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On Ohta's Hypothesis: Most Amino Acid Substitutions Are Deleterious

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Abstract. Ohta's hypothesis that most amino acid substitutions are deleterious grew out of a class of population-genetics models called shift models. Recently, shift models have been shown to be biologically unreasonable and have been replaced by a more plausible house-of-cards model. In this paper, the simplest form of the house-of-cards models is shown to be incompatible with most of the major features of protein evolution. Moreover, this model is shown to not be a model of exclusively deleterious-allele evolution, but rather to be a model with an equal mix of deleterious and advantageous substitutions.

Key words: Neutral theory — Nearly neutral theory — Protein evolution — Deleterious alleles — Molecular evolution — Mutational landscape

Introduction

By 1971, five generalities about molecular evolution and polymorphism had emerged, providing the motivation and support for both Kimura's neutral theory of molecular evolution (Kimura 1968; Kimura and Ohta 1971) and for Ohta's nearly neutral theory (Ohta and Kimura 1971; Ohta 1972). These generalities were:

1. The protein clock runs in real time rather than generational time (Zuckerkandl and Pauling 1965). Creatures with short generation times evolve at about the same rate as those with long generation times. For example, Ohta and Kimura noted in their 1971 paper that the number of substitutions in cytochrome c leading to humans (29) and *Drosophila* (20) are not very different despite a hundredfold difference in generation times.
2. The DNA clock runs in generational time rather than real time (Laird et al. 1969; Kohne 1970). Creatures with short generation times evolve faster than those with long generation times. As this observation came from DNA hybridization studies, it was assumed that the measured DNA rate corresponded, in the main, to noncoding regions of the genome.
3. Different proteins evolve at very different rates. From the earliest sequencing studies it was clear that the rate of substitution of amino acids, whether measured on a per-locus or per-site basis, varied by several orders of magnitude among proteins.
4. DNA evolves faster than most proteins. Again, the DNA rate came from hybridization studies.
5. Average protein heterozygosities, as measured by gel electrophoresis, fall in the surprisingly narrow range of from 0.05 to 0.15. (Later studies have shown that the lower bound was incorrect. In fact, a histogram of heterozygosities across species exhibits a mode at zero [Nevo et al. 1984].)

The neutral theory, circa 1971, could account for protein evolution or DNA evolution, but not both. Kimura and Ohta (Kimura and Ohta 1971) chose to apply the theory to protein evolution rather than DNA evolution, even though they needed an untenable assumption about the clock-time dependency of mutation rates to do so. At the same time, Ohta and Kimura (1971) introduced the nearly neutral theory, which could account for all five generalities. The nearly neutral model was, in many ways, more revolutionary than the neutral theory as it hypothesized that the vast majority of all amino acid substitutions were deleterious, but it, too, had an unten-

able assumption. In this case, the assumption concerned the assignment of fitnesses to mutations, but the problem escaped notice for 19 years, during which time the nearly neutral theory became the dominant paradigm of molecular evolution.

Recently, Ohta and Tachida have written a series of papers introducing a new version of the nearly neutral model, one with a more realistic assumption about the assignment of fitnesses (Ohta and Tachida 1990; Tachida 1991; Ohta 1992). These papers change from a "shift" model for the assignment of fitnesses to a "fixed" model. (See the next section.) However, the papers do not spend time examining the new model in the context of the generalities that motivated the nearly neutral model in the first place. Moreover, they contain little discussion on one vital point: Are the fixations that occur under fixed models deleterious?

The purpose of this paper is to explore the new model in light of the five generalities and to describe the nature of the substitutions. The conclusions are rather surprising: The new model does not appear to be a robust candidate for molecular evolution as the main parameter of the model must fall in a very narrow range. Should the parameter fall in this range, then only half of the substitutions are deleterious; the other half are advantageous. The reason for the slowdown in protein evolution (when compared to DNA evolution) under the new model is not the difficulty of fixing deleterious nearly neutral alleles; rather it is because evolution has taken proteins to such exalted states that mutation rates to alleles that are candidates for substitution drop to near zero. Finally, the model is fragile. That is, a small change in its assumptions usually leads to a qualitative change in its dynamics.

A Tale of Two Theories

Before describing Ohta's theory, some background on Kimura and Ohta's neutral model as described in 1971 will be helpful. This version of the neutral theory, which addressed only protein evolution and polymorphism, made two fundamental assumptions:

1. The neutral mutation rate, measured in real time, is constant across species for a given locus. Thus, the neutral mutation rate per generation in creatures with short generations will be lower than in creatures with long generation times.
2. Mutations come in two flavors: neutral and "very" deleterious. The very deleterious mutations are under sufficiently strong selection such that they do not participate in molecular evolution.

The first assumption was needed to account for the clock-time dependency of the protein clock. At the time, clock-time mutation rates were not generally accepted. Thus, the neutral theory did not, as is commonly claimed, "predict" the molecular clock. Rather, a biologically unrealistic assumption had to be introduced to bring neutrality and the protein molecular clock into agreement.

The second assumption was introduced to account for the variation in substitution rates among loci. The neutral mutation rate was often written as $f_0 \nu$, where f_0 represented the fraction of mutations that were neutral and ν represented the total mutation rate. Thus, $f_0 \approx 0$ for histones and $f_0 \approx 1$ for fibrinopeptide. Of course, ν was assumed to vary across loci as well.

With these two assumptions, the neutral theory nicely accounted for the two protein-specific generalities. One might quibble that the number of assumptions equals the number of generalities, but we will leave that for philosophers of science. The theory cannot, however, account for the generational clock of DNA evolution or any of the other three generalities. Ohta's theory did that.

Ohta made the following five assumptions in her generalization of the neutral allele theory to the "nearly neutral" theory:

1. The mutation rate across species is constant per generation rather than per year. (This is a significant departure from the assumption of the Kimura and Ohta paper.)
2. The great majority of all amino acid mutations, even those that are nearly neutral, are deleterious.
3. The generation time is inversely proportional to the population size.
4. Mean selection coefficients of deleterious amino acid mutations are locus specific.
5. A significant fraction of DNA mutations that do not change amino acids are neutral.

The first four assumptions are contained in the 1971 paper by Ohta and Kimura. They were clearly uncomfortable with the clock-time dependency of mutation rate assumed in their other paper of the same year and were trying to come up with an alternative model.

The main implication of the first two assumptions is that the rate of amino acid substitution per year is

$$k \propto \frac{\nu}{N_e s g}$$

where N_e is the effective population size, s is the selection coefficient of deleterious mutations, and g is the generation time. Unlike the neutral model, the rate of substitution under the nearly neutral model depends on the population size. The dependency is such that evolution proceeds more rapidly in smaller populations, an observation they attribute to Mayo (1970).

As written, the formula for the rate of substitution is inversely proportional to the generation time. That is, it implies a generation-time effect. To remove the dependency, assumption 3 is involved: $g \propto 1/N_e$. Now we have

the clock-time protein clock without the awkward assumption that mutation rates are clock-time dependent. This was the "great leap forward" of the nearly neutral model.

Assumption 4 addresses generality 3. Proteins that evolve more slowly experience stronger selection against their mutations. They are more "constrained."

An immediate consequence of the fifth assumption is that DNA evolution should be faster than protein evolution and that DNA evolution should exhibit a generation-time effect. (I am sticking with the vocabulary of the time: by DNA evolution I mean silent or noncoding evolution.) The complete package of assumptions may be found in Ohta (1972).

Thus, the five assumptions are adequate to explain the four generalities about protein evolution. The fifth generality fits much better with the nearly neutral theory than with the neutral theory. In fact, much of the development of the nearly neutral theory in the seventies concerned its implications for polymorphism rather than substitutions.

There can be little doubt that Ohta's nearly neutral theory is the dominant paradigm for molecular evolution. Our vocabulary of "constraints" and "purifying selection" points to a world dominated by deleterious mutations and substitutions. Kimura himself finally turned to the theory in 1979 (Kimura 1979) and used it for his book on molecular evolution (Kimura 1983).

The second of the five assumptions will be a major focus of this paper. I will argue that the great majority of nearly neutral mutations are not deleterious. Rather, only a small majority are deleterious. Most importantly, I will show that of those that are fixed, precisely one-half are deleterious and one-half are advantageous. These and other results will come from an examination of the models that form the scientific basis of Ohta's theory.

The Models

The theories of the previous section are laid out without any explicit population-genetics models. As the theories stand, they are ambiguous on many points. When we examine the models of molecular evolution used to explore the dynamics of the theories, the ambiguities are resolved.

The original nearly neutral models did not address the distribution of selection coefficients. Rather, selection coefficients were represented by the letter s as in our formula 1. The first use of a distribution appears to be in Ohta (1977). There she cited Alan Robertson (Robertson 1967) as justifying the use of an exponential distribution. She provides the following quote from Robertson's paper: "I hold the view that the distribution of gene effects will probably be of an exponential kind (so that the smaller the range of effect specified, the greater the total number of loci concerned)." Curiously, Robertson is discussing the distribution of effects across loci while Ohta is discussing the effects within a locus.

Using the exponential distribution of deleterious effects, Ohta showed that the rate of substitution is inversely proportional to the population size and commented that the result echoed in our formula 1 may not be very accurate without the exponential assumption. The use of this model, which has come to be called the *exponential shift model,* shows that Ohta felt that the fraction of mutations that are deleterious is so high that we will not be in serious error in assuming that all mutations are deleterious. She is not denying the existence of progressive evolution, of course. She is choosing an assumption with the consequence that most of the substitutions that we observe—and whose dynamics we study—are deleterious.

Two years later, Kimura (1979) suggested that a gamma distribution with shape parameter 1/2 is preferable to an exponential distribution. He was concerned that the exponential distribution did not admit enough mutations in the neutral region $0 < s < 1/N_e$. The gamma shift model, as it came to be known, was the model of protein evolution used in his book (Kimura 1983).

Shift models entered the literature without much fanfare. The only justification for the assumption that most nearly neutral mutations are deleterious comes from statements like:

> "Since it is generally accepted that a great majority of new mutations are deleterious, it is natural to assume that a great majority of borderline mutations are deleterious as well" (Ohta 1977, page 149).

This error of logic should have been challenged at the time, but wasn't. The conflict with Fisher's (1958) view, based on an abstract model of phenotypic evolution, that the fraction of deleterious mutants should approach one-half as the strength of selection acting on mutations approaches zero, went unnoticed.

Even on their own terms, shift models are not realistic models of molecular evolution. Shift models require that all mutations be deleterious. Thus, when a deleterious mutation fixes, all subsequent mutations must be less fit than it, not simply less fit than the allele it replaced. The frame of reference of mutant effects is constantly shifting, hence the name. Advantageous substitutions are forbidden, even the substitution of the more fit allele that was replaced by a deleterious allele. As evolution drives the fitness of a protein downhill, the probability of fixation of an advantageous mutation remains zero.

In the 1990s, Ohta changed from shift models to *fixed models* for her nearly neutral theory. Fixed models are more frequently called house-of-cards models in the population-genetics literature (Kingman 1978). The change from shift to fixed models appears not to be motivated by the problems with the shift models alluded to above, but

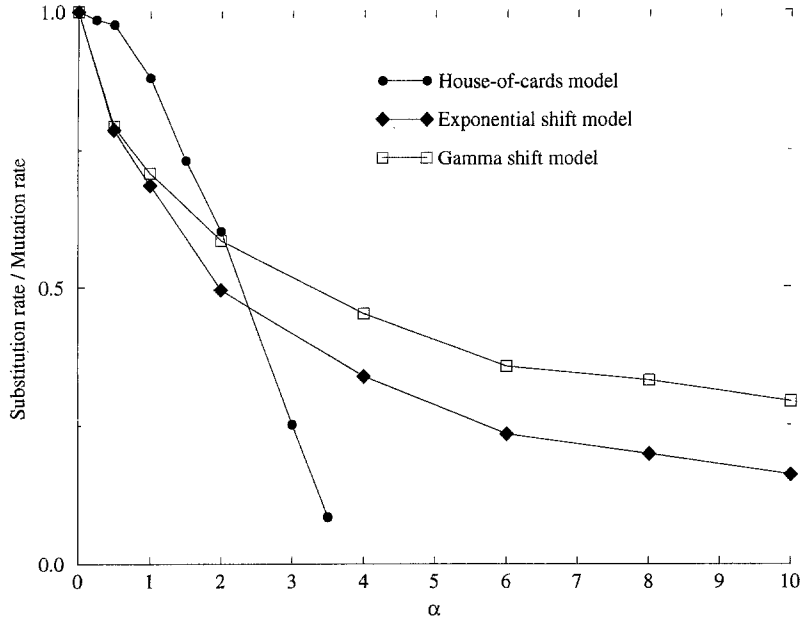

Fig. 1. The rate of substitution as a function of the strength of selection, α, for three models with $4Nv = 1.0$ and $N = 1{,}000$. Each point is based on 5,000 events.

rather by the following argument (Ohta and Tachida 1990):

> In the shift model, proteins can improve (deteriorate) indefinitely by successive advantageous (deleterious) mutations while their chance of improvement diminishes (increases) as advantageous (deleterious) mutants are fixed in the population in the fixed model. The shift model appears to be unrealistic since there must be a limit for the improvement of proteins. (page 220)

It is as if the problems with shift models of advantageous mutations are used to discard shift models for deleterious alleles.

There are reasons enough to discard shift models. Under the house-of-cards model, a natural replacement for shift models, each new mutation is assigned a random selection coefficient from a normal probability distribution (mean zero and variance σ^2) independent of that of its parent allele. It is important to remember that the model only applies to mutations of relatively small effect. The full distribution of mutations, including lethals and other mutations of large effects, is irrelevant to molecular evolution and our discussion.

The significance of the house-of-cards model is that it mimics very closely a view of progressive evolution held by many evolutionists. The population evolves with substitutions of alleles of successively higher fitnesses until such time as the selection coefficient of the common allele falls well into the right-hand tail of the normal distribution. As these substitutions occur, a greater-and-greater fraction of mutations become deleterious. (The fraction increases because of the "fixed" assumption that the distribution used to assign selection coefficients is independent of the selection coefficient of the parent allele.) Eventually, evolutionary progress stops at a point where "a great majority of all new mutations are deleterious." But, are "a greater majority of borderline mutations deleterious as well?"

The house-of-cards model is difficult to study mathematically, particularly in the context of a model of the gene such as Watterson's (1975) infinite-sites no-recombination model. Consequently, I have used computer simulations to explore the main feature of the model as well as those of a number of other models of deleterious alleles (Gillespie 1994). Here I will report two findings from that study that are directly relevant to Ohta's hypothesis.

The first finding concerns the steady-state rate of substitution under the house-of-cards model. Figure 1 illustrates the rate as a function of the strength of selection as measured by $\alpha = 2N\sigma$. (The rates in the figure are divided by the neutral substitution rate, which is equal to the mutation rate.) The substitution rates for the exponential and gamma shift models are given for comparison. Two things should be noted. The first is that the rate of substitution is a decreasing function of the strength of selection for all three models. Thus, all three behave as models of deleterious alleles should. The second is that the rate is a concave function of α for the house-of-cards model but a convex function for the shift models. Thus, there is a qualitative difference in the house-of-cards model when compared to the models it replaces. The difference is significant: the house-of-cards model behaves like the neutral model for α less than about one. When α is between one and four, it exhibits its unique dynamics. When α is greater than four, evolution stops! Thus, there is a very small window for the combined parameter $\alpha = 2N\sigma$ where the house-of-cards model is relevant. There is no reason why this parameter, which is

free to range over many orders of magnitude, should be in the narrow range of one to four.

As an aside, we might be curious why evolution should stagnate for large α. The reasons comes right from the theory of records (Glick 1978). In order for a mutation to be fixed, it must be more fit than the previous fixed mutation. As the fitnesses are drawn from the same distribution, the number of mutations that are more fit than all previous mutations after, say, n mutational events is the same as the number of records in n successive draws from the same probability distributions. An obvious property of sequential draws is that the waiting time for successive records increases with each record. It is known that the mean number of records in n trials is asymptotically equal to $\log n$. Thus, we would expect 14.39 records in one million draws, and only 0.69 new records in the next one million draws. Although the applicability of these results to evolution is confounded by the fact that not all advantageous mutations are fixed, the analogy with the theory of records makes the evolutionary stagnation for large α understandable.

The second finding concerns the fitness of the mutations that do fix, should α happen to be in the narrow range one to four. The results here are also surprising and best explained by example. Consider the case $\alpha = 2.5$. The average selection coefficient of the most common allele in this case is $\alpha/2 = 1.25$. At equilibrium, 99.3% of all mutations are deleterious. But only 65% of those mutations that are candidates for molecular evolution are deleterious. (A candidate is an advantageous mutation or a deleterious mutations whose selection coefficient differs from that of the most common allele by $1/N$ or less.) Thus, the observation that a great majority of all mutations of large effect are deleterious does not logically lead to the assumption that the great majority of all those of small effect will be deleterious as well.

More importantly, of those mutations that fix, precisely half are advantageous and half are deleterious. In other words, the house-of-cards is not a model of exclusively deleterious-allele evolution. Rather, it is a model with an even mix of advantageous and deleterious fixations. The reason for the slowdown in evolution is not the difficulty of fixing deleterious alleles—they are as likely to fix as are advantageous ones. It is because the mutation rate to candidate alleles has dropped to near zero. For $\alpha > 4$, it effectively drops to zero.

Complications and Conclusions

We are now in a position to see if the house-of-cards model can explain the generalities listed in the introduction. The initial problem concerns the clock-time dependency of the protein clock. For a theory based on the exponential shift model, the point of departure is the inverse relationship between k and N as given in equation 1. However, the house-of-cards does not admit such a simple relationship. In fact, Fig. 1 suggests that real populations will find themselves in one of two domains. In the left domain ($\alpha < 1$), the house-of-cards model gives way to the neutral model. In the right domain ($\alpha > 4$), there are not substitutions. Thus, as we survey a series of species with different population sizes, two modes of evolution should be seen: neutral evolution with a strong generation-time effect in species with small population sizes, and no evolution in species with large population sizes.

In other words, if primates evolve rodents should not. However, rodent proteins do, in fact, evolve and should evolve faster because of their shorter generation time. In fact, as no mammals appear to exhibit stasis, all must be in the left-hand domain. If so, we would expect to see a strong generation-time effect for mammalian proteins. We do not. Thus the house-of-cards model does not appear to be compatible with the clock-time dependency of the protein molecular clock.

The same problem occurs with the other protein generality. The house-of-cards model predicts that proteins should fall in two categories: those that evolve and those that do not. In fact, Ken Wolf (this conference) showed that the largest mode in distribution of protein rates sits in the middle. Although there is a mode on the left, the distribution is entirely different from that predicted by the house-of-cards model.

In its simplest form, the house-of-cards model is clearly incompatible with the generalities about protein evolution. Thus, the move from the shift model to the house-of-cards model, a move toward greater biological plausibility in assumptions, makes Ohta's original hypothesis appear untenable.

Two additional assumptions could be added to the house-of-cards model to enrich its dynamics and possibly increase its fit to the data: fluctuations in population size or in fitness. The former has been repeatedly mentioned by Ohta as a necessary part of any model of deleterious-allele evolution. The effects of population-size fluctuations on the model have not been thoroughly investigated, but we can speculate on their effects. If the population size fluctuates rapidly, we need only substitute for N the harmonic mean of the population size—or some related mean—and proceed as above. Nothing will change except for the value of α.

If the population size fluctuates very slowly, say on the time scale of molecular evolution, then the following scenario may occur. When the population size is small enough ($\alpha < 1$), alleles will fix that would be too deleterious to fix in larger populations. When the population size increases, a small burst of substitutions of advantageous alleles will replace the allele fixed in the small population. This scenario does not involve the fixation of any deleterious alleles and is precisely the scenario that I have called the "mutational landscape" (Gillespie

1984, 1991). There are a number of other factors besides a reduction in population size that may set off a burst of evolution such as hitch-hiking events and environmental changes. Ohta has often called the substitutions that occur in the burst "compensatory" substitutions as they fix up the problems caused by the substitution of the neutral allele that later became deleterious. More work needs to be done to see if this scenario is accurate.

The effects of fitness fluctuations are much more complex and will not be discussed here other than to point out that models with environmental fluctuations are compatible with most features of protein evolution (Gillespie 1991).

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