From population genetics to evolutionary genetics: a view through the trees

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This is a personal and impressionistic account of the situation in evolutionary genetics and molecular evolution, as it seemed to one participant in theoretical and methodological developments in those fields. It is based in part on a Presidential Address delivered to the Society for the Study of Evolution at its 1993 annual meeting in Snowbird, Utah.

Theory reaches its zenith

During the years I spent in the Lewontin lab (1964-1967), population genetics was a quite different field than evolutionary genetics is now. Theoretical population genetics had a tradition dating back before Hardy and Weinberg, and was a reasonably mature field. We spent our time making various evolutionary forces collide with each other, as experimental physicists do with particles. The intention was to try to discover general laws about which force would prevail. There was a great need for total generality, for a simple reason there were almost no data to be had, so that we could not constrain our conclusions to particular parts of the parameter space. As we could not know how large selection coefficients were, or what were the patterns of gene interaction, we needed results that would be general across all of them. The theory was beautiful indeed: the Kolmogorov equations as masterfully employed by Kimura, and the variance components machinery of Fisher and Wright, burnished to a high polish by Kempthorne and Cockerham.

Outside of this beautiful but small world was the frightening reality of interspecies differences, about which we could say almost nothing. In about 1970, evolutionary biology looked to us as it does in Figure 1. Our central obsession was finding out what function evolution would try to maximize. Population geneticists used to think, following Sewall Wright, that mean relative fitness, w, would be maximized by natural selection. This is true for one locus, as long as the relative fitnesses remain constant. But by 1966 work on multi-locus systems had advanced from its inception by Kimura (1956) and Lewontin and Kojima (1960). We now knew (Moran 1966) that w could decline as a result of natural selection and recombination, even decline steadily. It had already been known to Fisher and Wright that if the fitnesses changed secularly or as a result of gene frequency dependence, the result would usually be a failure to maximize mean relative fitness. But Morans result was shocking the failure to maximize w had invaded even the case of constant relative fitnesses.

If only we could find out what function other than w was maximized by natural selection in the presence of recombination, we would have some insight into what compromises occur between the genetic machinery and natural selection. We might then also be able to see what selection would to the genetic

system itself, and to what extent mean fitness, and hence adaptation, would be expected to be increased in evolution.

Two technological revolutions came to the field. One was the availability of computers. They were first employed in biology in solving the selection-migration cline (Fisher 1950; see Wilkes 1975). In 1954, the original and eccentric Nils Aall Barricelli was the first to use them for genetic simulation (Barricelli 1954), partly because he was one of the first to have access to a computer. Barricellis work was not published until later in English, and so it fell to Fraser (1957a, b) to do the first widely-noticed genetic simulations. By the early 1960s genetic simulation, numerical iteration of deterministic equations, numerical solution of equilibria in deterministic cases, and numerical evaluation of equilibrium distributions in stochastic ones, were all standard tools. They made the investigation of multi-locus natural selection possible.

Of course, in the absence of data to constrain the parameters, we really needed general theorems, not computer simulations. The late 1960s and early 1970s were therefore also the time that mathematical rigor first arrived in population genetics. Previous theoretical work had been to an engineering standard of rigor rather than a mathematical standard. Approximations were made, and small quantities tossed out of the expressions, seemingly arbitrarily. This often worked well, but sometimes we divided 0 by 0 and deluded ourselves. Now, however, we had serious mathematicians like Samuel Karlin heavily involved in population genetics. It would surely be only a matter of time before general results flowed forth, including the long-sought maximization principle. The journal *Theoretical Population Biology* was at the cutting edge of this revolution.

But in spite of the best efforts of a lot of fine mathematicians, and the earnest fumblings of the rest of us, the general results we hoped for did not turn out to be accessible. Most of the proofs that emerged were demonstrations that particular proposed generalizations were *not* correct, leaving us all still frustrated. It is fairly clear that these failures were not the fault of the researchers; the problems simply did not have tractable solutions.

There is no theorem that says that mean fitness, or even mean relative fitness, must be improved when natural selection acts on Mendelian genetic systems. Mendelian genetic systems are not optimally designed to be the ones most friendly to adaptation. The genetic phenomena, such as recombination, can make fitness decline. Nevertheless, it is equally clear that adaptation does occur a reasonable fraction of the time. If it did not, we would not be here to read this volume. Our genetic system is not optimal, it is merely good enough. Its ability to adapt effectively may not be perfectly general, but may depend on which part of

a parameter space of biological systems we live in. If gene interactions were more complex, perhaps we would rarely be able to adapt. The average progress in adaptation of Mendelian genetic systems may be something which we could not know without knowing more about where we are in the space of potential gene interactions. Wagner and Altenberg (1996) have raised the issue of the evolution of adaptability" and shown an example of an evolving system tending to move into regions of parameter space that allow it to successfully adapt.

The area in which theoretical population genetics succeeded best during this period was in the study of life history evolution. Following the lead of W. D. Hamilton and John Maynard Smith, particular genetic phenomena in life histories received theoretical treatments that expanded our understanding of how the life cycles of organisms have come to differ. While some phenomena, such as the evolution of recombination, have remained mired in controversy, others have yielded to theory. Some of the high points for me have been Hamiltons (1967) investigations of variations in equilibrium sex ratios, Maynard Smiths cost of meiosis" (1971), Brian Charlesworth solving the mystery of why Y chromosomes degenerate (1978), and insight from Marc Feldmans lab into modifier evolution (cf. Altenberg and Feldman 1987).

These undoubted successes have, however, affected only life history traits. They leave us without any better understanding of the elephants trunk, or the Pandas thumb. Some of the monsters" who lurked outside the species barrier in the 1970s were actually morphological evolutionists, who roamed the hallways of our departments, desperately seeking someone to help them analyze their data. Among ourselves, population geneticists used to boast about how little help we had given. Fred Bloggs came to see me with his meaningless snail data. I threw him out." (laughter)

Under these circumstances, morphologists could be forgiven for concluding that population genetics theory was useless to them, and for trying to find a different evolutionary theory that spoke to their concerns. One of the attractions of species-selection forms of punctuationism, for example, was that in one stroke they demoted all within-population genetic phenomena to the mere generation of variability, with the interesting natural selection occurring above the species level. This was a satisfying response to unhelpful population geneticists.

And yet, population genetics *had* already delivered insights into morphological evolution. The theory *has* succeeded in giving us a quantitative feel for the relative strength of various evolutionary forces. We can use it to show how slow change would be if driven only by mutation, how small selection coefficients must be to have their effect swamped by random genetic drift, and how much migration would be needed to

overwhelm local adaptation. All of these might also have been attempted using another theory of inheritance, such as the blending inheritance theory of Fleeming Jenkin (1867), but the results would have been very different. Our current understanding of when migration, mutation, or genetic drift can be invoked to explain an unusual pattern derives directly from the mathematical implications of Mendelian inheritance, as assimilated into evolutionary theory during the period of the Modern Synthesis. The fact that we could not be of much practical help to morphological evolutionists has tended to obscure this fact.

Enter the data

What was needed was data, and here a second technological revolution had a more dramatic effect. When I was a graduate student, Lewontin and Hubby (1966) were working on some obscure project involving electrophoretic gels. It seemed a bit odd, so we other members of the Lewontin lab felt fortunate in not being made to work on it. As soon as their paper had appeared, it was immediately obvious that the field was transformed: where there had been little data there was now a lot. A wave of excitement swept population genetics, and extravagant promises were made. If we could only compute the right statistic from them, electrophoretic gene frequencies would solve the problem of discriminating between neutrality and selection. But this was not to be, and as the years wore by, both lecture audiences and granting agencies became wary of the claim that we would solve this problem Real Soon Now. The reason for the failure was simple natural selection, with millions of generations and vast population sizes, can detect far smaller selection coefficients than we can hope to in any experiment. It should therefore have been anticipated that we were not going to be able to discriminate between neutrality and weak selection as well as evolution could.

Nevertheless, the revolution wrought by the arrival of molecular biology has been vast. It was now relatively easy to generate multiple-locus gene frequency data within species by electrophoretic techniques.

The need for methods to analyze these data was great, and population geneticists responded. One landmark was Ewenss (1972) derivation of the likelihood for a single locus sample from an infinite-alleles model, with the surprising conclusion that the number of alleles was the sufficient statistic for the parameter $q = 4N_e\mu$, the scaled product of effective population size and neutral mutation rate. As real data are likely to also contain some deleterious alleles as well, we cannot actually draw from this the conclusion that gene frequencies do not matter in this estimation. Watterson (1975) put forward the infinite-sites model and explored the estimation problems that it raises, suggesting the Number of Segregating Sites statistic as useful for estimating q.

Another landmark was Nei's (1972) genetic distance, which became an important tool near the species level. Even if we could not resolve the neutrality-selection controversy, we could use tools based on neutrality to ask other questions about population structure and history. In fact, one might say that the failure to resolve the neutrality-selection controversy actually strengthened the use of these tools. For if there was no easy way to discriminate between the gene frequency patterns expected under neutrality and under selection, one could simply use the neutrality theory to generate expectations, secure in the conviction that the presence of selection would change these patterns very little.

Twenty years on, we can see that these methods of data analysis had a greater effect than the pure theory done during the same period. To a considerable extent the change from "population genetics" to "evolutionary genetics" has been a change from a field that was theory-driven to one that is driven by data analysis.

Into the trees - the rise of molecular evolution

While population genetics was turning into evolutionary genetics, other kinds of molecular data and other uses of computers were also revolutionizing the analysis of between-species data. With molecular sequences available, precisely comparable data at the genetic level became available across multiple species. Previously, there had been some chromosome banding data, but only in certain groups. Morphological data suffered from having an unknown genetic basis, so that it could not give information at the genetic level.

It took only a few years from the first protein sequences to the first sequence comparisons between species. Zuckerkandl and Pauling (1962) were the pioneers. They foresaw a chemical paleogenetics" in which ancestral protein sequences could be reconstructed from contemporary comparisons, and in their work the molecular clock was first postulated. As the 1960s progressed, protein sequences for multiple species accumulated. The publication of the Atlas of Protein Sequences by the late Margaret Dayhoff (Eck and Dayhoff, 1966) was important in bringing evolution to the attention of molecular biologists, and molecular biology to the attention of evolutionists. Dayhoff took an uncompromisingly evolutionary approach to the data she compiled; the Atlas was, from the start, full of phylogenies and discussions of phylogenetic methods.

Suddenly, molecular biology started answering open questions about the evolutionary history of organisms. In the hands of Morris Goodman (1963a 1963b) Vincent Sarich, and Allan Wilson (Sarich and Wilson 1967), humans were relocated within the great apes (rather than being a sister group to them), and within the African apes, and finally made the nearest relative of the chimpanzees by Sibley and Ahlquist

(1984). The date of human-chimp divergence was reduced to 5 million years by Wilson and Sarich (1969). Similar revolutions affected other groups. Woese and Fox (1977) separated the Archebacteria from the Eubacteria, alough the Archaebacteria subsequently proved to be a bit less archaic than their name implied. In many other groups, molecular evidence had a less disruptive effect, confirming the historical reconstructions of morphologists more than it contradicted them. But especially where morphology faltered, molecular evolution was magically effective.

Numerical methods for molecular evolution

As the number of species in molecular data sets increased beyond a few, it became necessary to come up with a numerical methology for reconstructing phylogenies. This literature had started with Edwards and Cavalli-Sforzas paper of 1964, which is one of the true landmarks in the phylogenetic literature. Sadly, it is very little known among people making phylogenies, because of the misapprehension that this literature sprang from the work of Willi Hennig (1950, 1966). The availability of computers in the 1960s led to the development of methods for inferring phylogenies. The parsimony and likelihood methods were introduced by Edwards and Cavalli-Sforza, for blood group polymorphism gene frequencies. Camin and Sokal (1965) described the first discrete-characters parsimony method, and Cavalli-Sforza and Edwards (1967) one of the first distance-matrix methods. Molecular evolutionists were involved in this process very early: Eck and Dayhoff (1966) carried out the first molecular parsimony analysis, and Walter Fitch (Fitch and Margoliash 1967) used cytochrome sequences to pioneer distance matrix methods. Sanger had produced the first protein sequence in almost the same year that computers became widely available to scientists; ten years later, all of the major methods of analysis of molecular sequence data had been introduced, and most had already been applied to molecular data.

At first, these methods did not seem to be making any assumptions about evolution. But as distance matrix methods, and later likelihood methods, became more popular, it was apparent that they necessarily involved models of evolution. This point has not been easily appreciated by systematists it has taken outsiders to bring models to their attention. It is noticeable that, of the originators of the major methods of inferring phylogenies, only a few were trained as systematists. The others were population geneticists (Cavalli-Sforza and Edwards), biochemists (Walter Fitch and Margaret Dayhoff), medical microbiologists (Peter Sneath), or statisticians (Jerzy Neyman). The mathematical perspective did come easily to systematists.

Unilluminating models

The simplest models in molecular evolution (e.g, Jukes and Cantor 1969) were models of "random hits", in which each site was equally likely to change, with alternative nucleotides equiprobable when it did change. These models were based on the observation of an approximate molecular clock, but without any specification of what evolutionary forces were creating these changes.

Lewontin and Hubby (1966) had already suggested neutral mutation as a possible mechanism for electrophoretic polymorphism. It was Kimura (1968; Kimura and Ohta 1971) who first integrated within- and between-species molecular observations by suggesting that both electrophoretic polymorphism and sequence evolution were the consequence of the same process, neutral mutation. This had the effect on evolutionary genetics of bringing more data to bear, but an even greater effect on molecular evolution. It supplied it with a theory, ready-made.

It was immediately apparent that natural selection was acting as well. Some proteins were evolving rapidly (fibrinopeptides, for example) and others slowly (histones). These differences could easily be accomodated in the neutral mutation theory, which did not postulate that all mutations were neutral, only that all that caused polymorphism were. There could be a fraction of all mutants that did not cause polymorphism or sequence change because they were deleterious. It could vary from site to site and from locus to locus. It was to be expected that critically important regions of sequence would have most mutants be deleterious, and that these would be eliminated by "purifying" selection. Models of molecular evolution have undergone modification in recent years to take these heterogeneities of rate of evolution into account.

Most evolutionary geneticists and molecular evolutionists have agreed that most noticeable heterogeneities of rate of evolution were caused by the presence of this purifying selection. Both committed neutralists and zealous selectionists have been in agreement on this. Where they would disagree was whether the polymorphisms that were seen, and the sequence changes that were seen, resulted from natural selection or genetic drift. So far, there has been little progress in telling these forces apart, either from within- or between-species data. This has been frustrating, but, as we have noted, it has had the happy effect that data could be analyzed using modified neutral mutation models, without much threat from the inadequacy of the model.

The result has been that the models used in molecular evolution are particularly simple, but in some respects disappointing. They are primarily models specifying what does *not* happen. Since we do not know whether the changes that do happen are the result of positive natural selection or neutral mutation, there is no way to predict how much natural selection is expected to improve molecular function. I often find that

when people outside the field ask what theory predicts about molecular evolution, they are disappointed to find that its predictions are so negative.

Sequence samples from populations

While molecular evolution was maturing as a field, the same molecular technology was slowly invading evolutionary genetics. The invasion has been slow because so much evolutionary genetics was supported by the aptly-named NSF[1] . It is still much cheaper to do electrophoretic surveys at many loci than to get one population sample of molecular sequences. It is noticeable how long it has taken, since Kreitmans pioneering studies (1983), for population samples of nuclear molecular sequences to become common.

A major exception must be made for one tiny piece of eukaryotic DNA. Mitochondrial population samples have been widely used. They got much attention with the famous mitochondrial Eve" study of Cann, Stoneking and Wilson (1987). At first, mitochondria were used because their DNA was easy to extract. That ceased to be an advantage with the development of PCR, but other advantages (maternal inheritance, haploidy, and rapid rate of evolution) have been cited as additional reasons to concentrate on the mitochondrial genome.

Initially, few of the researchers using mitochondrial data related their trees to population processes. The presence of an Eve" seemed to be an unusual consequence of maternal inheritance. But every nucleotide in the genome should in principle have its own Eve" or Adam", as a result of the coalescent process at that locus. How far along the genome one can go and still find that same coalescent tree, so that every gene copy comes from that same ancestor, depends on the rate of recombination and the effective population size. The techniques of molecular evolution have invaded evolutionary genetics as people began to infer these gene trees". At the same time evolutionary genetic theory turned out to provide the theoretical framework for analyzing these gene trees. The basic theoretical work was done by J. F. C. Kingman (1982a 1982b). His work generalized Sewall Wrights result (1930) for two copies. Wright pointed out that two copies of a gene in a closed randomly-mating population would have a common ancestor an average of $2N_e$ generations ago, with the distribution being approximately exponential. Kingmans n -coalescent" process (Figure 2) is the generalization of this to n copies. The time during which all n copies have had distinct ancestral lineages is exponentially distributed, with mean $4Ne/n(n-1)$ generations. Before that, there were n-1 copies, to which the same rule applies, and so on.

[1] In the USA, NSF means National Science Foundation, and is alsothe abbreviation on your bank statement when you have Not Sufficient Funds

Kingmans work is mathematically elegant, but easily applied in practice. We do not need to use Kingmans technical machinery to understand and use the distribution of trees that his work predicts. The name coalescent" has come to be applied to any gene tree. Kingmans prediction allows us to make a statistical analysis of sequence samples from populations. In principle, when developed enough, this will allow a statistical analysis of the evidence which discriminates between the Out of Africa" scenario for human evolution with the Multiregional Hypothesis". At the moment, there is not a truly statistical methodology for analyzing the data in this controversy.

The key to analyzing population samples of sequences is not to focus too obsessively on finding the true gene tree. One will usually have too few varying sites in the molecule to make an accurate estimate of the gene tree. It therefore becomes essential to take into account the noise in our estimate of the gene tree. There are two main sources of statistical error in these inferences. One is the randomness of the coalescent tree itself, which is drawn from Kingmans distribution. The other is the error in our estimate of the coalescent tree, which depends on the randomness of the mutational processes at molecular sites.

The fundamental equation for likelihood inference in coalescent trees is (Felsenstein 1988)

$$L = \Sigma_G \text{Prob}(G|a)\ \text{Prob}(D|G, \mu)$$

where L is the likelihood (the probability of the data given the parameters), a is the collection of parameters for the population processes (effective population sizes, migration rates, etc.), and μ is the neutral mutation rate per site. The summation over G sums over all possible coalescent trees that could connect the observed sequences, counting not only tree topologies but also branch lengths. Interestingly, the first term inside the summation is the Kingman prior (or its analogue in the particular case); the second is the standard likelihood used when phylogenies are being evaluated.

Although these two quantities are easily computed, the sum looks like an impossible one to compute. There are vast numbers of forms of the coalescent tree, and each has an infinite number of possible branch lengths. For example, with only 10 sequences, to compute (1) we need to compute $2.571 * 10^9$ dimensional integrals! However, two groups have developed methods that use random sampling to take a Monte Carlo integration approach to this sum. Griffiths and Tavaré (Griffiths 1989; Griffiths and Tavaré 1994a 1994b) have taken in place of G the set of all mutational and coalescent sequences of events, without times of the events. Equation (1) then can be written in a recursive form. They compute the resulting sum by sampling paths down through the recursion. This can be extremely fast for sequences that obey Wattersons infinite

sites model, where no mutation ever recurs. It is less clear how rapidly it can be computed in a finite-sites model, such as the ones in standard use in studies of molecular evolution.

Our own lab (Kuhner, Yamato and Felsenstein 1995) has used a Metropolis-Hastings sampler to wander through the space of all possible coalescent trees (Figure 3). We do this by starting with a reasonable tree, and gradually modifying it. This is an example of a Markov Chain Monte Carlo method. Such methods are gaining wide acceptance for solving complex statistical problems. The Metropolis-Hastings sampler works by acceptance or rejection of the resulting tree, depending on the value for it of the quantity being summed in equation (1). After a large sample of possible trees has been built up, an estimate of the likelihood surface can be made. Mary Kuhner and Jon Yamatos COALESCE program, available in our LAMARC package of coalescent likelihood programs[2] , seems to compute and maximize L effectively on workstations and fast microcomputers with large enough memory.

In effect, the mathematics of likelihoods on phylogenies, and that of coalescent priors, has come together to provide a set of tools for likelihood analysis of population samples of sequences. I suspect that these will become the standard methods for analyzing these data.

The Metropolis-Hastings sampler averages over our uncertainty about the coalescent tree, taking our knowledge of that tree into account properly. One might imagine that this could be made unnecessary. Suppose that there was little uncertainty about the coalescent tree. For example, if we sequenced entire mitochondria, we could be very sure of its tree topology and fairly sure of its branch lengths. In such a case, that would eliminate the need for the Markov Chain Monte Carlo sampling. Yet it would not solve the problem. Looking at a large number of sites does eliminate the uncertainty that comes from the mutational variability. But it looks at only one coalescent tree, since the whole mitochondrion shares one coalescent. In addition, there is the risk that hitchhiking" natural selection could affect that coalescent, biasing our estimate of the effective population size.

To eliminate the noise due to the variability of the coalescent itself, we must look at many regions of the genome, each of which would have its own coalescent. That implies that we need population studies of nuclear DNA. And that raises the issue of recombination. It is not hard to generalize Kingmans formulas to allow for recombination (cf. Hudson and Kaplan 1985). In the presence of recombination, the coalescent tree becomes a collection of trees (see Hudson 1990), or a network with loops in it. However, both Griffiths and

[2] Available on World Wide Web at http://evolution.genetics.washington.edu/lamarc.html

Tavaré and my own lab have developed random-sampling methods for trees with recombination. When these methods are available, they will make it possible to analyze population samples of nuclear DNA. That will in turn allow evolutionary geneticists to subdue the variability of the coalescent tree itself, not just the uncertainty of our estimate of it.

Enter genomics

These same molecular and computer technologies have brought modern genomics into existence. As it is a high-budget technology, it has been even slower to show up in evolutionary biology, but its presence is now noticeable. Although many of the current genomics projects in evolutionary biology are for organisms that have economic importance, it is becoming possible for evolutionary biologists to search for major genes affecting traits that they study.

One does not need to be very daring to predict that this trend will continue. Of course, if traits are controlled by large numbers of loci of small effect, it will fail to explain them. To the extent that it succeeds, the classical variance components techniques will recede from view. In some cases the identification of QTLs can resolve old dilemmas. For example, suppose that we see a trait differing between two populations. Has this difference arisen because of genetic drift, or selection? And if selection, was it acting on this trait or on some correlated trait?

If genetic drift acted, we would expect all the loci involved to have differentiated in gene frequency to approximately equal extents, though in varying directions. If natural selection acted, we would expect the loci of largest effect to show proportionately more differentiation than those of smaller effect, but all loci to show differentiation in similar directions. If natural selection acted on a correlated trait, we would expect differentiation only in those loci that affected the correlated trait.

Figures 4 and 5 show the two natural selection cases, in an imaginary example. The first one is the result of natural selection on that character, the second one the result of natural selection on another character, which is only affected by the loci that are shown with asterisks. Of course, in reality the analysis is more complicated than this: there is expected to be a certain amount of heterogeneity in all these patterns as a result of genetic drift, and we have to be able to do the appropriate statistical analysis given that. But the point is that, without the genomics, we could not hope to untangle whether the trait in question was the actual target of natural selection. With the genomics, it is at least possible to ask the question. Other confounded evolutionary forces may be separated for the first time as well.

There has also been an explosive growth of comparative genomics. The existence of genetic maps for multiple species gives us data to discuss rates and processes of genome rearrangement. Sankoff and Goldstein (1989) have begun the process of developing probabilistic models of genome rearrangement. We will surely see many more uses of these in evolutionary biology.

Enter morphometrics?

Computer technology has led to the development of methods of image capture and analysis, and those to the development of morphometrics. Under the leadership of Fred Bookstein and Jim Rohlf, methods are being developed for analysis of outlines and landmarks, and even both at the same time. At the moment, this activity has had little impact on evolutionary biology, but that will surely change. When genomics can be done on quantitative characters affecting shape, morphometrics will be needed to discover which aspects of shape each locus is affecting. In between-species comparisons, there would seem to be much room for use of morphometrics.

At the moment, morphometrics suffers from its methods being purely geometric. Developmental biology has had no impact on morphometrics, as developmentalists are unable to provide parameterized models of the development of the characters. Thus one has to choose as the basic variables of interest ones which may not correspond to the developmental parameters. In fact, the information may flow in the other direction. Morphometric studies of within-population variation may suggest which aspects of form are varying indpendently, and which in a correlated direction. The difficulty with this program of study is that both mutation and natural selection will affect which aspects of form vary in a correlated fashion. This is simply a reworking of the old controversy between quantitative geneticists and morphologists over whether constraints are important in evolution. With genomics available, there could be some resolution in particular cases.

Really big trees

As phylogenies grow in size, further questions open up. It will become possible to statistically assess the role of species selection, for example. Kirkpatrick and Slatkin (1993) have studied statistics for testing imbalance in phylogenies. With such statistics, we could see whether there was significant evidence that a trait had been affected by species selection. If those clades that had higher values of the trait also seemed to be more speciose, this would be evidence for a role of species selection. We need to have large phylogenies to even attempt this, so it will not be happening very soon. It is possible to take a large phylogeny and

search for that linear (or even nonlinear) combination of characters that shows the greatest correlation with clade size.

If the data of paleontologists could be recast from survival of taxa to phylogenies that have quantitative characters on them, many questions about long term trends in evolution, as well as natural selection above the species level could be addressed.

Evolutionary biology in the 1990s therefore no longer looks like Figure 1. The species boundary holds few terrors, and within- and between-species variation can be studied together (Figure 6).

Continuing frustrations

In some areas there does not seem to be the progress that we need. We need a developmental biology that finds repeatable patterns, and hence canonical models, among different developmental processes. But if development, like life, is just one damned thing after another", then general models of developmental biology will lend little aid to evolutionary biology. The same is true of ecology. Although it is intrinsically a more important field than evolution, it is noticeable how little successful theory is available in ecology. When I was a graduate student, Robert MacArthurs work seemed to promise that strong ecological models of the Lotka-Volterra type would be available. Those models have succumbed to skepticism, leaving ecology as one of the few biological fields where postmodernism has made any inroads[3] . We are thus unable to connect our molecular and morphological inferences with strong and stable developmental or ecological models.

Emergent properties?

In the absence of developmental generalities, one interesting approach has been to seek generalizations that will apply to large classes of developmental systems. Stuart Kauffman has form many years been investigating the evolutionary implications of randomly connected developmental systems (summarized in Kauffman 1995). His NK model has two parameters that allow the complexity of the gene interaction to be varied. There is certainly reason to believe that it does not resemble the structure of real biological systems, but it is a daring attempt to pose the question more generally. It is just beginning to get the attention it deserves from evolutionary theorists. Years ago, I made (Felsenstein 1978) another long-term evolutionary model, an attempt to model the energetics of an evolving ecosystem. The crucial result, that the total energy content of the evolving ecosystem would rise linearly with time, depended on one tail of the initial

[3] (joke)

distribution of the efficiency of energy retention being a power curve. Even if this is realistic, which is open to considerable doubt, there is no way at present to connect it to a mechanistic model of the organism. It would be wonderful if models like these, that attempt to model the long-term course of evolution, could be interconnected and placed in a more general context. If such efforts fail, we can always fall back on the hope that there are some empirically valid generalizations that can be made about developmental and ecological interactions.

A new synthesis?

After the success of the Neodarwinian Synthesis in the period 1920-1950, connecting evolution and genetics in powerful ways, it is tempting to try to discern another new synthesis. There is certainly a synthesis of molecular biology and evolutionary biology going on. Nor does the influence run in only one direction, as molecular biologists have discovered that population variation can help them find out which regions of a genome are under constraint against change. But at another level there is no new synthesis. In the original synthesis, the formal structure of genetic systems came to evolutionary biology, and resulted in the mathematical theory of population genetics, easily the most elaborate body of theory in biology. In the present syntheses, no new theory has yet arisen. The forces invoked are those that we already knew about within populations. We have a post-neo-Darwinian synthesis without a post-neo-Darwinism or, at least, without a new theory. We have many new statistical and computational methods, but all use the pre-existing theory as their base.

Making such a theory is a major challenge. It probably involves asking a new set of questions, and seeking generalizations at a higher level. It may need to wait for developmental biology and ecology to come up with some new generalizations. Or it may simply need a new generation of evolutionary biologists who can ask a new set of questions.

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Figure Captions

Fig. 1 A cartoon of the state of population genetics in 1970.

Fig. 2 Kingmans coalescent process.

Fig. 3 The Metropolis-Hastings sampler.

Fig. 4 A genetic map showing differentiation of gene frequencies of loci drift.

Fig. 5 A genetic map showing differentiation of gene frequencies of loci affecting a quantitative character, when population divergence is by natural selection on a correlated character.

Fig. 6 A cartoon of the state of evolutionary biology in the 1990s.