The Neutral Theory of Evolution

Geoffrey K. Chambers*
School of Biological Sciences, Victoria University of Wellington, PO Box 600, Wellington 6140

Introduction

Today most people are perfectly happy to accept Charles Darwin's ideas about '*evolution by means of natural selection*' as the dominant paradigm in biology. So many of us may be quite surprised to know that this has not always been the case among professional biologists. First, the very idea of evolution as '*descent with modification from ancestral forms*' predates Darwin (see below). Second, during his own lifetime Darwin's account was overshadowed in the imagination of the Victorian public by Robert Chambers' 1844 speculative work *Vestiges of the Natural History of Creation*. This book invokes quite different processes driving evolution – sometimes called a mixture of magick plus the '*inheritance of acquired characteristics*' (and following Jean-Baptiste Lamarck in this latter idea). However, it was Darwin's version that the scientists of the day preferred. His greatest achievement became recognised as his hypothesis of '*natural selection*' being the most rational explanation of the process driving evolution. This makes the notion of evolution *per se* logically acceptable as accounting for the history of life on our planet.

So, it is almost unthinkable that during the succeeding century Darwin's ideas would face serious challenges and even outright rejection from biologists. Even more so that this happened twice! Indeed, today it is well and widely understood that evolution will still proceed even in the absence of natural selection.

Biologists and philosophers now recognise that a key vulnerability in Darwin's writing was his very sketchy knowledge of genetics. Specifically, it is our later knowledge of mutational processes and the distribution of naturally occurring genetic variants that led to conflict with Darwinian thinking. This article is concerned with the second of these periods of controversy arising from Motoo Kimura's so-called *Neutral Theory*. The author of this present article devoted a large part of his early career to participation in laboratory investigations around this question and these experiences form the basis for this account. But, before one can begin to explore this topic, it is necessary to examine its origins.

*Correspondence: geoff.chambers@vuw.ac.nz

Historical background

The first few decades of the twentieth century did not start well for Charles Darwin's ideas about the underlying mechanism of evolution. In contrast, the idea of biological evolution itself survived intact and perfectly acceptable. It remained pretty much as first formulated during the Enlightenment Period (Box 1).

Box 1 The truth of evolution

French enlightenment-period scientists, notably Buffon, Cuvier and Geoffroy, prepared the way for the acceptance of the whole idea of evolution based on new information about the fossil record and new studies on anatomical relationships between living organisms. The emergent argument goes along these lines:

1. The earth and rocks are filled with the remains of strange plants and animals, some enormous in size.
2. These organisms were alive in the past but are now genuinely extinct (*v*. simply hiding behind a bush in the local park waiting for someone to stumble over them.
3. Those creatures presently living are clearly different from those living in the past but do resemble them in many ways.
4. Remains of these modern organisms are not (for the main part) found among fossil strata.
5. There must be some process by which these old and now extinct creatures were replaced, or there would be nothing walking on the face of the Earth today.
6. Therefore, it is contingent upon these facts for scholars to think that these new creatures have replaced the old ones and are derived from them by some means or other.

And amazingly enough, there it stops. Nobody came up with an explanation for how one set of beings evolved into another. Attempts were made, including by Louis Agassiz, who postulated up to 50 episodes of '*special*'* creation at the hand of the Almighty. Charles Darwin is the person who first described the causal process of Natural Selection to explain biological succession.

*Special in the sense of not included in The Bible.

Geoff Chambers is an Alumnus Scholar in the School of Biological Sciences at Victoria University of Wellington. He is a molecular biologist with research programmes in Human Ancestry and Health and in Wildlife Conservation. His early training in protein chemistry at Leeds University, UK, and fascination with naturally occurring variants of metabolic enzymes led him to take up postdoctoral fellowships in the USA and Australia and delivered him right into the middle of the debate that forms the subject matter of this article.

Remarkably, Darwin's central concept of natural selection was rejected by both of the two major British schools of biological thought. The Naturalists (holistic thinkers) and Experimentalists (reductionist thinkers) had both fairly readily adopted Mendel's ideas about genetic laws following their earlier rediscovery. The biologists' problems stemmed from new knowledge about '*mutations*'. Typically, these involved single genes, were of big effect and nearly always caused bad things to happen to living organisms. This meant that mutations imposed a sort of '*genetic load*' on populations in terms of losses through mortality. Hence, it seemed unlikely that a mutational mechanism would create the sorts of advantageous changes that natural selection was thought to favour. Darwinian thinking fell out of fashion and was relegated along with Lamarckian thinking (aka '*inheritance of acquired characteristics*') as most unlikely candidates as causal mechanisms responsible for directing evolutionary change (see Mayr, 1982, p. 547–548 for some fairly alarming quotations from this period).

Darwin was saved only by a scientific revolution known as '*The Modern Synthesis*'. This was an exercise in theoretical population genetics which came up with single-gene models to explain how mutational variants could remain in populations even when they were deleterious (aka '*balancing selection*'). Their models were couched in terms of '*selection coefficients*' (more about these later). This new '*Neo-Darwinian*' programme began well because biologists like Theodosius Dobzhansky and others were able to measure selection coefficients associated with chromosomal inversions in *Drosophila* and successfully test the theoretical predictions by running competitions between selected lines of fruit flies in devices called '*population cages*'. So, everything seemed to be going along fine until molecular methods made it possible for biologists to begin to measure levels of genetic variation in natural populations. The first step was known as the era of '*allozyme*[1] *electrophoresis*' where the so-called '*gel jockeys*' measured the rates of migration of enzymes and other proteins in an electric field. This was a technical proxy measure to estimate variation in DNA sequences. They argued that the sequence of bases in DNA determines the amino acid sequence of their gene products such as metabolic enzymes. The chemical structure of these protein catalysts determines their shape and surface charge, which in turn dictates how fast they will move in electrophoretic gels. At the end of each experiment their position in the gel (aka their '*mobility*') can be revealed by special histochemical staining techniques by taking advantage of their catalytic properties. From 1966 the earliest pioneers (notably Lewontin, Hubby, and Harris) upset the applecart forever by finding unexpectedly high frequencies of allozyme mobility variants (*see* Lewontin, 1974). This implied a much higher genetic load than had ever been anticipated. The situation was all made much worse by the prompt realisation that the basic electrophoretic method probably delivered an underestimate due to '*hidden*' variation; i.e. an unknown number of amino acid substitutions that did not affect mobility. Not to mention the fact that these protein-based analyses very much underestimate the underlying level of nucleotide variation in DNA, as later established. The wheel had turned full circle and created the second serious threat to Darwinian orthodoxy. Ironically it turns out that genetic load was a problem both in its own right initially, and later became one via its dismissal as insignificant!

Thus, salvation of a sort seemed to arrive in the form of Kimura's '*Neutral Theory of Evolution*'. This concept escaped the concerns about genetic load by pushing natural selection to the sidelines. In short, Kimura argued, as a primary hypothesis, that these protein variants were associated with selection coefficients[2] that were zero or close to it. Hence, they were not subject to balancing or directional selection (see later for more on these terms). In the following sections this article briefly explains the mechanics of this theory and its contentious reception by population geneticists of the time. It concludes by asking, now that the dust has settled, if this theory really did deal a mortal blow to Darwinism.

What is Neutral Theory?

In my view, the real genius of the Kimura hypothesis comes with the recognition that evolution is still possible under neutrality because (allozyme) allele frequencies will change from generation to generation simply as a result of random sampling through differential reproduction. Because natural populations are finite in size and not all individuals have the same numbers of offspring, then the genetic make-up of the population will change from generation to generation by what is described as a '*Poisson sampling process*'. New alleles will arise from time to time by mutation and their frequencies will wax and wane over subsequent generations. Some of them will inevitably increase in frequency to approach 100% (aka '*fixation*'). When this occurs the gene (and protein) sequence will have changed forever and evolution will have taken place. The species will now be permanently differentiated from all others. From there Kimura and his colleagues developed a body of mathematical theory with increasing sophistication (Box 2) to calculate rates of evolution under various assumptions.

Box 2 Types of Neutral Theory

The following list gives a brief chronological summary of the development of Neutral Theory models.

1. The Infinite Alleles Model: assumes that each new mutation is genuinely novel. This proposal was made to help to make the mathematics tractable.
2. The *k*-Allele Model: new mutations create one of a limited set (*k*) of possible varieties, an idea developed in response to criticisms of the infinite alleles model by making it more biologically realistic.
3. The Step-Charge Model: here new mutations change the mobility state of an '*electromorph*' by +1 or -1 step. This approach was taken to model typical allozyme electrophoresis data which often produced uniform '*electromorph ladders*'.
4. The Slightly Deleterious Alleles Model: a mathematical demonstration that even mutations conferring a slight fitness disadvantage on their host organisms could be maintained in populations for considerable periods and even reach fixation.

These models start from the recognition that neutral evolution is a '*stochastic*' process; i.e. goes along in a step-by-step

[1]A variant form of an enzyme that differs structurally and has a different electrophoretic mobility from other forms but may or may not differ functionally from them with respect to biochemical properties.

[2]These parameters describe relative ability of particular variants to survive a selection process. Their mathematical properties are described later in the article.

fashion and conditions at the start of one generation lead to the outcome in the next in a non-deterministic manner. So, by assuming that each mutation was the result of an entirely novel, never to be repeated, event and using what is known as the '*diffusion theory approximation*' algebra, Kimura was able to model iterative sampling over many generations and obtain end-state predictions about outcomes. The fascinating result was that his equations produced a startlingly simple formula for the rate of evolution, i.e. the rate at which one allele is entirely replaced by an alternative form. This rate = $4N_e\mu$ where *Ne* is the '*effective population size*' and μ is the '*mutation rate to neutral alleles*'[3]. Already by this time it had been well established that each protein evolves at its own characteristic rate and that these rates (or their reciprocal *Unit Evolutionary Period,* UEP) may differ by a factor of 20 or more, say cytochrome c (slow) *v*. fibrinopeptides (fast) – see Wilson *et al.* (1977) – and remain constant over very long periods (aka '*the molecular evolutionary clock*' after Zuckerkandl & Pauling 1962). It was clear that such UEP differences must represent differences in mutation rate and selective constraints inherent in protein molecules themselves; i.e. there are many essential amino acid residues in cytochrome c and few in fibrinopeptides. This seemed to match what was then known about the biological functions of these protein molecules. The parameter, *Ne*, was more difficult to come to grips with. Clearly, it would be less than *N* (the census population size) because not all reproduce successfully or to the same extent. The size of *Ne* would also depend on the ratio of males and females in the population. In some special cases this effect could be calculated or approximated. However, a core difficulty could not be overcome; both *Ne* and μ are quantities of uncertain magnitude and hence the compound property $4N_e\mu$ or '*neutral rate of evolution*' was even more uncertain. This is not to say that Kimura's theory did not make testable predictions. It did, but as we shall see later, they proved surprisingly hard to test.

One might think that such an elegant body of work would have been well received. On the contrary, it caused intellectual outrage among a wide group of Neo-Darwinian biologists because it denied that directional selection promoting advantageous variants was what drove evolution. Previously, this was a generally unvoiced, but apparently deeply-held conviction. So, the written response was sharp and biological scholars once again became divided, this time into the '*Selectionist*' *v*. '*Neutralist*' schools. We will next see how this all played out.

How population genetics sees natural selection

This picture is derived from the single gene-eyed view taken by the theoretical infrastructure of the Modern Synthesis. It visualises competition between variant alleles in terms of '*fitness*' and '*selection coefficients*'. The idea of fitness (Box 3) is seen in strictly evolutionary terms and reflects differential reproduction.

Thus, the selection coefficients are the relative fitness differentials between alternate genotypes viz:

Genotype	AA	AB	BB
Fitness	1–*s*	1	1–*t*

In the above formulations (after Chambers 1988) if both *s* and *t* have positive values, then the two homozygotes AA and BB will be less fit than the AB heterozygote and both alleles will be maintained in the population by balancing natural selection at frequencies dependant on the ratio (*s*/*t*) of these values. In contrast, if *s* is positive and *t* is negative (or vice versa), then directional selection will favour allele B (or A) and it will move towards fixation; as shown later in Figure 1. As we have already seen, this all worked perfectly well for Dobzhansky's chromosomal inversions, but what about allozyme variants?

Box 3 The various meanings of fitness

The English word 'fitness' has several meanings which might seem pertinent to evolution as was captured later in the popular '*survival of the fittest*' conceptualisation and which followed long after the publication of *On the Origin of Species.*

1. Physical Fitness: gazelles that run fastest don't get eaten.
2. Match to the Environment: in the sense of 'fitting in well' or well-suited to a particular ecological niche.
3. Most Deserving: a sort of spiritual view that those who are rated most virtuous will survive.
4. Most Fecund: those leaving the highest number of descendent offspring are said to have the highest Darwinian fitness.

It is only Definition 4 that directly applies to evolution (although admittedly advantages under both Definitions 1 and 2 may be seen to contribute). Those with highest fitness in this sense are the ones who leave the largest number of offspring who themselves contribute to the next generation.

Thus, Captain James T Cook may be said to have had high single generation fitness because he had several children in his lifetime, but rates zero overall because none of them had any surviving children of their own.

Measuring selection in molecular terms

Surprisingly there turned out to be several approaches available to resolve the Selectionist v. Neutralist controversy[4]. The first is to match experimental data to neutral models. Biochemist Walter Fitch began by asking if rates of protein evolution were as expected under neutrality. Early tests rejected the neutrality hypothesis, but these depended on having data available from multiple sequences for a single protein from a variety of species (available only rarely in those days) and these early findings must be rated as indicative at best. Another data matching exercise is to see if heterozygosity within and between populations is as predicted. In summary, a simple direct concept was ultimately compromised by a lack of sufficiently discriminating statistical tests. An elaboration of this idea is to match the numbers and frequencies of all alleles in a population to neutral theory models. Here at last there was an available statistical method, '*The Ewens-Watterson Test*' with sufficient power to discriminate. Sadly, it was by then also recognised that the data properly had to include, or allow for, all the electrophoretically cryptic variation. Only a few such data sets were ever obtained and these only via heroic laboratory exercises running gels under

[3]This is the rate at which selectively equivalent (neutral) alleles arise in the population.

[4]Selectionists held that most if not all allelic variants were associated with non-zero selection coefficients. In contrast, the Neutralist School held that majority of allelic variants had very small (effectively zero) selection coefficients. They did not dispute that a small fraction of alleles in natural populations might be maintained by balancing selection or even positively advantageous.

many different conditions (e.g., see Keith *et al*., 1985). This work rejected neutrality, but the general case is hardly overwhelming with so few examples.

The second approach was to seek causal explanations for the maintenance of enzyme variants via biochemical models. A small number of quite elegant studies were carried out to explain geographical patterns of allozyme variation in terms of kinetic constants etc. and balancing selection mediated via environmental factors. These cases are themselves limited and have a further problem. When one begins to test for biochemical differences between enzyme variants one often finds that they differ with respect to everything that gets measured. Hence it is always going to be difficult, if not impossible, to tell which differences in properties are significant and which are merely correlated properties resulting from structural differences (see Gould & Lewontin, 1979 for more on this theme).

The third approach is empirical. Neutral processes differ from those shaped by natural selection in that they are not directed. Hence, they are not often expected to result in apparently ordered patterns that persist over long periods of time or over vast geographic regions. They are never expected to produce congruent patterns repeated over time or space. Several studies including some of those described immediately above showed large-scale clinal geographic patterns of variation and others reported parallel clines[5] in different places. Overall, a slight majority of the systems examined turned out to show exactly such patterns, including correlation with environmental variables (e.g., Oakeshott *et al*. 1982). Subsequent work has shown that there may be other explanations underlying some of these observations. For instance, the apparent clines within a single species might alternately be a large hybrid zone between two closely related species or subspecies.

Finally, one has the option of following Dobzhansky's excellent example and measure *s* and *t* directly in population cages, with or without including variable environmental factors such as food type, or temperature etc. Despite an energetic following amounting almost to a cottage industry, this research programme proved to yield equivocal results. Values returned were small and highly variable, researchers gained conflicting views of the mode of natural selection even in single allozyme systems. This dilemma is captured in the visual model presented in Figure 1 and shows how difficult it is likely to be to gain an unambiguous outcome in such situations.

As a brief extension to this story the author is keen to point out that this present account is mostly concerned with protein level variation, reflecting the leading analytical technology at the time of the debate. It is now known that these protein coding genetic differences turn out to be just the tip of the iceberg. Even the very first DNA sequencing surveys showed that nucleotide substitutions were much more abundant than amino acid substitutions. This arises in part from the degeneracy of the genetic code where as many as six different triplet codons may encode a single type of amino acid. At first sight it might look as if natural selection would be blind to synonymous nucleotide changes, i.e. those that simply change one codon to another coding for the same amino acid. However, this is not necessarily a given because the t-RNA species corresponding to one codon may

[5]A cline is measurable gradient in a single character (or biological trait) in a species across its geographical range.

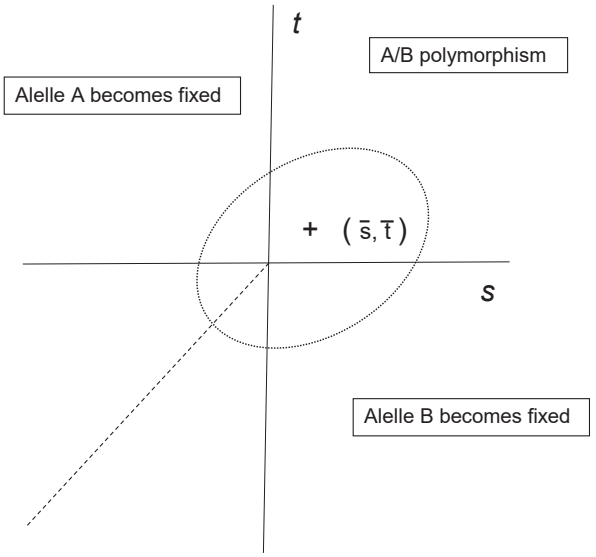

Figure 1. Representation of a simple two-allele (A, B) genetic polymorphism (see text) showing balancing selection (aka overdominance or heterosis). The axes are selection coefficients s and t for the two homozygotes AA and BB respectively. The point + marks the global mean value of s and t with a 95% confidence interval shown by the dotted ring marked around them. The dotted line in the lower left quadrant marks the boundary of an unstable equilibrium (negative heterosis) anywhere away from this line one or other allele eventually goes to fixation as shown

be more abundant in cells than its partner(s) corresponding to the alternate type(s). It is recognised that this can lead to overall differences in protein expression in turn producing differences in catalytic capacity. Further there is also a type of hidden variation problem with nucleotides due to unseen multiple substitutions at a single site that ultimately restore the original sequence. However, having laid down all these disclaimers one notes that there are now more than adequate tools for generating DNA sequences and statistical tests of power capable of testing for neutrality within and between populations and species. This is a story for another day and does not end with the advent of this methodology alone. Geneticists have discovered that due to limitations of recombination on chromosomes genes cannot be considered in isolation, but rather exist as part of an extended '*haplogroup*'. Such set ups turn out to be prone to '*selective sweeps*'. Here, even a single newly arisen nucleotide variant at one position in a haplotype may suddenly become of marked selective advantage and rapidly increase in frequency. In the process it drags along all of the other variants in its immediate haplotypic region, regardless of whether they are advantageous, neutral or disadvantageous. The single gene-view of the world becomes a marked disadvantage under such circumstances.

Is Darwinism dead?

As stated earlier in the opening section of this paper, the Darwinian view of evolution has two main components. These are known as the fact of evolution (Box 1) and the hypothesis of Natural Selection as the force which drives it via differential fitness (Box 3) among organisms. The arguments in Box 1 all but carry the day for evolution as a process of descent via (genetic) modification. However, many people will not be convinced by such arguments unless they know exactly how the process works. This is exactly why Darwin's ideas about natural selection were

Box 4 The logic of Darwinian Evolution

The following scheme is summarised from Mayr (1982) p.479–481:

Fact 1: Most species have high fertility.

Fact 2: Nonetheless, population sizes remain generally stable.

Fact 3: Resources are limited and their supply remains generally stable

Inference 1: Because more individuals are produced than their environment can sustain, there must be a struggle for survival and reproductive space.

Fact 4: No two individuals are the same.

Fact 5: Much of the difference between individuals is heritable.

Inference 2: The outcome of the struggle for survival has a genetic basis (natural selection).

Conclusion: Over generations natural selection will induce gradual genetic change including the emergence of new species (evolution).

so important. The entire concept has been neatly unpacked by Mayr's (1982) '*five facts and three inferences scheme*' (Box 4).

Under Kimura's model the neutral theory process of '*genetic drift*' only replaces the struggle for resources and natural selection in guiding differential reproduction. The overall evolutionary scheme remains intact.

Closing summary

So an era of white hot debate might seem very much to have ended with a whimper rather than a bang. Some now might even say that the debate was not worth having in the first place – but the truth may lie far from it in the view of this author. In my opinion we are left with rampant genetic variation at the molecular (DNA) level, disappointingly small *s* and *t* values (say compared with those for chromosomal inversions) and processes additional to natural selection (including neutrality) as candidate forces directing evolution. True, there may have been no clear winner in the Selectionist *v*. Neutralist debate, but we have substantially enriched our view of mechanisms controlling biological history and the future.

Perhaps this was the right outcome because maybe there never was any real contest between neutrality and selection, except perhaps for some people's views regarding their relative significance in managing molecular genetic variation in populations. In fact, the two ideas can (and now do) rub along together perfectly well. The neutral process of genetic drift is an undeniable (and mostly undenied) fact of life for finite populations. The question now becomes: Is natural selection acting on such variants strong enough to overcome genetic drift or not? The answer very much depends on population size and structure, which are reflected in the magnitudes of *N* and *Ne* respectively.

In the last analysis we should ask: Is this what evolution (even at the molecular level) is really all about anyway? Have we, in fact, been seduced by the effectiveness of Mendelian ideas about inheritance and the fabulous success of the Modern Synthesis. The received wisdom view at the time of the Selectionist *v*. Neutralist debate may just have been too microscopic. There is indeed a bigger picture to consider. The legitimate focus of natural selection is on quantitative traits, running speed, endurance, etc. Wide experience of modern *Genome-wide Association Studies* (aka GWAS) has demonstrated that such traits are governed by very many genes, each individually of only small effect. Even genes with relatively large effects and which may have huge *p* values (i.e. *statistical probability*) for association may yet only account for 2% of the total variance in the trait. So, this is where a deeper truth may lie. Each of these gene variants will only be expected to be associated with small selection coefficients. In conclusion, and for the present, neutrality serves us best as an excellent null hypothesis. It does not exclude the possibility of natural selection very much in the way that Darwin first envisioned it.

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This is a historical review aimed at the wider NZAS membership. For this reason, the following list is limited to a small number of good sources for further reading. Those who are interested in this topic can use them to reconstruct the literature.

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