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Evolution of mutational robustness

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Abstract

We review recent advances in the understanding of the mutation-selection balance of asexual replicators. For over 30 years, population geneticists thought that an expression derived by Kimura and Maruyama in 1966 fully solved this problem. However, Kimura and Maruyama's result is only correct in the absence of neutral mutations. The inclusion of neutral mutations leads to a wealth of interesting new effects, and, in particular, to a selective pressure to evolve robustness against mutations. We cover recent literature on the population dynamics of asexual replicators on networks of neutral genotypes, on the outcompetition of fast replicators by slower ones with better mutational support, and on the probability of fixation at high mutation rates. We discuss empirical evidence for the evolution of mutational robustness, and speculate on its relevance for higher organisms.

Keywords: Mutation-selection balance; Mutational robustness; Neutral mutation; Quasispecies

1. Introduction

In 1966, Kimura and Maruyama [1] published an important result about the average fitness of an asexually reproducing population. They calculated that the average fitness depends only on the mutation rate μ, and is given by $e^{-\mu}$ (times the fitness of the fastest replicator in the population), irrespective of the details of the fitness landscape. This result, also referred to as the Haldane–Muller principle (for Haldane [2] and Muller [3] first found the approximate solution $1-\mu$), has been the basis of countless arguments in theoretical population genetics, most notably on the evolutionary advantage of sexual replication [4–11]. However, the result is not as general as is widely believed. Kimura and Maruyama derived it under an important assumption—clearly stated in their paper. They assumed that the fastest replicating sequence in the population did not have any neutral sites.

Recent results [12–14] show that Kimura and Maruyama's assumption is crucial. If neutral mutations are taken into account, the average fitness of the population depends on both the mutation rate and the details of the fitness landscape. Moreover, in this case, a selective pressure exists which pushes a population into those regions of genotype space where the density of neutral sequences is highest, thereby increasing the robustness against mutations in the individual sequences. Under certain conditions, the selective pressure to increase mutational robustness can be even larger than the selective pressure to increase replication rate, so that a population can raise its average fitness by increasing mutational robustness and simultaneously decreasing individual replication rates. These results are best understood in the framework of quasispecies theory [15–17], which postulates that selection at high mutation rates does not act on the individual, but on quasispecies, that is, highly structured

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clouds of closely related mutants. The quasispecies concept has been used extensively in the interpretation of experiments performed with RNA-based viruses [18–20], where mutation rates of the order of one per genome and generation [21,22] can be observed.

It is important to note that quasispecies theory is not an alternative, but rather an extension of standard population genetics. Its basic equations are very similar in structure to mutation-selection equations that are studied in the population genetics literature [23–25]. The difference between quasispecies theory and classic population genetics lies mainly in the explicit consideration of high-dimensional sequence spaces, and in the consideration of high mutation rates.

In the following, we review the arguments in favor of the selective pressure for mutational robustness, as well as the consequences of this selective pressure for population fitness, individual replication rates, and the fixation process in quasispecies theory.

2. Evolution of mutational robustness

The following simple thought experiment explains how robustness against mutations has an immediate influence on the reproductive success of a sequence. Let us first consider a situation in which all mutations are either fully neutral or lethal, and weaken this assumption later. Consider two different sequences (genotypes), A and B, that differ in the fraction of mutations that are neutral (lethal). For sequence A, let one out of ten mutations be neutral, while for sequence B, assume that one out of five mutations is neutral. If both sequences replicate with the same individual speed, and produce mutated offspring with a probability of 0.5, then sequence B produces approximately 10% more viable offspring than sequence A. Now, if all immediate and future descendants of sequence A have the same 10% chance that a mutated offspring sequence is viable (that is, if this neutrality fraction is inherited), and likewise all immediate and future descendants of sequence B retain their 20% probability of producing mutated offspring that are viable, then it is clear that a colony seeded by sequence B grows approximately 1.1 times faster than a colony seeded by sequence A. Below, we refer to the two colonies seeded by sequences A and B as strains A and B, respectively.

Two additional conclusions can be drawn from the above example. First, the advantage of strain B over strain A must be a function of the mutation rate. If, for example, the mutation rate is around one, that is, if almost every offspring carries some mutation, then strain B grows twice as fast as strain A. In general, the larger the mutation rate, the bigger the advantage of strain B. Second, a potential *advantage* in replication speed can be offset by this robustness against mutations. Assume, namely, that instead of replicating with the same speed, sequences of strain A replicate 1.5 times faster than sequences of strain B. For small mutation rates, strain A will obviously outgrow strain B. However, if the mutation rate is sufficiently large (for example, around one), the advantage in replication speed is offset by the larger number of offspring surviving a mutation in strain B, and strain B will outgrow strain A.

It is fairly straightforward to cast the above ideas into a mathematical model, and to work out exactly under what conditions strain A or B will win [26]. Assume that the individual replication speed of a sequence in strain A is given by σ_A, and that the probability with which an offspring sequence in strain A is viable is Q_A (Q_A is the combined probability that an offspring sequence is either unmutated, or, if mutated, has suffered only neutral mutations). Then the overall growth rate of strain A is $\sigma_A Q_A$, and that of strain B is $\sigma_B Q_B$. Clearly, strain A will outcompete strain B if $\sigma_A Q_A > \sigma_B Q_B$, and vice versa.

If the two strains compete in a flow reactor with overall limited population size, then the concentration x_A of A as a function of time is given by:

$$x_A(t) = \frac{x_A(0)e^{\sigma_A Q_A t}}{x_A(0)e^{\sigma_A Q_A t} + x_B(0)e^{\sigma_B Q_B t}}, \tag{1}$$

and that of B likewise by $x_B(t) = 1 - x_A(t)$. Here, $x_A(0)$ and $x_B(0)$ are the initial concentrations of A and B, and we have assumed that $x_A(0) + x_B(0) = 1$. In order to compare the growth of A and B at different mutation rates, we have to make an assumption about how Q depends on the mutation rate μ. A reasonable assumption is $Q = e^{-\alpha\mu}$, where α measures the probability with which a mutated sequence is viable [26]. Fig. 1 shows the relative concentrations of strains A and B as a function of time and mutation rate. For the given replication rates, strain B outcompetes strain A

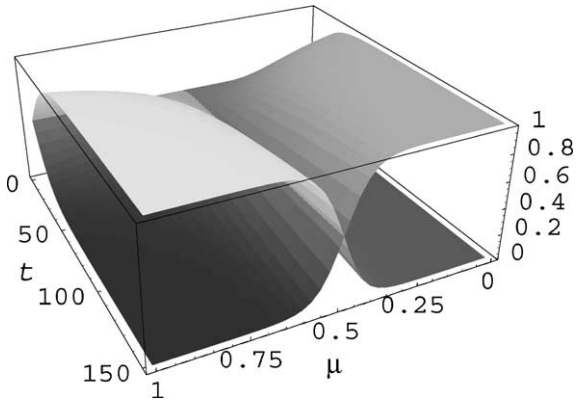

Fig. 1. Relative concentrations of strains A (dark surface) and B (light surface) as a function of both time t and mutation rate μ. Both strains are initially present in equal amounts, $x_A(0) = x_B(0) = 0.5$ ($\sigma_A = 1.1$, $\alpha_A = 0.4$, $\alpha_B = 0.2$).

at high mutation rates ($\mu > 0.48$), and loses the competition at lower mutation rates.

The frequency of neutral one-mutants relative to the total number of all possible one-mutants of a sequence is referred to as the sequence's *neutrality*. In the example above, all viable sequences within either strain A or B were assumed to have the same neutrality. This is a tacit assumption of population genetics, but it need not be so. In general, a neutral mutation will often alter the neutrality of a sequence. In proteins, for example, there exist so called suppressor mutations [27] which, once acquired, substantially increase the robustness of the protein structure against disruption by further mutations. The existence of suppressor mutations follows from basic biochemistry. If two amino acids at key positions in a protein interact particularly strongly, then in other positions of the polypeptide chain there can be substantial variation in the interaction strength between amino acids without disruption of the protein structure. On the other hand, if the residues at the key positions interact weakly, the correct protein structure can only be retained if residues at other positions provide exactly the correct interactions. Therefore, in general we must assume that each neutral substitution modulates the number of neutral substitutions possible in the future. How does this variation in sequence neutrality affect the growth of the two strains A and B from the above example? To answer this question, we have to discuss the growth of a single strain that consists of several closely related mutants with differing neutralities. Imagine that a strain is seeded by a single sequence with a particular neutrality ν_0. When the sequence reproduces, a certain percentage of the offspring sequences will be mutated. Each mutant sequence i has a neutrality ν_i, which is potentially different from the progenitor neutrality ν_0. When the unmutated and the mutated offspring sequences have further offspring, the number of viable offspring they produce depends on their respective neutralities, and the result is a complicated cascade of sequences replicating and mutating. After some time, the cascade reaches an equilibrium in the sense that the relative frequencies of the different mutant sequences remain approximately constant. The frequency of a particular sequence in this equilibrium state depends on the sequence's neutrality, and also on the neutralities and frequencies of neighboring sequences that occasionally produce this particular sequence via a mutation.

Mathematically, the cascade of replicating and mutating sequences can be described as a branching process [28]. If we are interested only in the equilibrium state after a long time and for a large population, we can make use of a result which states that asymptotically, the relative frequencies of the sequences are identical to those predicted from the quasispecies equations [29]. van Nimwegen et al. were the first to present a solution of the quasispecies equations for the case in which all mutations are either neutral or lethal [12]. Others have derived similar or extended results [13,14]. Here, we will only briefly sketch the main results of these studies.

In mathematical terms, the determining quantity is the connection matrix G_{ij} of genotypes, which contains an entry 1 if two neutral sequences i and j are exactly one mutation away, and an entry 0 otherwise. The frequency of viable sequences in the population is directly related to the population's average growth rate, and can be determined from the largest eigenvalue of G_{ij}. The equilibrium population structure, in turn, follows from the corresponding eigenvector. This somewhat abstract mathematical result has the following qualitative interpretation. In equilibrium, an adapting population contains only a small fraction of sequences with low neutrality, and consists mainly of sequences with high neutrality. This is because adaptation has moved the population into an area of sequence space where the density of neutral sequences is maximal. The overall growth rate of the strain depends on

this maximal density of neutral sequences. The denser the neutral sequences, the faster the strain grows, independently of the individual replication speed. A particularly useful aspect of this theory is that such a strain of closely related mutants—the quasispecies—can be described by a single number: its overall growth rate. Moreover, the growth rate consists of two multiplicative contributions: the individual replication speed (σ), and the fraction of viable sequences in the population (Q). Structurally, this situation is identical to the simple case we discussed initially, where all sequences within a given strain had the same probability of producing viable offspring. In other words, the above results for the two strains A and B remain unaltered in the case of variable sequence neutrality, if only we interpret Q_A and Q_B as the average percentage of viable sequences produced by the respective strains.

3. Survival of the flattest

The assumption of the previous section, that all mutations are either neutral or lethal, is of course unnatural. In general, the effects of mutations vary continuously from strongly deleterious over slightly deleterious to neutral and beneficial. In general, therefore, the overall growth rate of a strain cannot be expressed as a product of individual replication speed (σ) and fraction of viable sequences (Q). However, we can gain some qualitative understanding simply by discussing the distribution of mutants in sequence space as a function of the type of peak they inhabit. For example, consider the type of fitness peaks depicted in Fig. 2. In case A, we have a fitness peak that is relatively high but narrow, whereas in case B the peak is lower and wider (flatter). If the mutation rate is low, the population is in both cases concentrated towards the top of the peak, and strain A consequently grows much faster than strain B (because its replication rate is higher). However, at a higher mutation rate the picture changes. Now, in the case of A, most sequences occupy the slopes adjacent to the fitness peak, driven there by the strong mutational pressure. In the case of B, on the other hand, the flatness of the peak allows a larger percentage of the mutants to retain fitness values close to the optimum. As a consequence, the average growth rate of B exceeds that of A, and strain B can outcompete strain A although B's fitness peak is lower.

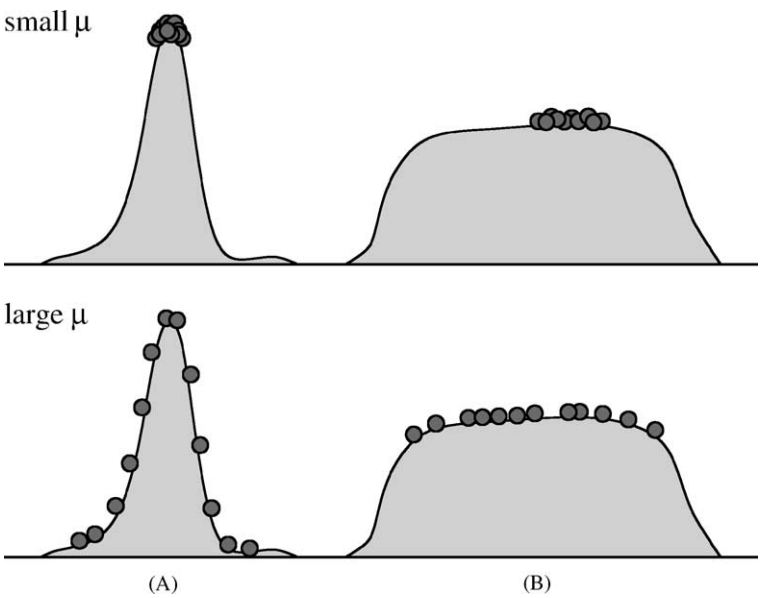

Fig. 2. Schematic drawing of the sequence distribution on a high, narrow peak (A) and on a low, wide peak (B) for small and large mutation rate μ.

This effect was first described in theoretical work by Schuster and Swetina [30], who studied the population dynamics on a fitness landscape with two peaks of identical or almost identical height. In the case of two peaks with identical height, they found that a population will always move to the peak with the stronger mutational support (that is, the wider and flatter peak in our terminology). If the peak with the stronger mutational support was slightly lower than the other one, the fate of the population depended on the mutation rate: the population would favor the higher peak at low mutation rates, and the peak with better mutational support at high mutation rates.

More recently, a similar effect was observed in experiments with digital organisms. Digital organisms are computer programs that are capable of error-prone self-replication, which leads to mutation and, because of limited availability of resources, to evolution [31]. Forty evolved strains of digital organisms were propagated for 1000 generations in two environments that differed only in their mutation rate: low and high [32]. After 1000 generations had elapsed, strains that evolved under a low mutation rate (the A strains) tended to have a higher replication rate than the corresponding strains evolved under a high mutation rate (the B strains). Twelve of these pairs of strains with the highest differences in replication rate were subsequently studied in direct competition at various mutation rates. Without exception, the A strain outcompeted the B strain at low mutation rates, but lost the competition at high mutation rates and was driven to extinction.

The mutation rate at which a B strain turns competitive can be predicted from measuring the decay in mean fitness as a function of the mutation rate in the two strains (Fig. 3). The A strain can hold its own as long as its *mean* fitness exceeds that of the B strain. (In other words, because mean fitness is a group property, knowledge of any individual fitness is insufficient to predict the eventual outcome of the competition.) It is worth mentioning that in this experiment, unlike the case considered by Schuster and Swetina, the difference in replication rate between the A and B strains was not small. In all 12 pairs of strains considered, the A strain replicated at least 50% faster than the B strain in the absence of mutations, and in 2 cases, the A strain replicated more than 10 times faster than the B strain in the absence of mutations.

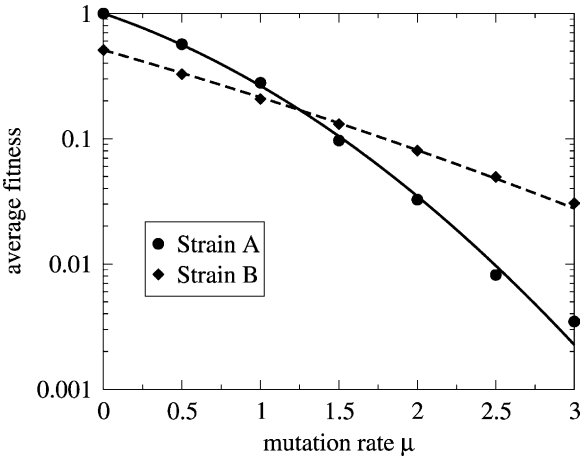

Fig. 3. Average fitness vs. mutation rate μ in two different strains of digital organisms. In this example, strain B starts to outcompete strain A at a mutation rate of about 1.25. Data from [32].

4. Probability of fixation

Above, we have outlined a theory that describes the circumstances under which a strain residing on a lower but flatter fitness peak can outcompete one that resides on a high but steep peak. However, the theory developed thus far can only describe the competition of two strains that are present from the outset in macroscopic quantities. It does not address the question of how a strain can move to occupy a lower but flatter fitness peak in the first place.

Imagine a strain in mutation-selection balance, located on a high but narrow peak. Now, imagine that by chance, a mutant ends up on a nearby peak which is lower and flatter. If this mutant managed to replicate a number of times, it would eventually grow into a new strain capable of replacing the strain on the high and narrow peak. But will the mutant be able to replicate a sufficient number of times? After all, its individual replication rate is lower than the one of the fastest replicator currently in the population.

Wilke investigated the invasion of a slower replicator with better mutational support *in silica* for RNA sequences replicating in a simulated flow reactor [26]. He found that such a mutant had indeed a positive probability of fixation, provided the mutant would eventually grow into a strain that could outcompete the previously dominant strain. Recently, progress has been made on the theoretical description of this inva-

sion process. We can calculate the exact probability of fixation with the aid of branching process theory [33]. One of the main qualitative results that we obtain from this theory is that the probability of fixation of a single mutant is indeed positive if and only if the strain the mutant will grow into has an advantage over the currently established strain. The individual replication rate of the invading mutant has no bearing on whether fixation is possible or not, and moreover, it has only minor influence on the actual fixation probability as well. Instead, the fixation probability is mainly determined by the mutational neighborhood of the invading sequence [33].

5. Discussion

Quasispecies theory is most suitable for the description of RNA virus evolution. High sequence heterogeneity [18,34,35] and high mutation rates [21,22] are well documented for RNA viruses. Therefore, the basic assumptions of quasispecies theory are met. However, to date, virologists have not succeeded in presenting clear experimental evidence for selection for mutational robustness or the outcompetition of faster replicators. One study that is frequently cited as evidence in favor of the outcompetition of a faster replicator by a slower one investigated the competition of different strains of vesicular stomatitis virus (VSV): de la Torre and Holland [36] found that a particular strain of VSV could rise to dominance if seeded at a fraction of 10^{-1} into its progenitor population, but would remain suppressed if seeded at a fraction of 10^{-3}. However, the experiment did not clarify whether this suppression was caused by quasispecies effects, by drift (that is, the strain simply did not rise to fixation at the low initial concentration), or by frequency-dependent selection. (Frequency-dependent selection has been observed repeatedly in viral populations [37,38], and is not included in the current quasispecies theory.) Because of the weak evidence in this and similar experiments, Holmes and co-workers concluded that there is currently no direct proof for the suppression of faster replicators by slower ones, and for quasispecies effects in general, in RNA viruses [39,40].

However, indirect evidence in favor of quasispecies effects—in particular for the selection for mutational robustness—abounds, and is not restricted to RNA-based viruses. We find tolerance against mutations to an extent that largely exceeds the level expected from mere coincidence in the genetic code [41] and in secondary structures of RNA virus genomes [42]. Genetic regulatory systems in eukaryotes are surprisingly robust as well [43].

Although the theory presented in this article explains why mutational robustness evolves, it does not explain how genomes become robust against mutations. Even for the digital organisms mentioned earlier, we do not have a good understanding of what makes a genome robust. The incorporation of non-coding regions into a genome is certainly not necessary to increase robustness. If the length of the genome is held fixed, digital organisms subjected to high mutation rates do nevertheless increase their robustness, typically at the expense of replication speed. It seems that robustness is achieved through a re-coding of the genetic information in a fault-tolerant manner, which can involve the introduction of redundancy and the loss of highly optimized functions. A similar increase in fault tolerance does occur in the evolution of the suppressor mutants in proteins discussed earlier [27], but in this case fault tolerance is not achieved through the introduction of redundancy. Within higher organisms, on the other hand, there exists evidence that particular genes have evolved that confer robustness on other particularly vulnerable genes, and therefore to the genome as a whole. Evolution of such robustness is usually called canalizing evolution [44], and has been discussed early on by Waddington [45] and Mather [46].

One candidate for such a canalizing gene is Hsp90, the heat shock protein that keeps unstable or metastable proteins functional by providing a scaffolding. Hsp90 is ubiquitous throughout the kingdoms of life, and is particularly important in signal transduction networks in metazoans (see [47]). Recent evidence suggests that Hsp90 has an additional role in preventing genetic variation from having deleterious effects on the function of polymorphic proteins [48,49]. In other words, Hsp90 may be a gene that evolved in response to selective pressure towards mutational robustness. Both in *Drosophila* and in *Arabidopsis* Hsp90 silences genetic variation which would have phenotypic effects in the absence of Hsp90.

Besides generating mutational robustness, a gene such as Hsp90 has an effect on the evolvability of

a genome [50]. First, a flatter fitness landscape in which deep valleys are filled in by the action of Hsp90 is more easily traversed, and distant peaks can be reached more easily. Second, phenotypic variation suppressed by the action of Hsp90 can be released under certain circumstances. In selection experiments involving *Drosophila* [48] and *Arabidopsis* [49], a phenotypic trait uncovered by the deactivation of Hsp90 was made to persist, through selective reinforcement, in the presence of Hsp90.

As discussed above, the genetics that underlie mutational robustness are not at all clear. One possibility is that gene duplication events lead to *redundancy*, which can confer robustness on the organism. Krakauer and Plotkin [51] recently pointed out that, if there is a cost for redundancy, a selective pressure to increase robustness via redundancy in higher organisms exists only for small population sizes. They claim furthermore that, for large population sizes, redundancy (but not necessarily robustness) is minimized. The results of an analysis of the genetic networks of yeast indicate that mutational pressure—rather than small population size—is responsible for the robustness found in this particular organism [43,52]. Robustness is mainly caused by the interaction of genes with unrelated functions; only very few duplicated, redundant genes contribute to the overall robustness of the genetic networks. Whether a similar argument holds for the protein Hsp90 is unclear. Should Hsp90 be regarded mainly as a redundant gene; as the integral part of a robust, interacting gene network; or is its main purpose rather the increase of evolvability, as suggested by Wagner et al. [50]? In the light of the above results and open questions, we believe that a thorough investigation into the origins and mechanisms of robustness in lower and higher organisms is warranted.

In the discussion of robustness in eukaryotes in the preceding paragraphs, we have tacitly assumed that the results derived in the framework of the quasispecies model are valid for recombining organisms as well. We have two reasons to justify this assumption. First, we can expect that the results derived for asexual replicators are directly relevant for the dynamics of adaptation within tightly linked stretches of DNA, for example, within single proteins, or, more generally, within individual haplotypes. Second, although the selection of mutational robustness under recombination has not been studied in detail so far, we believe that recombination will intensify, rather than weaken, this selective pressure. Recombination alone always creates sequences that are within the boundaries of the current mutant cloud [53]. Therefore, recombination has a contracting property, which should act towards a stronger concentration of the population in those regions of genotype space in which the density of neutral sequences is highest.

6. Conclusions

Recent advances in the theory of asexual evolution have revealed that the mutation-selection balance of asexual organisms is much more intricate than what was previously believed. The average fitness of an asexual population depends on the distribution of high-fitness sequences in sequence space, and there is a selective pressure to increase the robustness against mutations. Another way to express this result is to say that asexual populations reduce their mutational load by increasing the fraction of sequences in the population that have a high neutrality. In particular, at very high mutation rates, populations may achieve the largest reduction in the mutational load by increasing sequence neutrality at the expense of individual fitness. This can lead to the outcompetition of a faster replicating but less robust clone of sequences by a slower replicating but more robust clone. These results may provide an explanation for the ubiquity of mutational robustness across different kinds of model systems, from the genetic code to the structure of genetic networks of higher organisms.

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