Generative Entrenchment, Modularity and Evolvability: When Genic Selection meets the Whole Organism[1]

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Modularity and the Problem of Generative Entrenchment

There are in this book almost as many concepts of modularity as arguments for it. Anatomical elements in serial homology, chromosomes, super-genes, neural circuits, and traits have all been treated as modules. Now we have new kinds of things: HOX clusters, morphogenetic fields, and undefined *quasi-independent* traits that must be nearly independently selectable if evolution is to work). There are also the various detailed developmental-genetic dynamical structures and the hypothesized adaptive complexes they generate. Winther (2001) provides a taxonomy. We prefer the term *evolutionarily significant modules* for the units we discuss, because evolvability appears to require such modules, and because our argument concerning them derives from considering the nature of selection processes.

We begin with a class of models for the evolution of developmental structures which include an important and general dynamical structural feature: *Generative Entrenchment* or GE (Wimsatt 1981, 1986, Rasmussen, 1987, Schank and Wimsatt 1988[2]). To summarize where we will go: in 1988, we first described a new and puzzling phenomenon in some multi-locus selection models designed originally to test and modify models by Kauffman (1985, 1993). These systems generated interactions among alleles at different loci in cases where one might suppose they "should not" occur. This phenomenon had an interesting explanation involving heterogeneous fitness classes and a new kind of frequency dependent selection, but we were unsure then how to generalize it (Wimsatt and Schank 1988). We present and discuss more striking demonstrations of it here, whose analysis suggest that it is robust, and should apply quite broadly. Prima facie, this phenomenon should make significant evolutionary changes, including modular ones, more difficult. We are convinced that dealing with the processes described here must be a part of the solution for how modularity is possible. We would like to know what conditions allow or prevent the evolution of modular architectures.

Generative Entrenchment

Consider the phenotype as a generative structure: a smaller number of elements interact with each other and with inputs from the environment, producing a growing structure of interacting elements having a broadly adaptive organization. Circuits in the genome and their products fit this description (as elaborated and reviewed by Davidson 2001), as do particular genes (e.g., *bicoid,* Schmidt-Ott and Wimmer *this volume*). Different elements of the phenotype from individual genes through circuits, to developing morphological elements and behaviors should all fit this broad description. Environmental inputs, maintained conditions and periodic or other patterns of change (e.g., daily or seasonal variations) can also have significant generative roles in the developing phenotype (Greene 1989).[3]

The generative structure of the system (including the organism plus relevant aspects of its environment) has a characteristic set of causal interactions which could be variously represented. One of the simplest representations is a directed graph, where nodes are parts, processes, or events, and arrows are consequences of the presence or operation of nodes on other nodes. For each node, consider how many other nodes can be reached from it by following the arrows. This indicates how much of the phenotype is downstream of, causally dependent upon, or affected by a given node. We define the *generative entrenchment* (GE) of a node as the magnitude of this downstream dependency. (This is left deliberately vague—it could be operationalized in various

[2] Similar detailed arguments were first made by Riedl (1978). Arthur has been elaborating a similar account since 1982 (Arthur 1984, 1997). It is in most respects now the fullest available, though each approach has its own twists.

[3] Our research biases (Wimsatt 1980) are such that we should find proportionately more of these as our knowledge of developmental genetics progresses (Oyama et. al. 2001).

ways.) See Figure 1. GE is not limited to classical developmental processes. It spans the "extended phenotype" or the "recurrently assembled" resources reaching beyond traditional organismal boundaries as posited by developmental systems theory (Wimsatt 2001). Downstream consequences considered should be limited to characteristic effects, common and systematic enough that they could be consequences of selection.

Insert Figure 1 about here

Generative structures are found at various levels of organization and the interactions between them. Consider generative structures produced in self-organizing systems such as social insect colonies. Several species of army ants go through emergent reproductive cycles of over a month in which they enter statary and nomadic phases (Schnierla 1971). Nomadic phases are driven in part by the development of larva (nodes) and emergence of new workers (nodes) both chemically and tactilely interacting (connections) with adult workers (downstream nodes) "agitating" them and initiating the nomadic phase with colony level patterns of behavior and spatial relationships (colony level phenotypic patterns of behavior). Here the rules of individual interactions (between nodes, larva, newly emerging workers, and workers) become entrenched as do mating rituals, more generally, in animal systems of reproduction.

All other things being equal, nodes with more downstream connections should be more evolutionarily conservative because there are more consequences of their activity, which can be disrupted if connections are changed arbitrarily. Thus there is a much greater chance that something will go wrong if they are changed. In the simplest (multiplicative) selection models, the probability that a modification exhibits improvement over variant(s) already present declines exponentially with increasing number of downstream nodes. The probability of *strongly* deleterious or lethal consequences also increases. In simple models, perturbations earlier in development should be more likely to have widespread downstream effects. This conceptual model strongly suggests a probabilistic analogue of von Baer's law that differentiation proceeds from the general to particular, with taxonomically more general things expressed earlier in development. This also suggests a cone of increasing variation with progress through development. Greater generative entrenchment yields greater evolutionary conservatism, which leads to greater taxonomic generality (Riedl 1978, Arthur 1984, Wimsatt 1981, 1986, Rasmussen 1987, Schank and Wimsatt 1988). This is confirmed, with some qualifications, in all of our studies, including the simulations reported below.

Various critics have proposed a different pattern to the "cone of variation": developing from a single celled zygote to the variously called "neck" of an "hourglass". Elinson (1987) compared early stages to a "funnel", with a now famous figure adding the diverse earlier stages left out by Haeckel to his widely copied drawing of diverging later stages of diverse vertebrates. The waist of the hourglass has been called variously the "phylotypic stage ," (Sander 1983) the "Bauplan," and the "zootypic stage" (Slack 1993, Duboule 1994). These terms have different theoretical associations and different mechanisms are proposed for the "neck", but each is taken to correspond to a specific and significant reduction in variation at an intermediate developmental stage across specific animal groups and possibly more generally across taxa of intermediate generality. Raff (1996:208) refers to this pattern as the "developmental hourglass". This pattern has recently become a center of debate over how rigidly constrained development is at various earlier stages in ontogeny, and the possible evolutionary consequences of different patterns of constraints. And how, above all, is the early developmental variability consistent with the

downstream dependency we expect? (All concerned appear to accept GE as acting and explaining patterns of variability downstream of the neck.)

None of the models above deal adequately with complications arising from gene (or other) duplications, functional redundancy, changing degrees of modularity, or canalization at different stages of development, at least not if these structural organizational parameters are free to vary independently. (They probably cannot, but this cannot be assessed until we have models incorporating them.) Modularity, duplication, and functional redundancy should each decrease entrenchment by reducing interdependency between (for modularity) or dependency upon specific system components (for duplication or redundancy), but assessing how all of these factors interact demands both more empirical and theoretical work. Nonetheless, GE does suggest a prima facie constraint acting in development, and developmental geneticists now commonly infer from evolutionary conservatism or phylogenetic breadth to greater causal centrality, and conversely. It remains a good default inference for later development in spite of various still unexplained kinds of exceptions showing greater divergence in earlier developmental stages of amphibians, ascidians, and echinoids (Raff 1996). Some or all of these exceptions may be due to the above lacunae in the models. If so, the problem lies not with GE per se, but with overly simple models or inaccurate conceptualizations for evaluating GE in complex organizational structures.

Darwinian processes should almost inevitably give rise to generative structures (Wimsatt 2001)[4]. However, we are still left with two perplexing questions: How can complex adaptive systems evolve and continue to evolve in any other than a predominantly accretional way if their generative elements become increasingly entrenched with increasing complexity (Schank and Wimsatt 2000)? How does this permit continued modular evolvability?[5] It is no surprise, therefore, that a fundamental research focus of the evolutionary sciences is to figure out how complex systems can continue to evolve when evolutionary processes generically give rise to entrenched structures.

We suspect that elements of early stages are quite entrenched within a lineage—i.e., experimental manipulation of pre-Bauplan stages causes problems, but there are many ways of getting to highly similar phylotypic bauplan stages in different related lineages. On this hypothesis, the Bauplan stage is in effect canalized. Generative elements early in development are (1) more likely entrenched in complex adaptive systems, (2), more likely more entrenched, (3) more entrenched elements have lower probability of adaptive modification when changed, and (4) greater GE increases the probability of *major* disfunction when changed. These are *only* probabilities because of variations in network structure, in their adaptive design, and in detailed conditions that modulate consequences. Some entrenchments reflect differences in adaptive design at the species level or higher, some to genetic variation within species, and some to developmental noise and environmental variation. Specific solutions to the GE paradox may provide a starting point for a deeper understanding for how complex adaptive systems evolve despite expectations that GE grows nonlinearly with complexity. That early developmental

[4] Sterelny (in conversation) urges qualification. Darwinian processes give rise to GE globally, but this does not mean that in any given case, it will favor more exposed dependency over, say canalization. For a start on relations between generative entrenchment and canalization, see Wimsatt 1999, though much more is required.

[5] No simple answers to this question meet all necessary constraints. Some things early in development escape entrenchment, such as pseudogenes and code synonymy (Wimsatt 1986). Riedl 1978 considers other proposals. But pruning unentrenched branches from a tree still leaves a tree (albeit one growing with a slower exponent). So an hourglass of variation (Duboule 1994, Raff 1996) requires additional mechanisms, such as canalization at the "neck". These mechanisms and others likely all play hybrid roles in different specific cases, since phenotypes are assembled opportunistically by evolution.

changes are relatively common in amphibians indicates something systematic if they do not have a common origin: we want to know why so many exceptions to the GE paradox should occur there. Are these exceptions piecemeal fixes or do they indicate general principles or both? Much work clearly remains.

Simulating the effects of generative entrenchment

Our simulations were originally designed to test hypotheses by Stuart Kauffman (1985, 1993) on the evolution of gene-control networks. Kauffman argued that selection could not maintain more than a small fraction of the connections in a gene control network of realistic size in the face of mutation. His model networks were directed graphs, with genes as nodes, and arrows (connections) between them representing influences of one gene on another (Fig. 2). Mutations involved random reassignment of the head or tail of an arrow, changing gene interactions, and altering the connectivity of the network. Each gene thus had two ways to mutate, so *n* connections had 2*n* mutable "sites." The ratio of genes to connections determined connectivity of the network, and through this, many of its generic (overwhelmingly likely, and thus robust) topological properties (Kauffman 1985, 1993). Generic properties are (commonly statistical) properties which are widely distributed among members of an ensemble of systems constructed subject to certain constraints. Generic properties of networks of M nodes with N connections per node might include the average number of connections reachable from nodes, the number of closed cycles in the network, and the mean cycle length, expressed as functions of M and N.[6] We considered the robustness of his conclusions in more realistic simulations which included the effects of generative entrenchment. Differential GE is also a generic property of such structures, but one which Kauffman failed to model. (Schank and Wimsatt, 1987). At least some results from these models are more general, impacting any phenotypes whose genes or generative elements are subject to a diversity of selection forces of different magnitudes. This diversity may be produced by differential entrenchment, but need not be. Since having a non-homogeneous array of selection pressures is a robust property of machines and evolving systems generally (Wimsatt and Schank 1988, Wimsatt 2001) this should apply widely to virtually all phenotypes.

Insert Figure 2 about here

Simulation Procedure

In Kauffman's simulations, populations of "ideal" networks with an arbitrarily chosen "wiring diagram" were subjected to mutation, and allowed to reproduce with fitness determined by similarity with an "ideal" network. (The similarity measure was number of identical connections). This was a relatively simple "selection-mutation balance" model, though with many loci rather than just a few. Populations had 100 circuits (or "organisms"), each with 20 genes and 20 connections (so 40 mutable sites), a mutation rate of .005 per site per generation, and selection coefficients of .05 per connection. (Kauffman's selection coefficients were the same for all connections, a crucial assumption.) After 1000 generations of selection,

[6] What properties are generic and their generic values may (but need not) be quite sensitive to the exact specification of ensemble properties. Thus circuits with 3 connections per node (and more surprisingly, those with an *average* of 2 connections per node rather than exactly 2 connections per node) may have quite different generic properties. Thus the structural specification of the ensemble is at least as important as the determination of its generic properties. The claims we use about differential generative entrenchment are much less sensitive than many of Kauffman's to exact ensemble specifications. (Schank and Wimsatt 1988, Wimsatt and Schank 1988, Gelfand and Walker 1984).

approximately 45% of the "good" connections in a circuit were randomly replaced by others. With his assumptions, this yields a reduction in fitness of 45%, and more importantly, loss of any structure involving more than a few connections.[7]

These changes displayed the relevant properties of generic networks (Fig. 2). (A generic property is in effect a "high entropy" property—possessed by almost every member of the ensemble of structures possible for the parameter values used.) If mutations randomly sample the state-space of possible connections, mutations to structures already in generic states will likely remain generic while structures in non-generic states will likely change to more generic states. It should be harder to maintain structures in non-generic states the less generic they are. From the easy transition to generic states observed in randomly mutated circuits, Kauffman argued that selection could not maintain specific wiring diagrams in large circuits and thus that almost all relevant properties of large gene control networks would be generic and not explained by selection. For generic outcomes, any circuit would have a specific structure, but the specificity was irrelevant: what mattered was that the same outcomes would result also from a large variety of alternative circuit structures. It is good if important things can be "generic" (making them highly robust), but we were bothered by the strong limitations on selection suggested by his model, and particularly by some of the assumptions he made to get them.

Kauffman's assumption of equal fitness decrements for loss of any connection was simple but problematic. In virtually any circuit (random or not), different nodes or connections influence different numbers of other nodes. In Fig.2, the connection from 5 to 3 has no further consequences (no arrows leave 3), but the connection from 16 to 13 has many. From node 16, we can travel: $16 \rightarrow 13 \rightarrow 19 \rightarrow 14 \rightarrow 17 \rightarrow 5 \rightarrow 3$, with other divergent paths along the way. It is rare—essentially impossible for robust reasons—to find interesting networks in which all nodes have equal influence (Wimsatt and Schank 1988). Loss of connections through which many nodes are reached should cause more disruption than those leading to only a few. So larger selection coefficients should be assigned to nodes with more nodes and connections downstream. Our simulations paralleled and extended Kauffman's, with this crucial difference: in our models, fitness decrements for loss of a "good" connection was always at least a monotonically increasing function of the number of the nodes downstream of it.[8]

Initial Results and Discussion

We simulated various sized populations of circuits with different numbers of nodes and connections, connection densities, mutation rates, and fitness functions for different measures of GE. With similar parameters, we observed a decline in the proportion of good connections to equilibria of about 50% as noted by Kauffman (1985, 1993). Identical selection coefficients caused random loss of good connections in Kauffman's simulations, but our similar losses occurred almost entirely among connections with lower GE, with consequences elaborated elsewhere (Schank and Wimsatt 1988, Wimsatt and Schank 1988), and further below. Differential preservation of generatively entrenched connections occurred even with selection coefficients not much stronger than those lost: the effect was strongly non-linear.

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[7] Kauffman used high mutation rates to give observable effects with smaller populations of smaller circuits in shorter times. He makes arguments for how these effects should scale up to larger circuits in larger populations with smaller mutation rates. Our results (which point in unanticipated directions) raise questions about their relevance.

[8] The simplest such measure is the sum of downstream nodes. This treats all causal interactions as equally important, but this is not required. We have tried various measures of entrenchment, and various circuit architectures, some more conservative, with similar qualitative results (Schank and Wimsatt 1988). This robustness is surely due to the strong nonlinearity of the results reported here.

These simulations were designed to assess the effects of differential generative entrenchment of phenotypic traits, but this assumption was used only to assign different selection coefficients. Thus, the results are more general. Even relatively small differences among selection coefficients of genes (whether due to differential generative entrenchment or differential adaptive importance) were sufficient to bias the preservation of nodes towards the more strongly selected ones, even with quite strong losses due to drift.

A More Sophisticated Model

These results were confirmed and elaborated with a more realistic and adjustable model (Wimsatt and Schank 1988) allowing simulations with much larger circuits, and more revealing and useful graphical output. The two most significant changes were:

(1) Fitness decrements were *assigned* to connections, rather than *calculated* from their generative entrenchment.This was usually unproblematic because the model dynamics were affected only by the selection coefficients.[9] This throws away information but the ability to choose distributions of selection coefficients from scratch allowed crisper tests and demonstrations that were crucial below. Plausible distributions of selection intensities for randomly constructed networks could still be determined and then plugged into the model (as we did), but one could also test distributions of selection coefficients that might not occur in nature to determine their effects.

(2) Most critically, Kauffman's (1985, 1993) assumption that all fitness contributions summed to 1 was relaxed. This assumption seems initially plausible for relative fitness: we commonly let fitnesses of genotypes range from 0 to 1. Kauffman's model assumed something that looked equivalent, but is not: that the sum of fitness *decrements* for deleterious mutations in all genes summed to 1. This version is not realistic either for populations or for individuals. (So if there were a lethal mutation, then all other mutations would have to be neutral!) In our model, the maximum sum of fitness decrements for an individual (which we call the *exposure*) can be greater than 1. The value of this sum is a key parameter. Fitness decrements were calculated for each circuit in the population, and circuits with total fitness decrements (*realized exposure*) greater than or equal to 1 were assigned fitness 0. So on this model fitnesses still range from 0 to 1, but the sum of fitness decrements do not. (When you're dead, you're dead, but there are many ways to kill a cat, sometimes simultaneously.) All organisms and any complex machines (those with more than one part!) characteristically exhibit this kind of potential overkill.

This failure mode deserves to be a principle of meta-engineering. You're not even in the game unless most ways in which you could be dead won't happen and none of the others have happened yet. Some things you just can't mess with—those are protected before we ever start talking about *differential* selection. As we'll see below this fact preserves more genes, gene combinations, and adaptations than would be possible if Kauffman's picture were right. In effect, Kauffman's model is too optimistic: with his fitness assignments, in the worst possible case, you're dead—but just barely. But a "squashed bug" or "road kill" is not just barely dead.

[9] Detailed circuit connectivities affect how GE's and thus selection coefficients change with loss of connections. We treated selection coefficients as constant for the duration of a replication. More realistically they should be vectors with different components realized against different genetic and environmental backgrounds, and which thus change during the course of the simulation. This idealization underestimates epistasis in the network. Since we argue that unanticipated interactions are (already) causing troubles, underestimating interactive effects is conservative and should not undercut our conclusions. But keeping selection coefficients constant when they are changing as a result of changing connectivities forces us to forgo any conclusions about trajectories of change in such networks. For various reasons, the analyses given here are of only local validity, but for that they appear to be quite robust.

Truncation selection [in which any organism with phenotypic values on the wrong side of critical "thresholds" fails to reproduce] probably characterizes all real cases of selection.

In this model, phenotypic properties are characterized only in terms of their contribution to fitness—which may itself be thought of as a very abstract phenotypic property. The model here is a modified truncation selection model (with a threshold at fitness 0, and reproduction with probabilities proportional to fitness if it is greater than 0). It is more realistic than Kauffman's models (1985, 1993), and also more realistic than truncation selection models with selection as an all or nothing affair with 0 fitness below threshold, and 1 above. It allows lethals and a (kind of) conditional lethal. (A lethal is a mutation with a fitness decrement of 1. It is treated as unconditional. So even the fittest genotype (fitness 1) modified by adding a lethal mutation then has fitness 0. By extension, one kind of conditional lethal for a genotype (there are others[10]) is one whose assigned decrement makes the fitness of *that* genotype less than or equal to 0. It is conditional because there are other (fitter) genotypes for which that decrement would not be lethal. This kind of conditional lethal is also captured by the model). This model is also convenient for simulation of genetic load phenomena: the genetic load of a population is $1 - W$ (where W is the Mean Darwinian Fitness of the population) if maximum fitness of a genotype is set equal to 1. The effects of further simplifications in these models will be assessed in the discussion.

Simulation Procedures, Results, and Discussion

Figure 3 illustrates a model with these new assumptions (Wimsatt and Schank 1988), showing how connection frequencies change over time: 10 averaged runs ("replications") for 2000 generations with 100 haploid asexual circuits each having 100 genes with 100 connections assigned equally (20 each) to 5 fitness classes of .02, .03, .04, .05, and .06. The total exposure of 4 is contributed proportionally (.4, .6, .8, 1.0, and 1.2) by these different classes. [This is high enough to show the effects of higher exposures, but probably still much lower than found in nature. Higher exposure should allow maintenance of more genes by selection if the reproductive potential is high enough to handle the greater expected mortality.[11] This is a variant of the genetic load problem.] A mutation rate of .005 per connection end per generation yields a (plausible) average of 1 mutation per organism per generation, and a wait for back mutations comparable to the length of the simulation. Mean population fitness (plotted every 500 generations) declines to around 10-11%. Therefore a reproductive potential of 10 or more is required for the population to maintain itself.

Insert Figure 3 about here

The top graph is a continuous stacked bar graph showing cumulative frequency of good connections in all classes over time (incremented every 50 generations). (Separate data for different fitness classes was unavailable for graphs in Schank and Wimsatt 1988.) Cumulative frequency stabilized at about 70%, considerably higher than Kauffman's 50-55%. With circuits 5

[10] This generates a simple "additive" kind of context dependence for lethality which could be thought of as a kind of cumulative overload ("the straw that broke the camel's back"). It would not cover many important kinds of conditional lethality, e.g., of the kind suggested by polyploidy or other forms of redundancy, where the fitness decrement of a mutation were 0 or much less if there were a functional allele at the other locus.

[11] Expected mortality from this source can be reduced by reducing mutation rate, or by diploidy, tandem duplication or other modes of increasing redundancy, or canalization or other modes of regulation of effect (Wimsatt and Schank 1988).

times larger, 6-7 times as many connections were preserved as in the original Kauffman simulations. With an exposure of 4 rather than 1, circuits that lost even a few more connections were lethals. So Kauffman's (1985, 1993) low connection equilibrium was at least partially an artifact of not using a truncation-selection model.

Two particularly salient results emerged from these simulations:

(1) As with simulations for smaller circuits, a strong differential bias favors retention of more strongly selected alleles. This bias is visible in both top and bottom graphs of Fig. 3.

(2) A new phenomenon emerged in the bottom graph of Fig. 3—a bifurcation between the fates of connections with lower and higher fitness decrements. The two lowest fitness classes (.02 and .03) decreased in frequency throughout the simulation. The two top fitness classes (.05 and .06) also decreased initially, but reversed direction after generation 50 (the plotting interval) and went nearly to fixation. The middle fitness class (.04) seemed right on the fence: after generation 100 it fluctuated indefinitely at 76-80%. So there were very different fates for connections with relatively small differences in selection coefficients.

Why did this bifurcation of trajectories occur? Our explanation has broad implications for selection models. There are three contributing factors. The first is a kind of fitness rescaling which applies even in Kauffman's original simulations. Also necessary (but missing from Kauffman's models) is the existence of different fitness classes, which, with the fitness rescaling produced a dynamics which can yield trajectory reversals. The third factor, (also missing from Kauffman's models) is truncation selection, which amplifies effects of the first two.

Consider the fitness rescaling: start as Kauffman (1985, 1993) did with a population of identical organisms each with an ideal circuit having 20 connections, a fitness of 1, and a fitness loss of .05 per connection for mutated connections—a 5% loss in relative fitness. Now suppose after some generations with drift and random mutation, the fittest circuit has but 10 good connections, and a fitness of .50 (on the original scale). But the loss of a connection with.05 in fitness now represents a 10% loss in *relative* fitness. Thus as the Mean Darwinian Fitness of the population declines, the loss of an allele with a constant absolute fitness contribution yields a successively larger loss in relative fitness.

This is a new kind of *frequency dependent* variation in selection in what was supposedly a strictly additive model with constant fitnesses. A multiplicative model would scale appropriately to produce no changes in relative fitness if fitnesses asymptoted to zero, but not if the model is adjusted (as below) to allow for truncation selection. So if truncation selection is general, so is this mechanism. The additive/multiplicative assumptions and their effects in population genetics are discussed in Wade, et. al, 2001.

A trajectory reversal (causing a bifurcation) requires also more than one fitness class. Any continuous distribution of fitnesses would do, but discrete classes make the phenomena clearly visible.[12] Kauffman's original simulations where all connections have equal fitnesses can't do it. With multiple fitness classes, mutations and drift cause initially highly fit organisms to lose alleles, stochastically, at equal rates. Losses occur until those loci reach their respective selection-mutation equilibria. Classes with smaller fitness contributions lose proportionately more alleles because those alleles have lower equilibria with mutation. They all started at fixation so allele frequencies of lower fitness alleles have further to go, and take longer to get there.

[12] Surprisingly, classical additive quantitative genetic models assumed that all alleles contributed the same fitness increment, and so they missed the phenomenon which is so important here.

Alleles need not start at fixation in natural populations, but larger changes in selection coefficients will still lead to more rapid changes in the affected allele frequencies. Less strongly selected alleles cannot "keep up." A vector of changes in selection coefficients of different sizes acting on different alleles should have a similar effect to those demonstrated here. The processes discussed here are not limited to the particular initial conditions of these simulation experiments, and also not limited to selection for a single optimum circuit.

In these simulations, as the population's mean absolute fitness decreases over time, *relative* selection coefficients of alleles in all fitness classes increase by the same scalar factor ($1/W\max$) until the fitter ones stop declining in frequency, reaching their (temporary) selection-mutation equilibrium first. Alleles in the lower fitness classes have not yet reached theirs, continue to be lost, and in doing so further inflate all relative selection coefficients. Alleles in the higher fitness classes now reverse (in order of their selection coefficients) and increase in frequency. In effect, the population reached a rough fitness equilibrium (in Fig. 3, from roughly generation 500 on), after which evolution proceeded by the substitution of fitter alleles for less fit ones at other loci.[13]

Without these fitness differences, all classes would have reached equilibrium at the same time, with no selection reversals. With ubiquitous fitness differences at different loci in nature, occurrences like these must be common: this mechanism should be actuated by any environmental shocks or arbitrary perturbations from equilibrium. Indeed, this phenomenon raises new questions about whether it ever makes sense to expect frequency independent selection— broadening a conclusion Lewontin reached by another route in 1955.[14]

Further Validation of these Proposed Mechanisms

The simulations of Figure 3 had equal numbers of alleles in the different fitness classes, an unrealistic assumption. Evaluation of generative entrenchment for connections or nodes in circuits generated at random (Fig. 2) suggested a roughly exponential distribution, with geometrically increasing numbers of connections having lower fitness decrements. These are so-called "scale free" networks. Cellular descent trees or branching cascades in gene control mechanisms in which all cells or nodes downstream of a given event are affected (or even just a constant proportion of them) suggest similar relations (though redundancy, canalization, and differential modularity could affect these). We have investigated models with a variety of different exponential distributions of connection numbers (ratios of 2, 3, 4, and 5 of connections in neighboring fitness classes, giving various "rates of expansion" for the exponential.) They all showed similar kinds of interactions.[15]

The model circuits with the particular parameter choices discussed below had exponential distributions, and also elegant symmetry properties, which facilitated detection of patterns of inter-class compensation on several levels (Fig. 4 and subsequent figures). These circuits had 5 fitness classes contributing to a total exposure of 4, as above, but different fitness distributions

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[13] This mechanism make these systems striking demonstrations of Simon's (1962) ideas of near decomposability. They have the same association of force and relaxation times [stronger forces go more rapidly to equilibrium], but also the unusual and counterintuitive feature (which Simon's lacked) that *the long range behavior of the weak interactions dominates ultimate outcomes for the strong interactions*.

[14] Lewontin 1955 is the experimental basis and Lewontin 1958 the theoretical treatment. The idea used here is actually a broader concept than frequency dependent selection: rather than the frequency of single allele-types as individuated by sequences and linkage-relations, it involves the frequency of genes in a given fitness class, which should be the same for purposes of selection. Perhaps it deserves a different kind of name because the mechanism producing it is so different.

[15] The interactions could be detected most easily by the presence of trajectory reversals in the higher fitness classes, but should also impact the equilibrium ratios of fitness class frequencies.

and a larger number of connections. Crucial for symmetry properties, the connection-class fitness decrements were halved as the number of connections doubled, so that each class made the same total fitness contribution (of .8). The largest such circuit-type simulated had 8 connections with a fitness contribution or decrement of .10, and for the other fitness classes, 16 of .05, 32 of .025, 64 of .0125, and 128 of .00625, for a total of 248 connections with a 16-fold range of selection intensities ranging from very strong to quite weak.[16]

Is there any way to further test the form of fitness interactions suggested in the discussion of figure 3? Suppose that trajectories were products of equilibrating processes of selection and mutation operating both within *and* across fitness classes. (Standard mutation-selection balance models would cover equilibration within a single fitness class, but there is no reason to suppose that equilibration processes are compartmentalized in this way.) Such interactions were assumed in our explanation for the trajectory reversals above. The fact that two mechanisms, truncation selection and rescaling of relative fitness for alleles with constant "absolute" fitness contributions, both produce this effect should make it especially robust.

The proposed mechanism should be at least partially compensating as well: if one class fluctuated higher than its expected value while all others were at theirs, the higher mean Darwinian fitness would depress the realized *relative* fitnesses, relaxing all classes (stochastically) to lower levels under mutation pressure and similarly for deviations in the other direction. This suggests inter-class compensation for frequency deviations. Testing specifically for it would further validate this proposed mechanism. We want a situation in which if one fitness class deviates from its equilibrium trajectory, causing a net fitness deviation from equilibrium of a given amount, we can determine whether and how closely the sum of frequency deviations in the other classes produces an equal and opposite fitness deviation. In the model of figures 4-7 we produce this in a situation which also has a more realistic distribution of selection coefficients. We look for returns to equilibrium after the system is perturbed from it by "natural" fluctuations, produced by sampling processes.

Insert Figures 4 and 5 about here

In prior research we had focused on trajectory averages, accumulating individual replications to improve estimates. Smaller populations (only 10 rather than the 100 used earlier) showed much larger fluctuations in class frequencies, and lead to a crucial reassessment of the data—a figure/ground reversal. These fluctuations were remarkable, with nearly simultaneous visibly countervailing fluctuations in different classes. But instead of being noisier and more problematic for calculating averages, the character of these fluctuations became the targets of interest. If present, an equilibration process should be at work in each replication, with individual noise as "natural experiments" yielding different sets of perturbations on it. Averages were no longer data-goals, but became reference standards to calibrate perturbations in individual replications.[17] Fluctuations far from the equilibrium trajectory in one class should be opposed by

[16] Initial simulations with population sizes of 100 showed similar but less extreme trajectory reversals than those discussed below. Kauffman was puzzled by the reversals and proposed smaller population sizes to test whether they were a kind of sampling artifact. We were convinced they would not go away, but didn't anticipate how revealing this would be. Also, computation time was limiting. Single replications of N = 100 for 4000 generations took 26 hours (in 1989!), and could not be scheduled often. With populations of 10, we did 3 or 4 per night.

[17] This then became an (unplanned) near perfect example of a common model-building strategy found often in classical genetics: building a model which generates a pattern which can be compared with the data, not to confirm

fluctuations in one or more of the other classes. If evenly balanced, fluctuations in any class ought to be compensated by the sum of deviations in the other four.

Even with populations of 10, very few simulations had large fluctuations permitting easy analysis.[18] The most striking, replication #50, had massive fluctuations in an unusual and very useful pattern (Fig. 4): 2 out of 8 connections in the largest fitness class were lost by drift very early (by generation 100). These both subsequently back mutated, going rapidly to fixation—the first between generations 400 and 450, and the second between generations 1300 and 1350. This gave 3 of the largest possible perturbations: the double loss at the beginning, and two back mutations restoring connections to populations, which (by hypothesis) had adapted to their absence. (Back-mutated large connections (classes 1-3) go from 0 to fixation within the 50 generation sampling period of the data). The double loss in the largest fitness class was remarkable, and its presence in the first class allowed a fortuitous form of data aggregation by pairing the other four classes to show patterns not visible otherwise.

The analysis began with graphs output by the simulation: 2 each of the 50 run averages and the trajectories of replication #50 (Fig. 4). Pairing average and replication #50 trajectories (graphs #2 and 4 of figure 4) together, we could extract the deviations (Fig. 5). The deviations revealed clearly countervailing trajectories, with the deviations in classes 2-5 above the *x*-axis while the massive perturbation in class 1 was below. Classes 2-5 then recentered their (still oppositely directed) fluctuations twice, matching their compensation as the deviation in class 1 relaxed twice precipitously back to the zero-line.

Insert Figure 6 about here

We then plotted the sum of deviations in classes 2-5 with the primary deviation. The lumped sum was a noisy mirror image. Reversing the sign of the primary (Fig. 6) permitted direct comparison—and a remarkably good fit: the scale of the noise was small compared to the large excursions in the primary that it followed so closely. Summing all 5 deviations to see their net effect deviation from the equilibrium trajectory revealed a large transient excursion downwards made and reversed in the first 200 generations, followed by relatively close tracking of the zero-point with excursions usually smaller than those in any of the contributing five classes for the rest of the run (Fig's 5, 6).

So not only did equilibration of classes 2-5 occur with the massive deviations in class 1, but there also appeared to be equilibration among classes, as one would expect: no class should be privileged. How can we visualize this? Because each class made the same fitness contribution, relative frequencies of the different classes could be meaningfully graphed at the same scale, signifying fitness deviations. To understand what is happening, note the following:

(1) The number of connections affects loss rates in a given fitness class at two points: larger classes of alleles (in the lower-fitness connections) yields more expected mutations (and losses)

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the pattern, but to see the structure of deviations from it. The deviations are often more interesting than the original pattern. (Wimsatt 1987)

[18] Desirable fluctuations had unusually large losses in just one class. The quantized character of replacements in the higher fitness classes produce crisper shocks on the system. Run #100 was thus also interesting (Fig. 7): no losses in the first class but 5 (out of 16) in the second. These back mutated over the next 950 generations, (in generations 50, 150, 400, 650, and 950) producing a "sawtooth" pattern of deviation, with adjustment in the other classes and a smaller mean deviation for all 5 classes together than in run #50. But with the perturbation in the second class rather than the first, one could not lump classes 2+3 and 4+5 to see the clear scale effects we saw there.

though the proportional loss rate should be the same. But the class size affects the number of possible "correct" hits for back mutations restoring missing "good" connections.[19] Thus, in class 1, the first reversion (with 2 possible targets) occurred within 350 generations, but the second (with only 1 left) took another 900. For lower fitness classes with many more lost connections, "good" back mutations were virtually continuous.

(2) Since alleles in the more numerous classes had corresponding smaller fitness contributions, their finer scale adjustments in genotypic fitnesses were more likely to remain at intermediate frequencies in mutation-selection balance (not driven immediately to fixation if present), providing potential buffers for changes at other loci. Stepwise changes in frequencies in the top 3 classes across sampling intervals suggest movements from absence to fixation in one 50-generation interval, reflecting strong selection (Fig. 5). The bottom 2 classes wandered more continuously up and down reflecting smaller changes in total frequency if one went from absence to fixation, and many alleles at intermediate frequencies in fluctuating equilibria. This is expected given the larger number of alleles in those classes (with more back-mutation) and the weaker selection on them, allowing rapid (but weak and noisy) small-scale response.

Moreover, the fact that the perturbing fluctuations were in class 1, and the compensations occurred in 4 contiguous classes showing doubling of connections and halving of fitness contributions in successive classes allowed another data transformation:

For more clarity we lumped neighboring classes 2+3 and 4+5 to form two heterogeneous classes with larger differences in numbers and in fitness contributions,[20] making scale-dependent phenomena more visible with the 2 new composite classes than in 4 unlumped neighboring classes. The two compound classes now provided all possible sources for change counteracting deviations in the first class or each other, allowing direct visualization of opposing compensation in the deviations.

The 3 levels of primary perturbation in effect generated 3 experimental treatments for the other 2 classes at different stages in the trajectory. Several things now became visible in Fig. 6:

(a.) The primary perturbation is initially offset almost completely by the more frequent and smaller-effect alleles in class ***4+5***. Class ***2+3*** did not compensate significantly until after the first back mutation in class 1 (at generation 500). With expected mutations at 4 times the rate in *4+5*, and 4-fold smaller fitness contributions, fewer alleles would be fixed. With more alleles at intermediate frequencies, selective response is faster both because frequencies change more rapidly in the middle range, and there is no wait for a mutation. This is crisper confirmation of the explanation in (2) above, as are the other observations below.

(b.) From generation 550 to 1300 (the second back mutation in class 1), classes ***2+3*** and ***4+5*** compensated roughly equally, suggesting compensation proportional to their *exposure*. Even fluctuations in ***2+3*** seemed balanced by oppositely directed fluctuations in ***4+5***.

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[19] In this model genotype is just a list of pairs of numbers, for connections between pairs of genes, the second number the target of gene action and the first the actor. Random mutations would (stochastically) generate good connections in a class in proportion to the number of missing good connections.

[20] Each larger class had identical fitness contributions, with two sub-classes in a 1:2 connection ratio with 2:1 fitness ratio. Though heterogeneous, they were identical in structure, and differed only in size. With the nonlinearity of interactions, these strong similarities were crucial to clear demonstrations of the effects. Class ***2+3*** had 16 + 32 = 48 connections, and class ***4+5*** had 64 + 128 = 192 connections. These heterogeneous classes had respectively 3 times as many connections in them as classes 2 and 4, improving sample size. Numbers and fitnesses in the new heterogeneous classes now differed by a factor of 4 rather than 2, yielding much more pronounced scale effects.

(c.) From 1350 to 4000, the primary deviation had disappeared. Classes ***2+3*** and ***4+5*** compensated for each other alone, with oppositely directed fluctuations about 0.

(d.) Class ***2+3*** is significantly "grainier" in responses than ***4+5***, as expected. It moves less often, with visibly quantified "hops". With its fixations, and longer waits for rarer back-mutations, it acts as a secondary driver to 4+5.[21]

We thus see remarkably fine-structured adjustment in and between different fitness classes for deviations in any one of them, modulated by significant differences in the stochastic character of responses in different classes because of their sizes. The summed fit of classes 2-5 to class 1 deviations was so good (and tracked back mutations so well with changes on different scales) as to suggest a stochastic analogue to a Fourier series approximation to a square wave function. Stochastic fluctuations are endemic, but each large fluctuation was answered by countervailing ones in other fitness classes. With more connections and the smallest fitness scale, class 5 (or ***4+5***) was normally first to respond to a fluctuation elsewhere, and with the finest-scaled adjustments, with the others roughly in order as their increasing scale.

A Methodological Note

Why spend so much time discussing a single rare case? Isn't this a Monte Carlo simulation? And don't simulations establish credibility by showing that an outcome is very frequent in an ensemble as a whole, or at least under a desired array of parameter values? This objection would be misplaced. It was appropriate for us to do many runs to get good estimates for the average trajectories. And interclass compensation, if it occurs at all, must occur generally. But no matter how common these phenomena are, conditions allowing clear demonstration are rare. Any simulations with deviations large enough to see above the normal noise in the process exhibited apparent compensatory behavior. Seeing it requires larger fluctuations, and to analyze it, the fluctuations need to meet still more conditions. For those that did we saw compensation on all scales. Very few of our 100 replications were "natural experiments" which made "good" cases. Statistical analysis of the compensation would be a complex multivariate problem and not worth the effort given how clearly it is demonstrated in replications #50 and #100.

For clear demonstration we sought trajectories which (1) deviated substantially from their averages: larger deviations provide larger compensations, easier to detect and analyze. Desirable cases also have (2) most of the deviations concentrated in a single class, so its sign and magnitude could be compared with deviations in the other classes, (3) be in one of the top 3 classes, which characteristically produced "quantized" transitions—frequency changes large and sudden enough to generate noticeable responses in other classes, and (4), in the top class, so the other classes could be paired to better see scale-dependent effects and reduce the number of trajectories.

With all these conditions, a replication like #50 is like a rare and useful mutation. Only 3 others involved loss and back mutation of even a single allele in the top fitness class. The next best case was run #100, (Fig. 7) with loss of 5 out of 16 alleles in class 2, and their successive back-mutations over the next 950 generations. Figure 7 shows ("by inspection") nice and fine structured qualitative compensation involving all 5 classes. But location of the perturbation in class 2 prevented the class lumping which was so revealing in replication #50. Just eyeballing this case shows why it would be hard to analyze further.

[21] After generation 550, when equilibration is well-established, class ***2+3*** stayed in place in successive sampling periods for 42 out of 69 possible transitions for a transition probability of 39%, whereas class ***4+5*** did so only 8 times—a transition probability of 88%. Class ***2+3*** had 8 longer runs of 9, 6, 6, 6, 5, 4, 3, and 3 constant periods while class ***4+5*** had no constant periods longer than 2.

Insert Figure 7 about here

Discussion and Conclusions

Dilemmas of evolutionary modularity

Modular adaptive systems would seem to have great advantages over comparable systems with less modularity.[22] Herbert Simon (1962) pioneered arguments that "nearly-decomposable" systems modularly organized in "stable subassemblies" should evolve more rapidly than things that are not. Lewontin urges related principles of quasi-independence and continuity: with any variance for modularity, there should be selection for more of it (Lewontin 1978, Wimsatt 1981, Brandon 1999, Schank and Wimsatt 2000.) Wagner and Laublicher 2000 have recently offered a formal basis for this notion. Perhaps over the long-run selection or reduplication groups the architecture of gene expression and developmental interaction into units which can recombine together efficiently to address the combinatorial array of special problems organisms face in different environments. But Wagner and Altenberg 1996 consider such a mechanism, and on further analysis, raised doubts over its efficacy. The simulations here may raise further doubts. Thus the aims of this discussion are first to set some of the context and correlative complications, and second, to see how within the context of our results modular changes can most likely be established in evolution. Given the structure of our simulations, our results bear on the fate of changes in parts or modules insofar as they affect the fitness of the whole organism. They make no predictions directly of the relative strengths, numbers, or fates of intra- vs inter-modular interactions.

Modules must also function integratively (Wimsatt 1997, Schank and Wimsatt 2000). Here a tension emerges: Developmental biologists speak of anatomical or developmental modules, but the evolutionary modularity of the parts of a system—the ability to substitute and rearrange them without perilous fitness consequences—might seem at odds with the functional integration necessary to make something an evolutionary unit. What is the mapping between genetic organization and phenotypic organization such that evolutionary rearrangements or modifications of the genetic architecture doesn't scramble the functional organization of the phenotype?

To gene selectionists Dawkins' (1976) fable of evaluating rowers by interchanging them among crews so they perform against different backgrounds, and supposed parallels of this process with genetic recombination seem to promise that it is possible. Our simulations suggest that this may be misleading. Beyond issues of direct causal functional integration, an even deeper process binds even apparently unrelated elements that combine to build the phenotype, and results in a kind of unanticipated interaction spanning the whole organism. If the very processes that make evolution possible—variation and natural selection—poise organisms at the competitive edge of extinction, then our simulations suggest that evolutionarily significant modules become tightly coupled. But do they stay tightly coupled? If they do, the answer may lie

[22] This supposes that ability to adapt rapidly to environmental change is advantageous (and that such rapid environmental changes are relatively common over evolutionary and ecological time). Spatial and temporal patchiness has been accepted as common since the population biology of the 1970's, and Van Valen's (1973) "Red Queen" hypothesis assumes and provides evidence for constant environmental degradation for a species as a result of escalation by its competitors, predators, and parasites. In a long static environment, high integration could have bigger advantages. But not in all cases: modularity (e.g, the ability to lose and regenerate limbs) could well be beneficial for some evolutionarily stable aspects of the environment.

in entrenchment processes. We reify modules at various levels, but do we yet understand how such modularity is evolutionarily possible? We suggest below that such evolutionary modularity may be transient and rare, and made possible by relaxation of selection from either external or internal sources. But even so, this may suffice to generate significant evidence of what we call modularity.

What is a modular change? We assume it should be a change that can be made without affecting numerous other things in the phenotype as a whole. (We have seen that this may not be often possible for fitness, though that says nothing definite about the frequency of modular change thus conceived.) Reversibility is also evidence for relative modularity, because it suggests independent modifiability, and consequently, less fitness epistasis of that element with others in the phenotype. But also, many things that look modular and internally integrated may have been products of rapid elaboration of smaller pivotal changes which led to a bifurcation of divergent trajectories. If this involves changes in localized (but not decoupled) components of phenotype, we would have apparent modular change in at least one of two corresponding highly integrated subsystems of divergent species.

Summary of conditions under which modular replacements could occur

All of our simulations of generative entrenchment showed an almost overwhelming bias favoring retention of strongly selected or of entrenched connections. Yet in the simulation just analyzed, we had loss of two major connections. How is this possible? We believe these simulations show conditions relevant to the transitory appearance and disappearance of modularity. Under what conditions can such major losses occur? There are four, all important, and none surprising. But their conjunction permits conclusions which seem both surprising and important.

First, *as important as the connections were, their losses were not lethal.* They were conditional lethals, but lost early in the simulation when those conditions were not met—before other losses which would have made them lethal. (Their competitors were likely in comparable states.) Many deeply GE'd features would be lethals under broader or even all conditions, so *changes in evolutionary modules should not disrupt deeply entrenched features*. Modularity tends to decrease the effect and scope of entrenchments (Schank and Wimsatt 2000).

Second, *events like those in replication #50 were rare*, even though we manipulated conditions to make them much more common.[23] But how common must modular changes be on what are very long time scales for population geneticists (say 10^5 to 10^8 generations) to appear moderately common in the paleontological record? The answer, we suggest, is "not very." (Such changes don't commonly flip back and forth, for example, though reversibility would strengthen the case for modularity, because it suggests independent modifiability.) The lovely example provided by Wimmer (Wimmer et.al. 2000, Schmidt-Ott and Wimmer *this volume*) of how *bicoid* could entrain and integrate existing control circuits provides a plausible way of making changes deep in development while preserving the downstream consequences whose disruption would be lethal. But this change became irreversible through loss of once useful but now redundant control linkages—another expression of generative entrenchment. And even if most such deeper changes employed similar scenarios, they would not be common in absolute terms.

Third, *population sizes were very small*: only then could we expect to lose important adaptations or make major changes through drift. In addition, the more we learn about

[23] Thus we used very small populations and artificially high mutation rates, but the absence in our model of recombination and population structure in a patchy environment would act in the other direction. But such changes don't have to be common, even on paleontological time scales.

evolutionary history, the more such bottlenecks in population size (or local ones, leading to local differentiation of populations) seem common.

Finally, *you have a better chance of losing a crucial adaptation, or surviving a seriously deleterious mutation (or a potentially beneficial but poorly integrated one) if conditions are very good—presumably both genetically and in the environment*, as they were early in the simulation. Then traditional "hill-climbing" tinkering can improve the fit. The pivotal importance of this condition (first proposed and developed by Wallace Arthur 1984, 1997) emerges much more strongly here than before. Why? Because only with the excess slack cut by very good conditions do we have room for quasi-independence to work on modules of any appreciable "size". We return to this below.

Could such a simple model possibly have anything to tell us?

This model might have seemed quite unrealistic for its positing a single "best" genotype and the consequences of mutational loss from that point. But there are important and generalizeable things to learn from it. Properly construed, this is not a single-peaked optimizing argument, but a satisficing one (Simon 1962) about behavior just above the rising flood. The prime function of modeling a population as composed of a focal adaptive phenotype is not to provide a realistic model of variability and focal adaptation in nature—it isn't—but to show the consequences of mutation-selection balancing processes under conditions where a population starts with reserve reproductive capacity and degrades to just making it. And the loss of smaller-contributing elements documented here and their effects suggests that degradation. With the occurrence of the major adaptive back-mutations, these simulations also show the consequences of sudden relaxations in selection intensity. And this may be to the domain of attraction of a quite different adaptive peak: what is scored as a back mutation to an original state is characterized only in terms of its fitness, and could just as well be another important mutation to an alternative state, and the release of variability with the relaxation of selection an exploration of new modifiers to the new state. Virtually every empirical population genetic study tracking stasis or small adaptational reversals or local genetic differentiation confirms this as the common picture. Losses of weakly selected alleles and anchoring of more strongly selected alleles says things about maintenance, but also about evolution, which can pivot around the stronger alleles. The effects arising through back mutation to restore stronger alleles can also just as well stand for the establishment of new fitter alleles, which then permit further changes, both through the relaxation of selection (like following exploitation of a new niche) but also through the accumulation of newly possible specific modifiers to improve performance or remove deleterious consequences of these new mutations.

The stratification of allowable replacements near a truncation selection threshold (in which deleterious mutations in only a narrow window of selection coefficients can be tolerated) suggests the remarkable picture of "neutral percolation" through a high-dimensional adaptive topography for RNA configurations presented in Huynen, Stadler, and Fontana, 1996. A neutral mutation is one that is (selectively) neutral relative to the allele it replaces, not one that is silent or does nothing. Then substitution of one allele for another in the same fitness class is, for these purposes, a neutral substitution. So their qualitative picture of unconstrained motion from one attractor to another through neutral percolation should apply also in the context of our model. And addition of small ranges in selection coefficients as "nearly neutral" broadens the scope of their picture.

Indeed, consider this model as a partial description of a much more complex system, rather than as a complete model of a simple system. If we treat the 248 locus model as a lower-dimensional slice—a sampling from the loci and alleles in a much larger system—the chance of

back-mutations for any of the alleles is effectively zero, and with losses and back mutations we are doing a random walk through a very high dimensional genotypic space. Indeed, this is a far more realistic interpretation of this model as applied to nature. From the point of view of these selection models, it doesn't matter if the allele that "reappears" (as defined in the model) is a genuine reappearance or just an appearance of another allele with the same selection coefficient as the one lost.[24] It doesn't even matter whether what appears is a gene or a change in fitness reflecting an environmental change. Fitness is a relational property between organism and environment, so conclusions drawn in our models about response of selection on genotypes to fluctuations in fitness apply equally whether those fluctuations are genetically or environmentally induced.[25]

Organismal selection revivified?

Most strikingly, these simulations suggest that the evolutionary outcome for an adaptive element in a phenotype commonly depends on the fates of other phenotypic elements which may be quite developmentally and functionally disconnected from each other save for just being parts of the same organism. This kind of interdependence reveals a new arena in which discussions of modularity in development and in evolution must take place. Selection is always selection of whole organisms: We have rediscovered what might be called the "last straw" principle. For an evolutionary unit close enough to the threshold of viable fitness, loss or compromising of almost any functional component (no matter how functionally decoupled from other components) can drag it under, and the more fitness "buoyancy" a unit loses the less it has to play with. And genetic load and "Red Queen" (van Valen 1973) arguments each independently suggest that organisms spend most of their time close to that threshold.

The interdependency among phenotypic elements emerging from these simulations seems remarkably non-specific. Though phenotypic and environmental details clearly impinge in determining fitness, what ultimately determines whether an evolutionary unit can lose a phenotypic component is the magnitude of its fitness contribution, and how close the evolutionary unit is to the critical fitness threshold. It should be emphasized that this is not necessarily loss of *modules* of different sizes, but of any parts whatsoever, modular or not, including ones which may be quite distributed in location and quite pleiotropic in their effects. Indeed, *what we appear to have demonstrated unusually clearly in these models is organismal selection*, for here organismal fitness still appears to matter enormously even in a case where we have removed all obvious sources of gene interaction. But that is ultimately illusory, for *the existence of truncation selection itself creates a maximally global form of epistasis. And truncation selection is ubiquitous.*

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[24] Though it should be (exponentially) more difficult to find allele-replacement in the same fitness class with increasing size of the change.

[25] The conclusions of this model do depend upon (1) the distribution of selection coefficients, (2) the closeness of the fitnesses of an organism and its deme to a truncation selection threshold, and the dynamics of (3) gain, (4) loss, and (5) fitness changes of alleles in genotypes near this boundary. Our conclusions are fairly robust under plausible variations in (1). Anything that could cause sufficiently large uncontrolled or unpredictable changes in 2 thru 5 during the course of the simulation (i.e., on a scale comparable or faster than the relaxation times of the equilibrating processes modeled here) could affect outcomes of these simple models. Three features of (endo-) genetic architecture require further analysis. Diploidy (and polyploidy), linkage, and sex, if present in a system seen only through this model, could appear to affect mutation rates and stability of selection coefficients in ways not predicted by this model. Whether (or when) these effects are significantly large needs analysis. Aspects of population structure (exogenetics?) and environmental patterning could also be important.

A strength of weak ties?

Another surprising result from these simulations is the role played by genes of small fitness effect. Things that are "nearly neutral" are readily lost. With lots of them (as we suppose there are), allowing populations to approach more closely to the critical fitness threshold and inflating the relative fitness contributions of all, their loss serves to better anchor the larger contributing elements more securely. Larger scale mutations act as constraints on smaller scale mutations because of their rarer occurrence on longer time scales, but they are in turn cemented in (and made more constraint like—in their presence or absence which must be adapted to) by loss of smaller scale mutations with negligible individual importance. Paradoxically, phenotypes with more homogeneity in the importance of their components (if they could exist) would have their parts less secure than comparably important components of phenotypes which had lots of "small change" adaptations.[26]

This shows up in our simulations. Compare Fig. 3 (100 loci, fitness range from .02 to .06), with Fig. 4 (248 loci, fitness range from .00625 to .10). The simulations depicted in these figures both had an exposure of 4, and the larger circuit's lower mutation rate offsets its larger size. The main difference between them is their equilibrium mean Darwinian fitnesses—smaller by a factor of 3 or more for the larger circuits (about .10 vs, about .03). The effect is clear and to be expected for the greater rescaling of relative fitness in the latter case (Fig. 4). Connections with assigned fitness of .02 and .03 in the first simulation go to *lower* frequencies than connections with assigned fitness of only .0125 in the second simulation, and the top three classes exhibit similarly divergent effects. This is due to the presence of more weakly selected alleles (connections) in the second simulation.

The consequences of breathing space

Gaining (or regaining) a larger-contributing element gives more "breathing space" and deflates relative fitness contributions. It should thus increase loss of smaller-contributing elements, *so the net effect is to generate a substitution of fitter alleles at some loci for less fit ones at other loci*. This is the lesson of the trajectory reversals in the upper fitness classes accompanying the losses in the lower fitness ones (Fig. 4). Even without epistasis as normally conceived, there can be significant global inter-locus interaction of the whole phenotypically embodied genome in the co-production of fitness.

But this "relaxation" phenomenon has another important consequence: *either a significant relaxation of environmental conditions improving fitness* (facilitating Arthur's (1984) "n-selection") *, or a mutation doing the same thing should lead to a wave of further possible (usually smaller) innovations*, and that for reasons apparently "internal" to the fitness architecture of the genome and design of the phenotype. Looking now to modes of phenotypic architecture which can yield these benefits, redundancy seems a natural, giving reserve room for experiment through simple duplication of function. Gene-duplication provides a "bottom-up" example. Or phenotypic architectures may provide highly context-sensitive and conditional arrangements, which work only contingently and transiently. An emerging and important example of this last is provided by the heat-shock proteins, whose dosage and activity can interact with environmental stress to allow developmental expression of an extremely diverse range of morphological variation (Rutherford and Lindquist 1998).[27] But canalization, maternal

[26] This is a thought experiment with a result that is more surprising than threatening: all real organisms should have lots of "small change" adaptations.

[27] The mechanisms they have uncovered would tend to be activated not in "relaxed" environments, but in various kinds of environmental stress (particularly but not only heat shock), presumably like those described by the Grants

effect, symbiosis, social support, and all of the modes of behavioral plasticity, as well as relaxation of competition provided by extinction of competitors or windfall fluctuations of environmental conditions can provide rich opportunities of the second sort, and all of them can open up new opportunities for elaboration or transformation of the niche. We need to look to the ecological dimensions of the problem.

If we look to the now best documented cases of selection in the literature, the extended work on Darwin's finches by the Grants (e.g. 1989) and their students (reviewed in Weiner 1994), we see lots of opportunities for the kinds of fluctuations in fitness suggested as important in our simulations. A few percent difference in beak depth in a bad year make the difference between life and death, in the next year between being mated or not, and in those and other years, in food preferences and niche differentiation. Yet in good years, with excess food, such marginal differences may have no impact on mating and survival. Even larger variants could survive, and with the right conditions, spread. Intense selection, with frequent reversals in direction of favored variants provide excellent opportunities for the importance of conditions suggested by our simulations in allowing relatively major and even modular changes, confirming at least part of Wagner and Altenberg's (1996) conclusions.

The significance of context independence

Finally, what kinds of adaptations make the relatively context independent contributions which make their relative fitness scale up when they approach the threshold of a truncation selection regime?[28] Our models tell us that these kinds of adaptations are important. They are adaptive elements whose contribution does not scale multiplicatively, or proportionately to the fitness of the organism, but tend to make a fitness contribution whose effect is at least partially independent of the other factors producing differential fitness. Any time we have threshold effects, or things whose losses are not readily compensated for, we violate multiplicative scaling, and can produce the conditions for these models to apply. And threshold effects are ubiquitous in organic systems.

Paradoxically, the things whose fitness effects are most insensitive to context are large and far reaching enough in effect that their loss is fatal, and so they never can be gained or lost independently. But this is not modularity: the context insensitivity is one-way: loss is unconditionally lethal. For modularity, we need a context-insensitivity at the other end of the fitness scale: interchangeability that does not disturb relatively high fitness too much, fitness of a well-functioning system. Only with design for modularity and for portability (as with plasmid bodies, or commonly commensal parasites) can we imagine true modularity—involving frequent gain or loss, or exchange of coordinated clusters of traits.[29]

Quasi-independence seems theoretically and observationally robust. This appears to be in direct conflict with generative entrenchment, unless we keep the scale of events in mind. Frequent events on a paleontological scale may be quite rare to a population geneticist. So perhaps our constraints do not prevent modularity on a macro-evolutionary scale. If we suppose that quasi-independence is highly context sensitive—suggesting that if there are modules, they

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(1989). However new morphological variations produced by HSP's may boost fitness, and thus generate what is for those variants a "relaxed" environment in the sense used above.

[28] To scale in this way, an adaptation must contribute a fitness component which is relatively context independent, and thus contributing, say, on average .1 offspring, rather than increasing reproductive output by 10%. The former contribution yields fitness of 1.1 and 2.1 for an organism to which it is added having fitnesses of 1 and 2. The latter would yield fitnesses of 1.1 and 2.2.

[29] Kim Sterelny's skepticism about an earlier incompletely and erroneously formulated version of this point was crucial here.

are shifting arrangements which show relatively high context sensitivity, or, while modular, are requirements for almost any living thing of that type—then such modules can, nevertheless, still be deeply generatively entrenched. So if we are right, modular changes may happen, but rarely, and require near "garden-of-eden" conditions to support these experiments.

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Figure Captions

Figure 1: Representations of life cycle, with developmental stages and generative entrenchment (a). Circles are features internal to phenotype, squares features in environment, both characteristic of normal development, and interacting causally through succession of developmental stages. Nodes in dotted circles are assumed to be changed or perturbed, with downstream consequences propagated along arrows to other lighter shaded nodes. Activation or change of features early in development characteristically has more pervasive effects as in (b), than later changes as in (d), but some early changes have relatively little effects, as in (c). Some may have no effects (as with a mutation in an inactive pseudogene). And many effects exit and reenter across organism/environment boundary.

Figure 2: A directed graph representation of a gene control network with 20 genes and 20 connections output by the program. Nodes are genes, and a directed arrow indicates action of the gene at the tail on the expression of the gene at the head of the arrow. Mutations act on connections, and may randomly reassign the gene at the heat or the tail of the arrow. With 20 connections, it thus has 40 mutable sites. This particular graph was produced by 100 mutation events acting on a closed loop model gene system of 20 genes and 20 connections. It is indistinguishable in generic properties from one constructed at random.

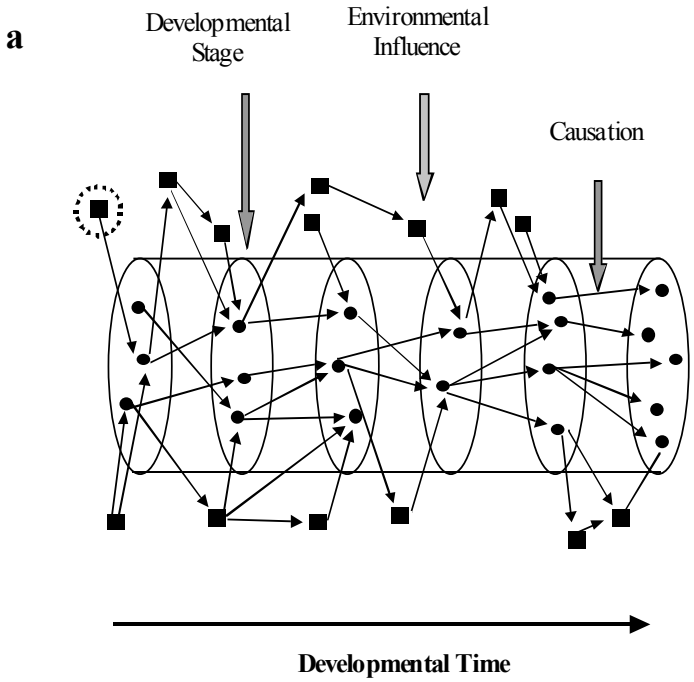
Figure 3: Evolution of connection frequencies (of good connections) in 5 fitness classes in gene control networks having 100 genes with 100 connections, so each network has 200 mutable sites. Population size is fixed at 100. The mutation rate is .005 per connection end (thus averaging 1 mutation per network per generation). The 5 fitness classes have 20 connections each of fitness decrements of .06, .05, .04, .03, .02. Total exposure, the sum of fitness decrements, thus = 4. Frequencies are averages for 10 runs. Mean population fitness (in % of start) indicated every 500 generations in top graph. Grayscale gives relative selection intensity of different classes, with black for greatest. Top graph: stacked bar of frequencies of connection types as a fraction of the total at the beginning. Bottom graph: class frequencies as proportions of their initial values. Their average frequencies through time follow the same order as their assigned selection coefficients. Note frequency reversals in top two classes, discussed in text.

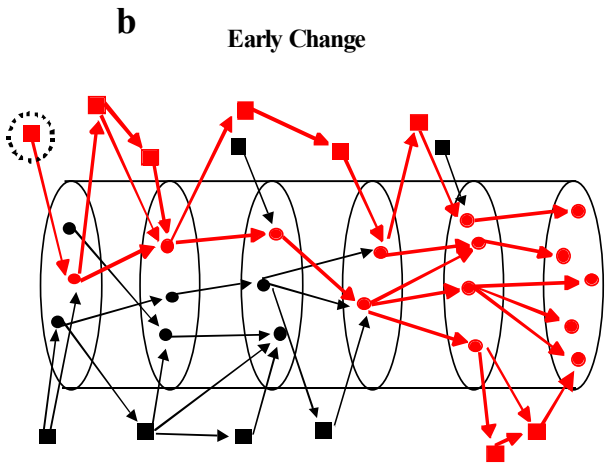
Figure 4. Gene-frequency trajectories for connections in 5 fitness classes in a 248 locus haploid model, with 8, 16, 32, 64, and 128 connections each having fitness contributions respectively of .10, .05, .025, .0125, and .00625. Thus each fitness class makes the same fitness contribution, of .8. Summed over all 5 classes this gives a total exposure of 4. Mutation rate is .0025 per connection end per generation. Top two graphs: run #50. Bottom two graphs: average trajectories for runs 1-50. Top graph in each pair is a cumulative stacked bar graph of frequencies in all classes. The bottom in each pair gives the relative frequency within each type by class. Note stochastic fluctuations in the top two graphs from average trajectories in the bottom two produced by use of small (N = 10) populations. Here there are anomalous trajectory reversals with initial decreases and later increases in top 3 fitness classes.

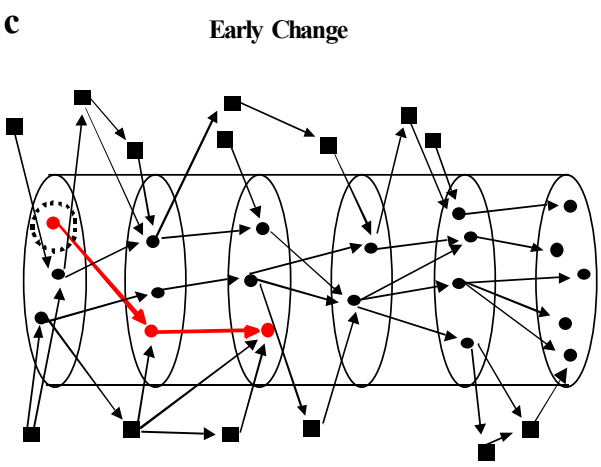
Figure 5: Combination of the data from the second and fourth graphs of the preceding figure to show deviations from 50-run averages of frequencies of different fitness classes of connections for replication #50, having an unusually large fluctuation in the first fitness class (loss by drift of alleles at 2 out of 8 loci). Larger fitness class frequencies (.1, .05, and .025) seem quantized after the first 200 generations (suggesting that back-mutated loci go to fixation within the same period they reappear, i.e., within 50 generations). Positive deviations in 4 smaller classes approximately offset negative deviations in the first class.

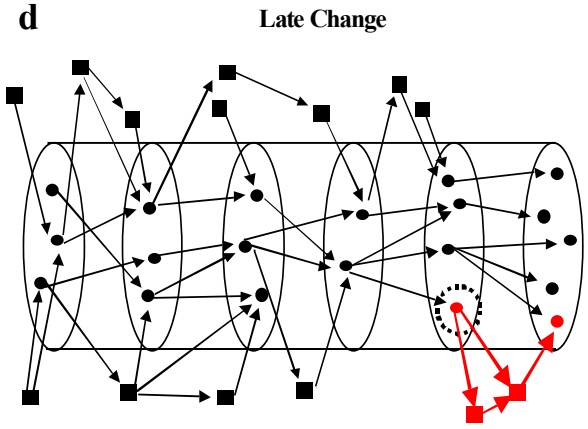
Figure 6: Equilibrating deviations in smaller fitness classes following a large perturbation with loss thru sampling error of 2 out of 8 alleles in the highest fitness class in the early transient stage of the first 200 generations of run #50. These alleles were reestablished by back-mutation after generations #400 and 1300 producing secondary perturbations. All 3 preturbations induced countervailing fluctuations in all other fitness classes. The 50 run average is used to estimate equilibrium tendencies of the system. The initial perturbing fluctuations in class 1 are shown here with negative sign (contrast preceding figure) to allow direct comparison with the sum of fluctuations 2-5, yielding a net deviation for all classes (Sum 1-5) which is less than deviations in any single class. Classes 2 and 3, and classes 4 and 5 are lumped together. Original losses occur within a 200 generation transient period, while decreases in mean fitness inflate relative fitnesses of all alleles, producing trajectory reversals for alleles in the 2 highest fitness classes. 248 alleles at loci in 5 fitness classes (.10, .05, .025, .0125, and .00625), having 8, 16, 32, 64, and 128 alleles respectively are subjected to mutation, and approach selection-mutation equilibrium frequencies determined by their fitnesses, the mutation rate, and population size.

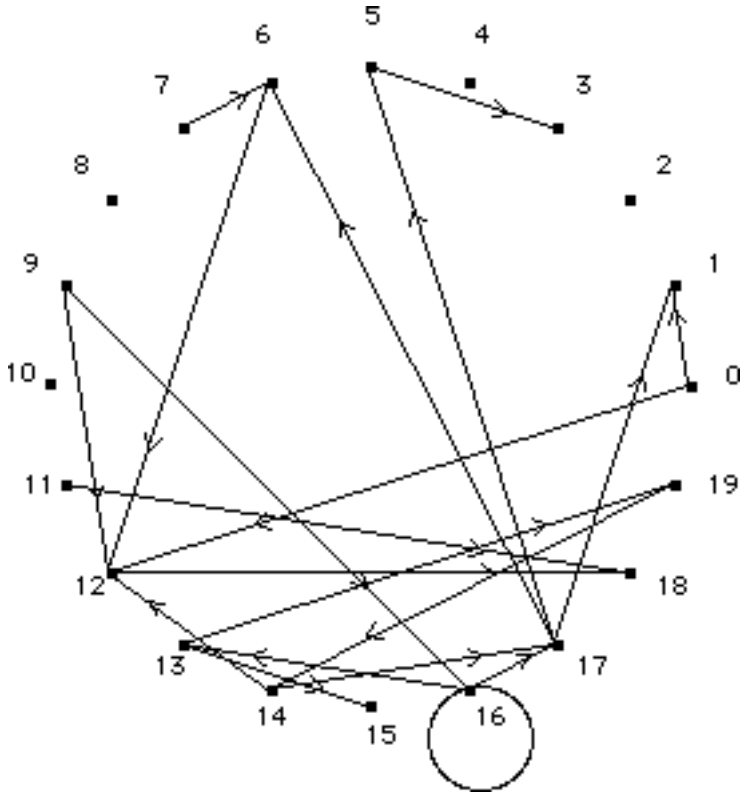
Figure 7: Equilibrating frequency deviations in fitness classes 1-5 from 100 run average for run #100, graphed with the total deviation of the 5 classes added together, showing compensation in classes 1, 3, 4, 5, for drift-induced perturbations in class 2. This is otherwise analogous to figure 6. The "sawtooth" trajectory of class 2 is a joint product of a sequence of back-mutations followed by quantal fixations (within the same 50 generation interval) occurring in generations 50, 150, 400, 650 and 950, and the exponential approach of the equilibrating average trajectory for class 2 (visible as the second trajectory from the top in the bottom graph of figure 3). Note particularly the countervailing deviations in 3, 4, and 5 after about generation 1200, when class 1 has gone to equilibrium fixation, and class 2 is asymptoting there. As a result, the sum of the 5 classes remains much more tightly bounded than any of remaining 3 fluctuating classes.










5
6
4
7
3
8
2
9
1
10
0
11
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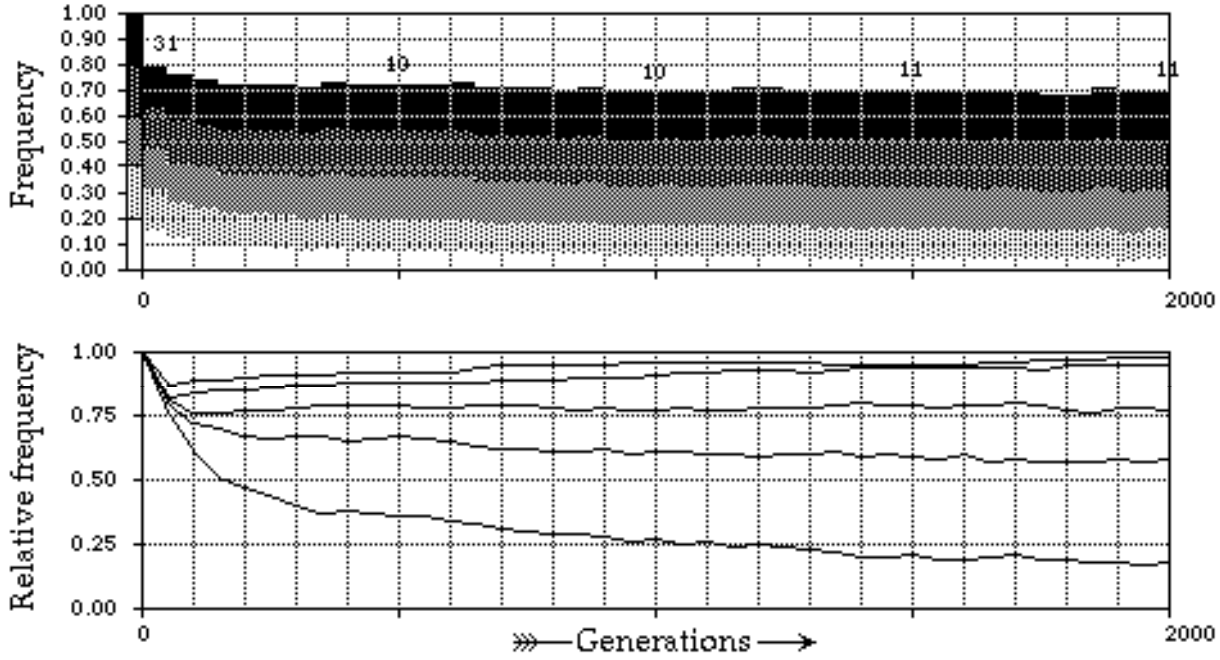
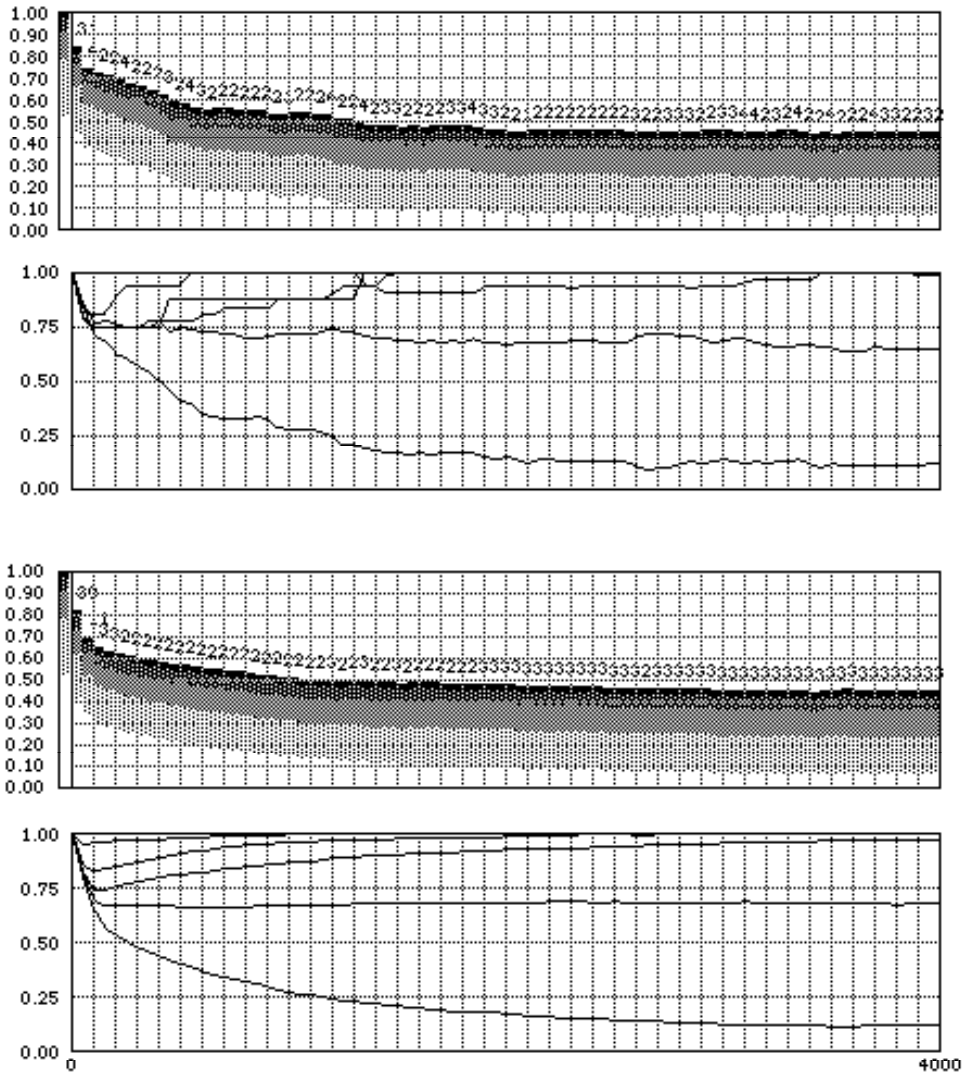
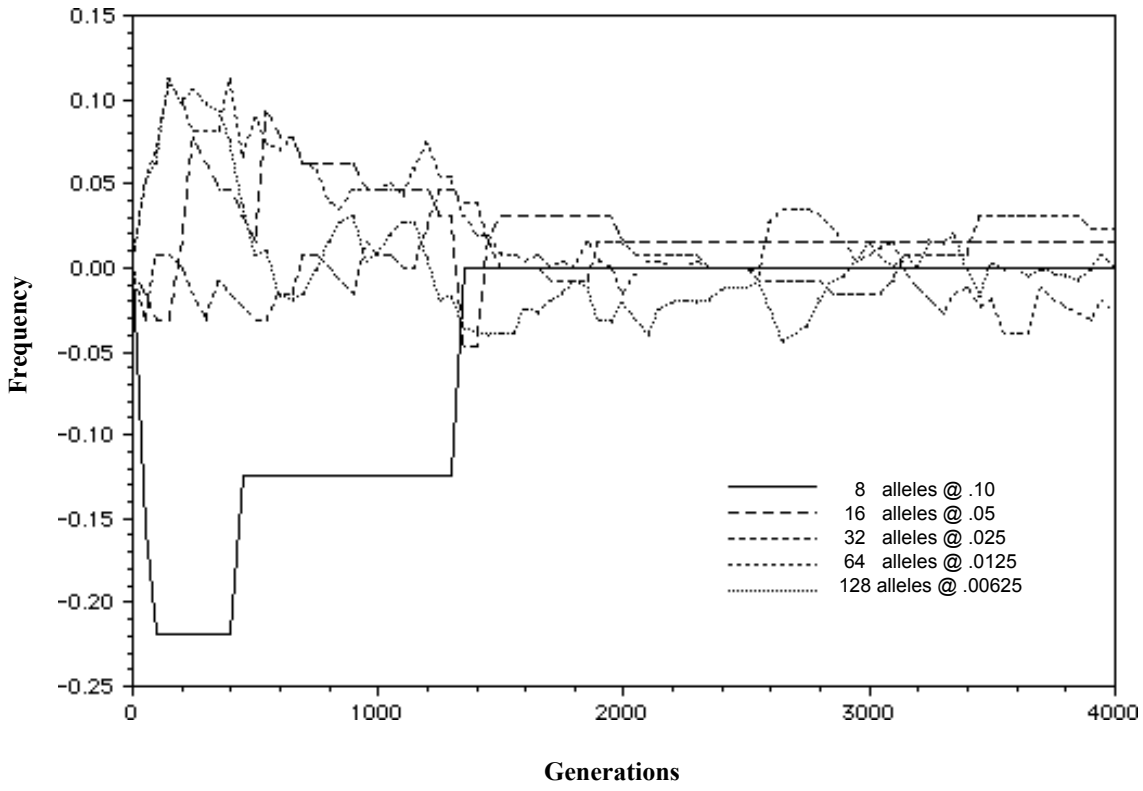
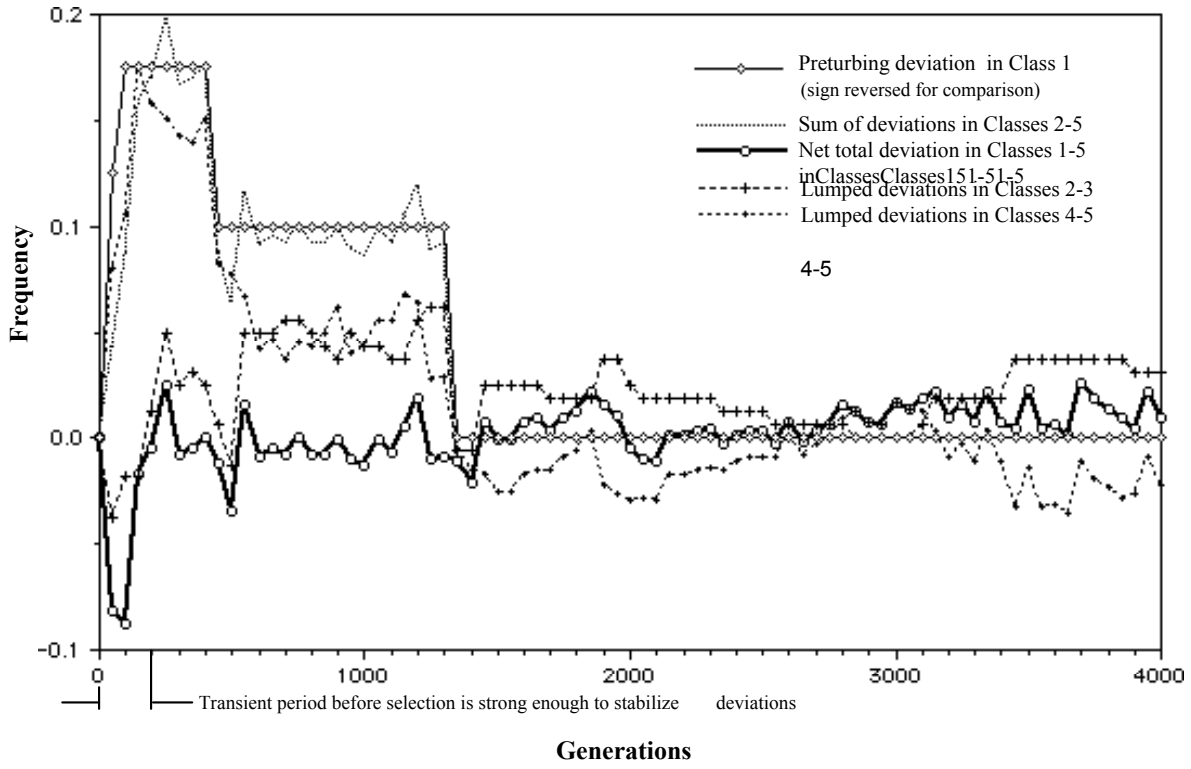
16

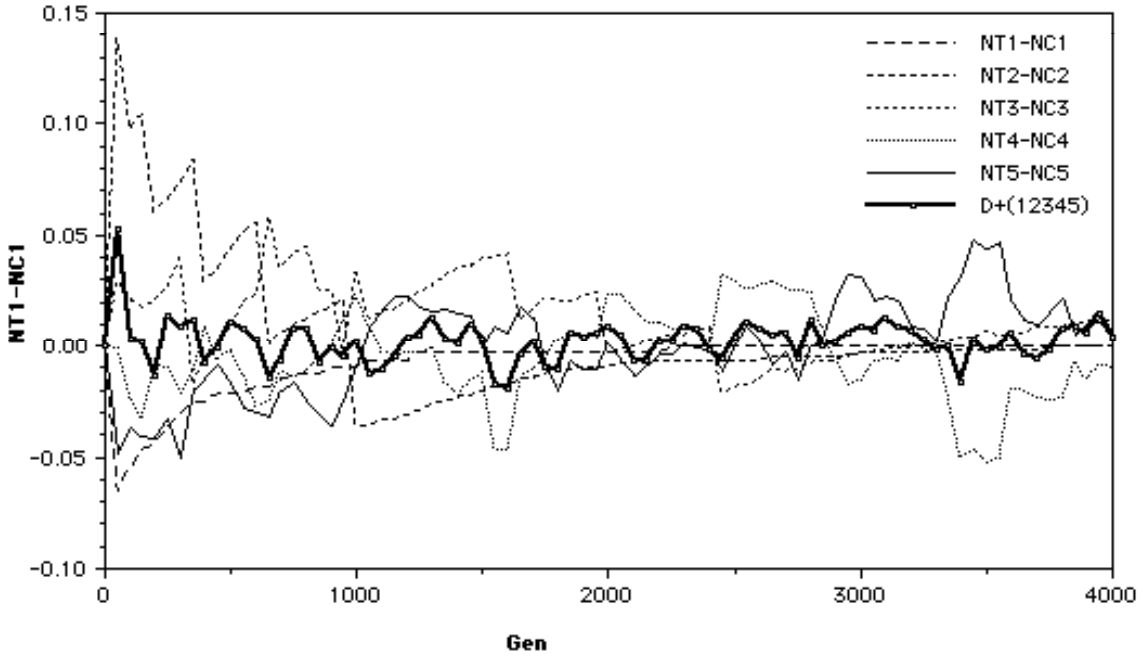

Frequency
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
31
10
10
11
11
0
2000
Relative frequency
1.00
0.75
0.50
0.25
0.00
0
2000
Generations



Fitness Types: 1: -0.1000 2: -0.0500 3: -0.0250 4: -0.0125 5: -0.0063
Genes = 100 Connections = 248 Mut. Rate = 0.0025000 Pop. N = 10


0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.20
-0.25
Frequency
0
1000
2000
3000
4000
Generations
8 alleles @ .10
16 alleles @ .05
32 alleles @ .025
64 alleles @ .0125
128 alleles @ .00625


0.2
0.1
0.0
-0.1
Frequency
0
1000
2000
3000
4000
Generations
Preturbing deviation in Class 1
(sign reversed for comparison)
Sum of deviations in Classes 2-5
Net total deviation in Classes 1-5
inClassesClasses151-51-5
Lumped deviations in Classes 2-3
Lumped deviations in Classes 4-5
4-5
Transient period before selection is strong enough to stabilize deviations


0.15
0.10
0.05
0.00
-0.05
-0.10
NT1-NC1
0
1000
2000
3000
4000
Gen
NT1-NC1
NT2-NC2
NT3-NC3
NT4-NC4
NT5-NC5
D+(12345)