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.

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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.

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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 Experiments (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 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 sequence of their gene products such as metabolic enzymes. 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).

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.

**What is Neutral Theory?**

In view of the above, it is not surprising that a few biologists (more about these later) took up the challenge and proposed that natural selection was no longer the only force determining evolution. A new 'neutral theory' of evolution was proposed by Motoo Kimura in 1968. By this it was argued that neutral evo-

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**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

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

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 chil-
dren in his lifetime, but rates zero overall because none of
them had any surviving children of their own.

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 differen-
tials between alternate genotypes viz:

<table>
<thead>
<tr>
<th>Genotype</th>
<th>AA</th>
<th>AB</th>
<th>BB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fitness</td>
<td>1–s</td>
<td>1</td>
<td>1–t</td>
</tr>
</tbody>
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In the above formulations (after Chambers 1988) if both s
and t have positive values, then the two homozygotes AA and

*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 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 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 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 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
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 level, disappointingly small in my opinion we are left with rampant genetic variation at the molecular level, disappointingly small. The neutral process of genetic drift is an unde-

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