - \frac{\mu_2}{\sigma_2^2} \right) \left(\frac{\sigma_1^2 \sigma_2^2}{N(\mu_1 p + \mu_2 (1-p))^2} \right) \right) \quad (1)$$

where p is the frequency of allele 1, μ_i is the mean offspring production of allele i , σ_i^2 is the variance in

offspring production of allele i , N is the population size, and higher order moments of the offspring production are neglected (See also Shpak & Proulx, 2007). This formulation holds when population size is fixed and culling is fair, but need not be described by binomial sampling. The rate of change represented in eqn 1 is scaled by the current allele frequencies because the effect of drift is greatest at intermediate frequencies, but the direction of change is affected by both the difference in mean fitness and the difference in the variance-scaled mean fitness. This second component is weighted by the inverse of the population size and is also a function of allele frequency, so that the net effect is context dependent. Equation 1 shows that the direction of short-term evolutionary change is affected by two aspects of the population context; population size and gene frequency.

Rice (2008) recently developed a different approach to address demographic stochasticity. He developed a stochastic version of the Price equation that defines a relationship between the mean change in gene frequency (or more generally, phenotype) and several aspects of variance and covariance of fitnesses. The expression for mean change in allele frequency can be written as

$$E[\Delta p] \approx \frac{\text{cov}(i, \mu_i)}{H(\bar{w})} - \frac{\text{cov}(i, \sigma_i^2)}{N\hat{w}^2} \\ = \frac{p(1-p)(\mu_1 - \mu_2)}{H(\bar{w})} - \frac{p(1-p)(\sigma_1^2 - \sigma_2^2)}{N(\mu_1 p + \mu_2(1-p))^2} \quad (2)$$

where \bar{w} is a random variable describing the per-capita offspring production in a given year, H represents the harmonic mean, \hat{w} is the expected value of average population fitness, and higher moments of the distribution of offspring numbers are ignored. The formulations of Rice and Proulx differ in the way that terms are grouped, but inclusion of all higher order terms in either approximation recovers the exact expression for the expected mean change in allele frequency. Rice's version highlights the fact that the variance in reproductive output determines the direction of change in allele frequency when the means are equal, and can also be used to determine the effect of differences in moments of higher order than the variance.

Regardless of the method of derivation, both short-term and long-term measures of selection depend on population size and gene frequency (Proulx, 2000; Shpak, 2005, 2007; Rice, 2008). Figure 3 (panel a) shows an example of this, the allele with both lower mean and variance in offspring production is expected to spread when population size is small or the frequency of the allele is high. In the region of population sizes where the isocline exists, fixation probabilities tend to be lower than p for low initial frequencies and higher than p for high initial frequencies, indicating that the common allele has an advantage simply because it is common. This will affect genealogical patterns, such as coalescence times, so

that even when all genotypes have equal long-term probabilities of success a signature of nonneutral evolution will be observable. Taylor (2009) has recently derived results for the coalescence process at eu-neutral sites linked to loci that affect fecundity variance. When the segregating loci meet Gillespie's (1974b) equivalence rule coalescence times are shorter than under drift and variance in family size alone. Thus, demographic stochasticity and environmental variation have opposite effects on the probability that a population will switch between two iso-neutral alleles.

Life-history trade-offs

A long-standing debate in evolutionary theory concerns the use of two related fitness measures, the Malthusian parameter r and the basic reproductive ratio R_0 . The Malthusian parameter incorporates the timing of reproduction, placing greater weight on earlier reproduction in growing populations, whereas R_0 simply sums up lifetime reproductive output. When population size is regulated, even by genotype-independent density-dependence (Metz *et al.*, 1992; Stearns, 1992), these two measures are not equivalent. Although it is not difficult to concoct different life-history strategies that achieve the same r , and only slightly more difficult to add the requirement that the two life histories share the same R_0 , these two measures of success are accurate only in certain idealized conditions (Charlesworth, 1980; Stearns, 1992). In general, the fitness measure that determines success depends on the whole life history, and two species cannot share these measures of success unless they are indeed identical. Different life histories can therefore represent another class of iso-neutrality. Several recent studies have investigated the joint effects of variation in population size and aspects of life history, including the lytic strategies of viral pathogens (Wahl & DeHaan, 2004; Hubbarde *et al.*, 2007; Alexander & Wahl, 2008; Patwa & Wahl, 2008a) and variation in the instantaneous rates of birth and death (Parsons & Quince, 2007a,b; Parsons *et al.*, 2008).

Parsons & Quince (2007a,b) define a model that incorporates both stochasticity in individual demography and changes in population size as a whole. Their model is similar to the Moran model in that strategies are defined by an instantaneous birth rate and death rate. By keeping the ratio of births to deaths constant among strategies, they define a scenario where the strategies can be thought of as neutral in that they have the same net reproductive rate (Parsons & Quince, 2007b). They were able to derive expressions for the fixation probability as functions of the initial allele frequency and population size which can be compared to the eu-neutral expectation.

$$U(p) = p + \frac{\lambda_2 - \lambda_1}{\lambda_1 + \lambda_2} p(1-p) \quad (3)$$

where λ_i is the birth rate of type i . This expression indicates that the fixation probability of an allele arising at

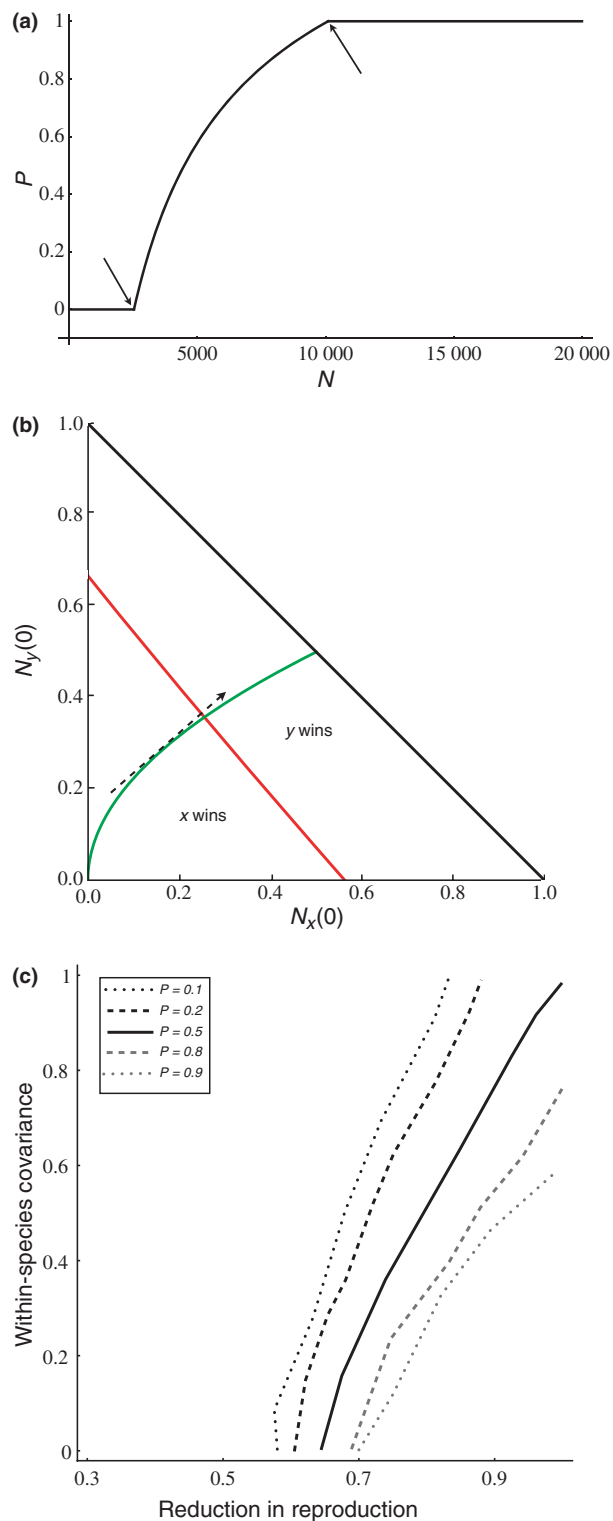


Fig. 3 Fitness isoclines for several scenarios. Panel a shows the isocline for demographic stochasticity as a function of initial frequency and population size. Allele 1 produces an average of 1 offspring with a variance of 1, whereas allele 2 produces a mean of 2

offspring with a variance of 10. To the left of the curve, allele 1 is expected to increase in frequency, whereas to the right of the curve allele 2 is expected to increase in frequency. The arrows indicate critical population sizes, below and above which the expected direction of allele frequency change does not depend on current allele frequency. Panel b reflects the situation modelled by Parsons and Quince. Type x has twice the birth rate, and twice the death rate, of type y . The population dynamics include a stochastic equilibrium where the total population size is scaled to unity, indicated by the solid black line. The green curve shows a sample of the mean trajectory of a population starting at low density, where the dashed tangent arrow indicates the direction. Below the carrying capacity, type x is expected to increase more quickly than type y . However, once the population nears the carrying capacity the greater variance of type x becomes a burden and type y is more likely to become fixed than a neutral variant. Below and to the left of the red line, type x achieve a large enough change in frequency to overcome this effect and is fixed with a probability greater than the initial frequency of type x . Once the population passes above and to the right of the red line, however, the deleterious effect of high variance overwhelms any tendency of x to increase in frequency before the carrying capacity is approached. In panel c, the contours show isoclines of the trade-off between reduced reproduction and reduced within-species covariance for a range of values of the initial frequency p . The contours show where the fixation probability is equal to the initial frequency when in competition with a species that has perfect within-species covariance and zero between-species covariance. At low initial frequency, fixation probabilities exceed initial frequency because of both the storage effect and the benefits of reduced within-species covariance. The top of the black dotted line where within-species covariance is 1 shows that a species can tolerate a 20% reduction in reproduction (pure storage effect), whereas the bottom of that line shows that the tolerable loss of reproduction increases to 40% when within-species covariance is zero (additional benefit of reduced variance).

frequency $1/N$ decreases as population size goes up, even though $U(p)$ is population size independent. This large population size limit of $U(1/N)$ is bounded above by $2/N$, and thus has a substantially lower bound than for deterministically selected alleles which fix with probability $2s$ in large populations (Crow & Kimura, 1970). Given this expression, a curve of iso-neutral behaviour can be defined (Fig. 3 panel b), where the strategy that has the higher birth-rate and higher variance prevails when the population is growing quickly but is dis-favoured when the population is already near carrying capacity. Here, the context of iso-neutrality is the gene frequency and current population size relative to the carrying capacity.

The storage effect and within-species covariance

Imagine the following scenario. In a survey of the *Arabidopsis* genome, a single nucleotide polymorphism (SNP) is found in the middle of a gene thought to have something to do with drought tolerance. Plants with different alleles do not have obvious phenotypic

differences, and careful measurements over the years in a wide range of natural environments show that carriers of two alleles not only have the same average fitness, but the same distribution of fitnesses (Kolmogorov–Smirnov test, $P > 0.2$). The allele, one might think, is neutral. Yet other evidence, via biogeographic studies and linkage analysis, points to rather strong balancing selection. What is going on?

In one case, one might find that the fitnesses are correlated with rainfall, and that one allele does better in dry conditions and the other in wet conditions. The distribution of environments encountered just happened to produce the same fitness distribution. Were we to redo the experiment under controlled conditions, one or the other allele would be favoured. Apparent neutrality would be the result more of low power to detect differences than of a lack of phenotypic effect.

But in another case, the two types might have fitness uncorrelated with rainfall or other environmental covariate measured (Clark *et al.*, 2007). It is only by looking at the fitnesses of multiple individuals with each allele that we notice that the fitnesses within genotypes in a given year are more similar to each other than expected. Within-genotype covariance exceeds between-genotype covariance. And this pattern of covariance is enough to maintain the two alleles through balancing selection. Although the allele does not affect the distribution of fitness, the pattern of covariance indicates that it somehow alters response to the environment. If neutral is as neutral does, this is certainly not neutral, yet a solitary individual seems unaffected by the allele.

The simulation results in Fig. 3c treat precisely this situation. We follow a population of constant size, where in each time step some fraction of individuals die to be replaced via a lottery by descendents of all individuals from the previous time step. The reproductive outputs of those individuals are created by exponentiating a normally distributed matrix of values with set values of the covariances within and between species. We show that low levels of within-species covariance can balance out intrinsically lower mean fitnesses. Although the variance of individual reproduction is the same, lower levels of covariance reduce the variance of reproduction of the whole species, affording the same advantage seen by reducing variance in reproductive success. These results at the individual level parallel those described by the storage effect at the population level. The storage effect produces long-term coexistence (and frequency-dependent probabilities of fixation) based on space-limited populations with different responses to environment and long-lived life stages that can persist through unfavourable environments (Warner & Chesson, 1985). Interestingly, this effect could not be detected by measuring the phenotypes of *individuals* because it emerges from the correlations between individual phenotypes within and between years.

Discussion

We have attempted to provide a classification scheme to aid in understanding the causal relationship between genetic and phenotypic variance and natural selection. In our framework, eu-neutrality represents the purest form of neutrality, where sequences do not differ in their function or their context, and therefore evolve following neutral patterns. Circum-neutral character pairs appear to be neutral, but do not behave neutrally because they differ in their circumstances. However, a pair of circum-neutral alleles that are artificially held in the same context would indeed become eu-neutral. Iso-neutral pairs of alleles have direct functional differences that lead to differences in phenotype, and yet sometimes (but never always) experience neutral changes in allele frequency. The behaviour of iso-neutral alleles depends explicitly on the ecological context.

Allelic substitutions that have a small effect on fitness also experience a context-dependent effect where population size determines the degree to which drift governs their fate (Ohta, 1992). The fixation probability of nearly neutral substitutions approaches the neutral expectation in the limit as population size becomes small rather than showing an iso-cline in parameter space. Nearly neutral substitutions can be considered iso-neutral as the ecological context determines whether or not they behave neutrally.

Evolution is a stochastic process (Lenormand *et al.*, 2009). The outcome of any evolutionary process is not a single result; it is at best a probability distribution of possible outcomes. Theoretical biologists attempt to corral the random aspects of evolution by separating the set of possible outcomes into qualitative results: ‘Allele *A* is more likely to become fixed than allele *a*.’ ‘The mean value of phenotype approaches *X*.’ ‘Species distributions are on average *Y*.’ Often, the goal of the theorist is to be able to boil down a set of parameters into a formula that allows a conclusion about the likely direction of short- or long-term change. If the characteristic feature of neutrality is that phenotypic change is not biased in a particular direction (Rice, 2008), then there are surprisingly few scenarios that produce it.

From the scientific perspective, the moving flag in the Zen koan at the beginning of this article is a marker of meteorological conditions, much as changes in genotype frequencies are markers of evolutionary conditions. The evolutionary meteorologist uses the movement of the flag to evaluate the present, and to predict both the relatively easily extrapolated near future and the potentially more chaotic distant future.

The success of neutral models to predict patterns in real data challenges us to rethink which twitches of the flag are worthy of attention. Our goal has been to argue for two main points. First, we need different definitions of neutrality so we can better understand what different researchers mean when they claim to be using a neutral

model. In particular, circum-neutral hypotheses should be distinguished from eu-neutral hypotheses rather than use the blanket term neutral to describe a modelled scenario where one aspect of phenotype is currently not under selection.

Second, we argue that neutrality is not as simple as it may look. Our analysis suggests that eu-neutrality is rare, perhaps vanishingly rare. Our review concentrated on models with simple genetics, but more complicated genetics are not likely to make the outcomes more simple (e.g. Lande, 2008). The often-heard claim that 'we used a neutral model because it is a null model' should be met with scepticism: A simple model does not necessarily derive from a natural assumption. Further, our exploration of iso-neutrality suggests that eu-neutrality is difficult to achieve. The most common claim of neutrality is that selection coefficients against a particular change are so small that selection is inefficient and drift dominates. But this is well known to be a context-dependent fact in that fixation probabilities are more similar to their eu-neutral values in small populations. Population size is just one ecological factor that can determine the context of the evolutionary process, and variation along the other ecological axes we have discussed will also shift traits between the neutral and the nonneutral column. While the nearly neutral view of molecular evolution requires that the fitness effect of substitutions be small relative to the inverse of population size (Ohta, 1992), iso-neutrality can hold even when alleles have grossly different phenotypes.

But our Zen monks concluded that the movement was in neither the flag nor the wind, but in the mind. Our minds, via conceptual or mathematical models, describe and communicate that movement. But, of course, there are variants and extensions of this koan. In one, it turns out that a monkey is moving the flag by shaking the rope. We must have the humility to realize that our proposed mechanisms, whether apparently simple or not, are indeed models that can be made utterly irrelevant by new information. We can never know how many ways monkeys can shake the rope to create precisely the 'random' fluttering characteristic of unchanging weather (Taylor, 2009). The modification of a eu-neutral scenario to more and more elaborate circum-neutral models runs the risk of becoming a storytelling exercise (Lynch, 2007; Hahn, 2008); a circum-neutral model with many parameters can always be made to fit observed data. The way to move forward is not to refute or accept a 'neutral model'; it is to test alternative hypotheses that differ in their assumptions about how selection acts.

In another ending, Miaoxin (the Zen equivalent of a graduate student) overhears monks discussing this koan, and after insulting their understanding explains, 'it's not the wind moving, it's not the flag moving, it's not the mind moving'. At this, all the monks realized enlightenment and left without visiting the Master they

had come to see. This level of enlightenment may exceed that appropriate for scientific discourse, and we hope that arguments over neutrality continue to be more than semantic exercises in which the lips move and the mind remains still. Our point in writing this review is not to develop a new rigid framework into which all forms of evolutionary change can be classified, but rather to encourage further exploration of the kinds of dynamical processes that produce evolutionary change.

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