Evolving Multi-Valued Regulatory Networks on Tuneable Fitness Landscapes

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Random Boolean networks have been used widely to explore aspects of gene regulatory networks. As the name implies, traditionally the model has used a binary representation scheme. This paper uses a modified form of the model through which to systematically explore the effects of increasing the number of gene states. These random multi-valued networks are evolved within rugged fitness landscapes to explore their behaviour. Results suggest the basic properties of the original model remain regardless of the update scheme or fitness sampling method. Changes are seen in sensitivity to high levels of connectivity, the mutation rate and the ability to vary network size.

Keywords: asynchronous; growth; mutation; NK model

1. Introduction

Gene regulatory networks (GRN) have long been cast as a form of discrete dynamical system through which to study their general properties, after [1]. In the vast majority of known cases, the underlying representation in the model is binary: genes are assumed to be either on or off at any given time and update using Boolean logic to capture the regulatory relationships between them. Such models have also been used to accurately predict aspects of the regulatory dynamics seen in mammalian cells [2], *Drosophila* [3], yeast [4], amongst others. However, the binary assumption is potentially a simplification and examples of increasing the number of gene expression states in dynamical system GRN vary from using the triplet low, medium, high (e.g., [5]) through to continuous values (e.g., [6]). Following [7], this paper adds a new parameter to the well-known random Boolean network (RBN) model [1] which enables the systematic exploration of the effects of altering the size of the alphabet (A) of the underlying gene expression state representation and logic. Moreover, the placement of gene regulatory networks within fitness landscapes is used to explore the effects of increasing the logic alphabet on evolutionary behaviour, specifically

within versions of the NK model [8].

Results suggest that a number of the basic properties of the original binary model remain, whilst aspects such as how fitness is sampled and how many genes contribute explicitly to the fitness calculation can significantly vary behaviour, particularly when network size evolves.

A comprehensive review of the significant body of work using other classes of gene regulatory network models – including ordinary differential equations, chemical reaction systems, Petri nets, etc. - is beyond the scope of this paper and the reader is referred to [9] for an overview.

2. The RBN Model

Within the traditional form of RBN, a network of R nodes, each with B directed connections randomly assigned from other nodes in the network, all update synchronously based upon the current state of those B nodes. As noted above, gene states are traditionally from a binary alphabet (A=2) and use a randomly assigned Boolean update function. Hence those B nodes are seen to have a regulatory effect upon the given node, specified by the given Boolean function attributed to it. Since they have a finite number of possible states and they are deterministic, such networks eventually fall into an attractor. It is well-established that the value of B affects the emergent behaviour of RBN wherein attractors typically contain an increasing number of states with increasing B (see [10] for an overview). Three regimes of behaviour exist: ordered when B=1, with attractors consisting of one or a few states; chaotic when $B > 2$, with a very large number of states per attractor; and, a critical regime around B=2, where similar states lie on trajectories that tend to neither diverge nor converge (see [11] for formal analysis). Note that the size of an RBN is labelled N, as opposed to R here, and the degree of node connectivity labelled K, as opposed to B here. The change is adopted due to the traditional use of the labels N and K in the NK model of fitness landscapes which are also used in this paper, as will be shown.

This paper uses a form of multi-valued logic (e.g., after [12]) over the original binary model: each node can exist in one of A states and is assigned a randomly created logic table for each of the A^B possible configurations (Figure 1). Figure 2 shows the typical number of nodes changing state per update cycle in such discrete dynamical systems where R=50, with various connectivity B and number of gene expression states A, using $0< B <6$ and $1< A <9$. As can be seen, in these random multi-valued networks (RMN) for low connectivity ($B < 3$) behaviour is not significantly changed with increasing A but becomes more chaotic with larger B thereafter. That is, significantly more nodes change state per update cycle than when A=2.

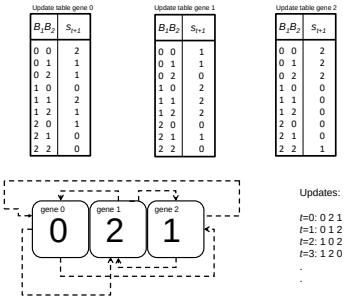
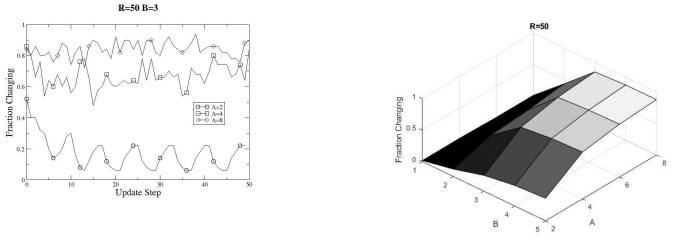


Figure 1. An example random multi-valued regulatory network model, with R=3, B=2 and A=3



(a) (b)

Figure 2. Showing the effects on the typical behaviour of the multi-valued regulatory networks with varying connectivity B and states A. Results are the average of one hundred randomly created networks per parameter configuration.

3. The NK Model

Kauffman and Levin [8] introduced the NK model to allow the systematic study of various aspects of fitness landscapes (see [10] for an overview). In the standard NK model an individual is represented by a set of N binary genes or traits, each of which depends upon its own value and that of K randomly chosen others in the individual (Figure 3). Thus, increasing K, with respect to N, increases the epistasis. This increases the ruggedness of the fitness landscapes by increasing the number of fitness peaks. The NK model assumes all epistatic interactions are so complex that it is only appropriate to assign (uniform) random values to their effects on fitness. Therefore, for each of the possible K interactions, a table of $2^{(K+1)}$ fitnesses is created, with all entries in the range 0.0 to 1.0, such that there is one fitness value for each combination of traits. The fitness contribution of each trait is

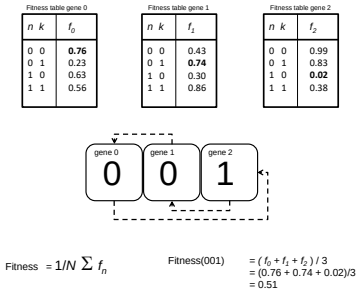


Figure 3. An example traditional binary NK model, with N=3 and K=1.

found from its individual table. These fitnesses are then summed and normalised by N to give the selective fitness of the individual. Exhaustive search of NK landscapes [13] suggests three general classes exist: unimodal when K=0; uncorrelated, multi-peaked when K >3; and, a critical regime around 0< K <4, where multiple peaks are correlated.

The traditional binary NK model has recently been extended to higher alphabets, i.e., fitness tables of size $A^{(K+1)}$ are created per gene, finding that the general properties of the landscapes are seemingly preserved [14]. This form of the NK model is here used to explore the evolutionary behaviour of the multi-valued regulatory networks introduced above – a version of the RBNK model [15].

4. The RMNK Model

The combination of the discrete dynamical networks and the NK model enables the exploration of the relationship between phenotypic traits and the genetic regulatory network by which they are produced [15]. In this paper, the following simple scheme is adopted: N phenotypic traits are attributed to the first N nodes within the network of R genes (where 0< $N \leq R$, Figure4). Thereafter all aspects of the two models remain as described above, with simulated evolution used to evolve the RMN on NK landscapes. Hence the NK element creates an explicitly tuneable component to the overall RMN's fitness landscape.

5. Evolving RMN

Simulated evolution has previously been used to design RBN, beginning with a simple feedforward network architecture [16] (see [15] for an overview). Following [10], the simple case of a greedy, genetic hill-climber is considered here. For a given RMN, mutation can either alter the logic function of a randomly chosen node or alter a randomly chosen connection for that node (equal probability).

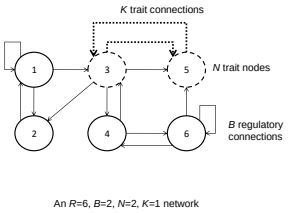


Figure 4. Example RMNK model. Each network consists of R nodes, each node containing B integers in the range [1, R] to indicate input connections and an A-ary string of length A^B to indicate the multi-valued logic function over those connections.

A single fitness evaluation of a given RMN is ascertained by first assigning each node to a randomly chosen start state (uniform in A) and updating each node synchronously for U cycles. Here U is chosen such that the networks have typically reached an attractor (U=50). At update cycle U, the value of each of the N trait nodes is then used to calculate fitness on the given NK landscape. This process is repeated ten times on the given NK landscape, repeated for ten randomly created NK landscapes, i.e., 10x10=100 runs, with the fitness assigned to the RMN being the average fitness. Then a mutated RMN becomes the parent for the next generation if its fitness is higher than that of the original (ties are broken at random).

5.1 Synchronous Updating

Figure 5 shows the typical evolutionary performance of R=50 RMN with various internal connectivity B ($0< B <6$) and logic alphabet A ($1< A <9$), on landscapes of varying ruggedness K ($0\leq K <5$) after 5000 generations. When N=10 (left column), fitness generally decreases with increasing B, regardless of K or A. That is, results for B=1 or B=2 are always statistically better (T-test, $p <0.05$) than for B=4 or B=5. When K=0, increasing A typically decreases fitness regardless of B. The relative decrease in fitness is highest when $A >2$ and $B >2$, with $B <3$ RMN seemingly most robust to increasing A. When $K >0$ and $B <3$ fitnesses increase with increasing A. Fitnesses are all roughly equally poor for $B >2$, regardless of A. Figure 5 (right column) also shows the effects of increasing the number of nodes by which fitness is explicitly calculated, with $N = R$. As can be seen, the same general behaviour as for N=10 emerges. However, the drop in fitness for increasing B from B=1 to B=2 is much larger and fitness levels are generally decreased for all B and A, regardless of K (T-test,

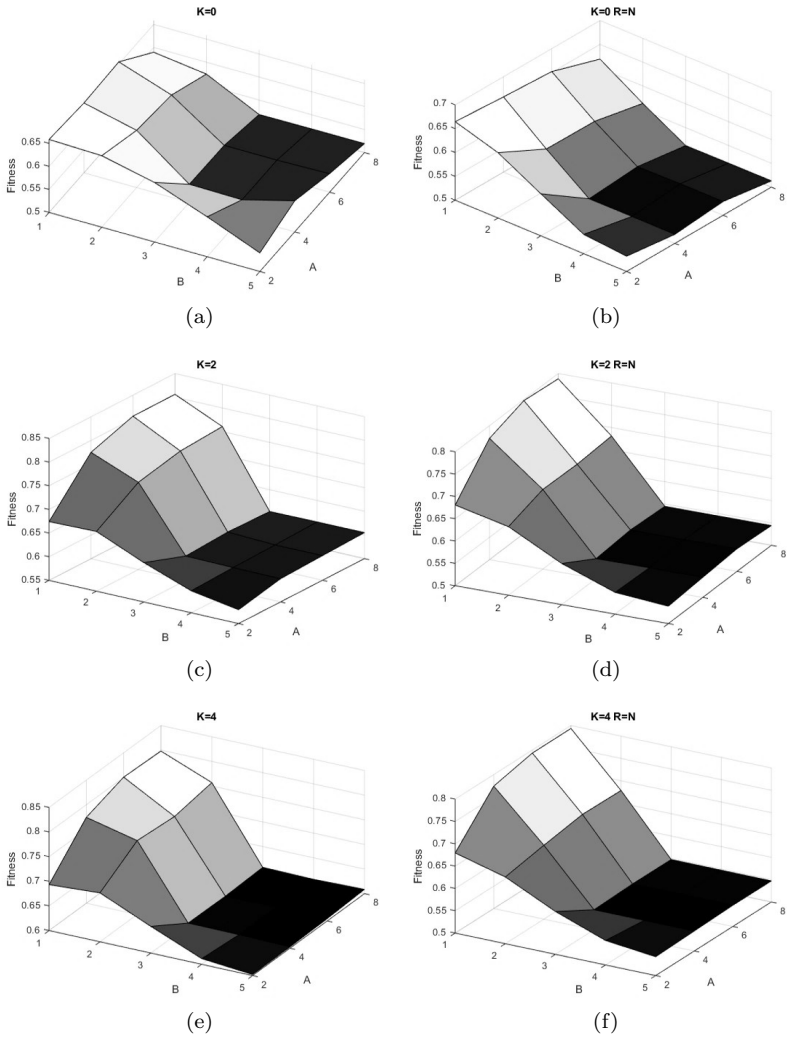


Figure 5. Showing fitness reached after 5000 generations for combinations of network connectivity (B), different logic alphabets (A), for various degrees of fitness landscape ruggedness (K). Left column N=10, right column N=50.

p <0.05 comparing each N=10 with $N = R$ cases). That is, it appears to be a significantly more difficult task, perhaps as might be expected.

In the above, fitness is calculated from the state of the N trait nodes on the step after U network update cycles, i.e., typically within an attractor. To explicitly consider the evolution of temporal behaviour, i.e., particular sequences of gene activity, the state of the RMN can be sampled on every update cycle, i.e., up to and including within an

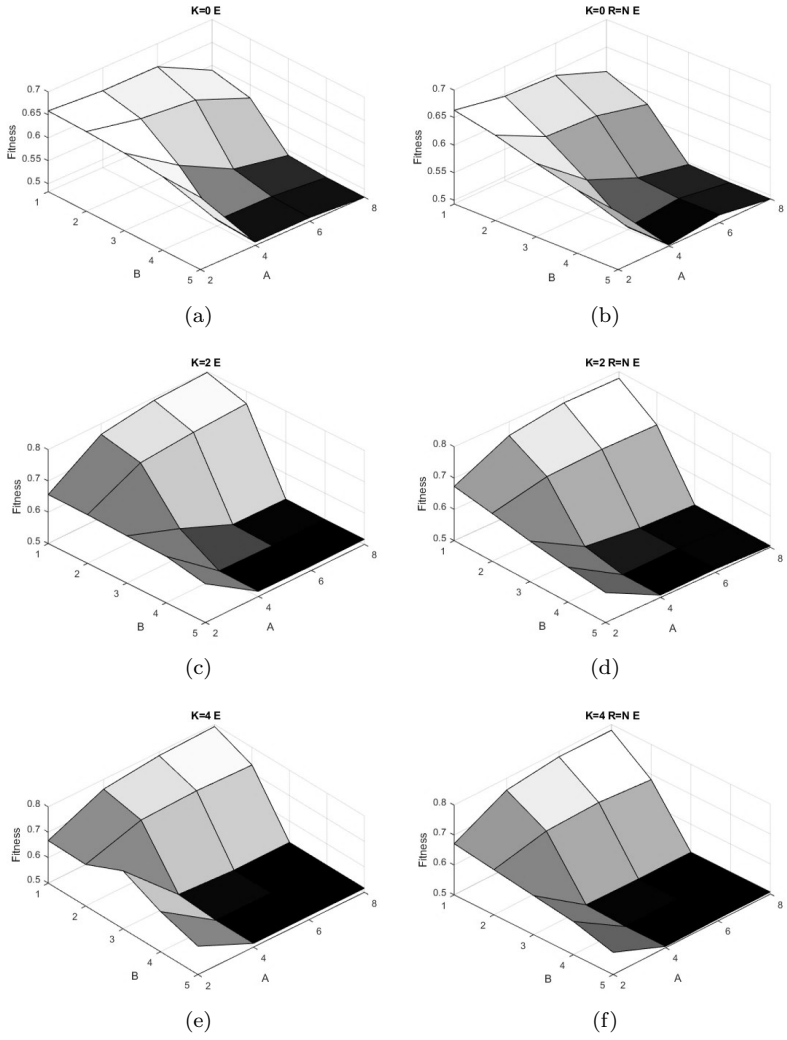


Figure 6. Showing fitness reached after 5000 generations for combinations of network connectivity (B), different logic alphabets (A), for various degrees of fitness landscape ruggedness (K) and number of explicit fitness calculation nodes (left column N=10, right column N=50) where the fitness is calculated as the average over each network update cycle.

attractor. Here total fitness is calculated as the average of the fitness of each successive state of the N nodes for U cycles. Thus, networks must evolve temporal behaviour which keeps them consistently within the high optima region(s) of the fitness landscape. Figure 6 shows examples of how the change causes a significant increase in fitness (T-test, $p < 0.05$) achieved with any K for $B > 2$ and A=2. Fitnesses are not significantly affected otherwise (T-test, $p \geq 0.05$). Figure 2 showed how the A=2 RMN, i.e., traditional RBN, experienced fewer numbers of nodes changing state for higher B compared to higher A.

The aforementioned work on the use of non-binary representations within the NK model reported some differences in the effects of varying the mutation rate between low and high A alphabets [14]. In particular, higher mutation rates (M) were found to be either neutral or beneficial for higher values of A in many cases: simply, the larger the search space, the more beneficial larger jumps in that space can become. Figure 7 shows examples of the effect of increasing the number of mutations, as described above, from one to three for N=10 and $N = R$. As can be seen, in comparison to Figure 5, the higher values of A benefit from the increased mutation when $B < 3$ and $K > 0$ (T-test, $p < 0.05$). Moreover, when A=2, the higher degrees of connectivity ($B > 2$) also appear to benefit from the increased mutation rate for all K when N=10 (T-test, $p < 0.05$). Again, it is assumed the greater percentage of updating nodes for the equivalent high A cases means the same improvement is not achievable. The same is generally true when fitness is calculated on every time step, as in Figure 6 (not shown).

5.2 Asynchronous Updating

Traditionally, RBN update synchronously, i.e., a global clock signal is assumed to exist. It has long been suggested that this assumption is less than realistic for natural systems and hence discrete dynamical models have also used asynchronous updating (after [17]). Harvey and Bossomaier [18] were first to present an asynchronous form of RBN wherein a node is picked at random (with replacement) to be updated, with the process repeated R times per cycle to give equivalence to the synchronous case. The resulting loss of determinism means such networks no longer fall into regular cyclic attractors, rather they either fall into point attractors (one state) or so-called "loose" attractors where "the network passes indefinitely through a subset of its possible states" [18]. Many forms of asynchronous updating are possible (e.g., see [19] for an overview) but the simple random scheme is used here to explore such updating in RMN. Simulated evolution has previously been used with asynchronous RBN, beginning with attractor matching to exhibit defined rhythmic behaviour [20].

Figure 8 shows the typical performance of asynchronous RMN over

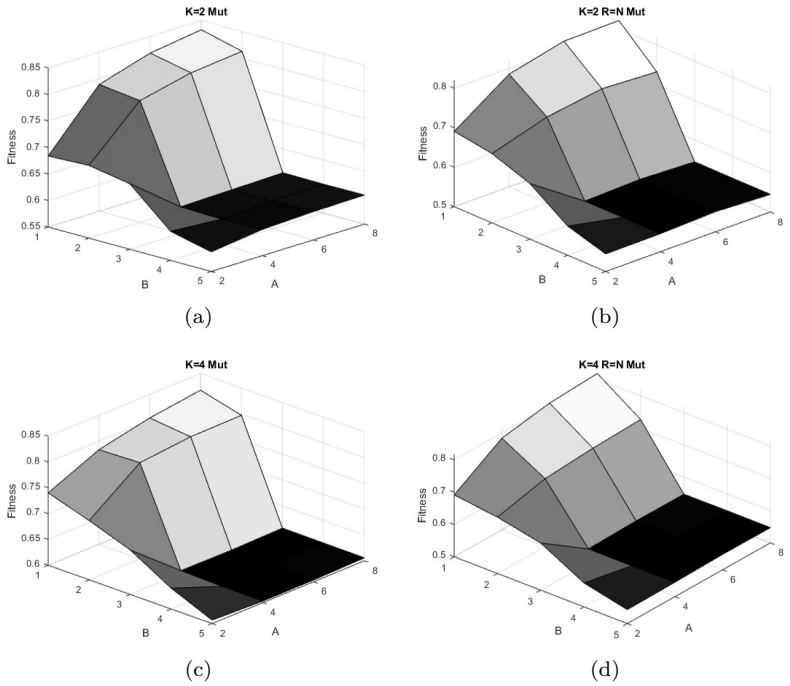


Figure 7. Showing fitness reached after 5000 generations for combinations of network connectivity (B), different logic alphabets (A), for various degrees of fitness landscape ruggedness (K) and three mutations per offspring production (left column N=10, right column N=50).

the parameter ranges used above. As can be seen, despite the change in the underlying update scheme, there is generally no significant difference in behaviour from that seen in Figure 5. Primarily, results for B=1 or B=2 are again always statistically better (T-test, p <0.05) than for B=4 or B=5, regardless of A. The use of fitness calculations on each update cycle, as in Figure 6, has also been explored with no significant changes observed (not shown). Similarly, the change in update scheme does not significantly alter the results reported above for an increase in the mutation rate (not shown): the higher values of A benefit from the increased mutation when B <3.

There is typically no significant difference in the fitness reached between the two updating schemes in all cases, with two notable exceptions: when B=2, for $N = R$ and K >0, for any A, the asynchronous fitnesses are significantly higher (T-test, p <0.05); and, when B=2, for any N, K and A, with the constant fitness calculation used, the asynchronous fitnesses are significantly lower (T-test, p <0.05). In the latter case it appears evolution finds it relatively harder to design such RMN

which must take consistently high fitness paths through the basins of attraction, presumably due to the stochastic nature of their updating. However, in the former case, when landscapes experience the highest levels of explicitly imposed ruggedness through the trait nodes, such stochasticity appears beneficial. It is here suggested a form of fitness landscape smoothing is occurring due to the randomness in the final attractors of the RMN reached; a typically low fitness RMN may achieve an atypically high fitness due to the loose attractors it exhibits, enabling evolution to move between peaks in the fitness landscape (after [21]).

5.3 Network Size

Novel sequences of DNA can originate through a variety of mechanisms including retrotransposons, horizontal gene transfers, during recombination events, whole genome duplications, etc. For example, it is estimated that over half the genes in GRN are the result of gene duplications (e.g., [22]), a process that may aid robustness as well as providing a mechanism for subsequent innovation through function divergence (e.g., [23]). Aldana et al. [24] examined the effects of adding a new, single gene into a given RBN through duplication and divergence. They showed the addition of one gene typically only slightly alters the attractors of the resulting RBN when B <3 but that attractor structure is not conserved for higher B.

The experiments reported above have been repeated with the addition of two extra "macro" mutation operators: one to delete a randomly chosen node (the N trait nodes cannot be deleted), randomly re-assigning all of its connections; and, one to duplicate an existing node, connecting it to a randomly chosen node in the network. These two operators occur with equal probability to the two previously described mutation operators above, i.e., one of four mutations are chosen to create the offspring per generation. The replacement process is also altered such that, when fitnesses are equal, the smaller network is kept, with ties again broken at random. Networks are initialised at size R, as before, and labelled as of size R' thereafter.

No significant change in the fitness of solutions is seen with the macro-structure mutation operators added regardless of whether N=10 or $N = R$ (not shown). However, as can be seen in Figure 9 (left column), when N=10, regardless of K, the networks decrease significantly in size when B <3 (T-test, p <0.05). The decrease in size decreases with increasing A. A=2 networks decrease in size when B <5. That is, not only do low connectivity networks evolve the highest fitnesses for all K and A, they are able to do so with a smaller number of nodes R'. It is known that both the number of states in an attractor and the number of attractors are dependent upon R within traditional RBN,

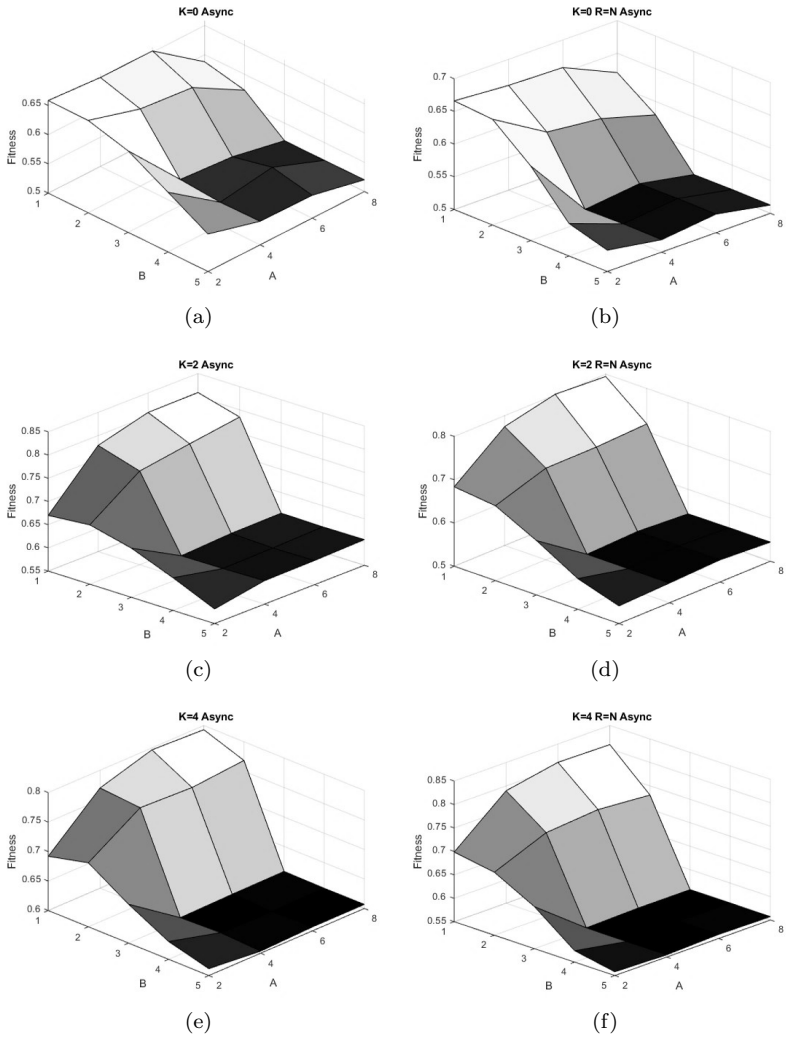


Figure 8. Showing fitnesses after 5000 generations for combinations of asynchronous network connectivity (B), logic alphabets (A), for various degrees of fitness landscape ruggedness (K).

and that the general form of those relationships changes for low and high connectivity. For example, when B=2, attractors are typically of size $R^{0.5}$, whereas, when $B = R$, attractors typically contain 0.5 x $2^{R/2}$ states (e.g., see [10] for a summary). Hence, regardless of A, the evolutionary process appears able to exploit the potential for ever smaller attractors for the low B cases, driven by the additional selection pressure for network size reduction, and to do so whilst maintaining fitness.

This result is somewhat anticipated by those of Aldana et al. [24] but is in the opposite direction and with $A > 2$: small reductional changes are maintained as the attractor space appears to be sufficiently conserved in both directions.

Figure 9 (right column) also shows the case when $N = R$, i.e., where there is no scope for network size reduction from the initial size. As can be seen, some growth occurs for all B and A, regardless of K ($R' > 50$). The largest growth is typically seen when $B=2$ and increases with A (T-test, $p < 0.05$). That is, $B=2$ connectivity appears to enable evolution to explore the space of larger networks without a drop in fitness: evolvability is increased under such conditions and further increased with increasing degrees of freedom in the gene state space A.

Figure 10 shows examples of the effects on network size explicitly considering the evolution of temporal behaviour by sampling the state of the RMN on every update cycle. Again, there is no significant effect on fitness (not shown) but there is a change in the type of growth seen from the single point (attractor) fitness sampling case. Regardless of N, A, and K, size is typically highest for $B < 3$. When $A=2$, networks are largest with $B \geq 2$. That is, significant growth occurs where the lower fitnesses emerge in such networks (see Figure 6). That networks do not decrease in size here for $N=10$ suggests that the removal of genes is more disruptive than the addition: when the path to attractors explicitly contributes to the overall fitness of the RMN, it seems gene deletion causes more change to the basins than addition. That is, gene deletion appears to affect the basins of attractors more than the attractors themselves since networks sampled after U updates experienced significant size reduction (Figure 9, left column).

Asynchronous updating gives the same general result as the synchronous case (i.e., as in Figure 9 with $N=10$ but does not show the significant increase in network size around $B=2$ when $N = R$, instead growth is very minimal for all B and A combinations regardless of K (not shown). Results are the same as for the synchronous case when fitness calculations are made on each update (not shown).

Thus, despite the selective pressure against growth used here, results suggest it is a relatively common occurrence during the evolution of GRN on rugged fitness landscapes. That is, fitness can be increased by the addition of a random gene due to the large number of relatively low optima typically experienced. Hence a population finds its progress "reset" within a higher dimension fitness landscape each time; new routes to optima in a bigger space become available on each gene addition. Another source of potentially more significant progress disruption is a change in the fitness landscape. That is, the movement of optima can cause a GRN to become less fit, increasing the likelihood of further novel genes being able to make a positive contribution to fit-

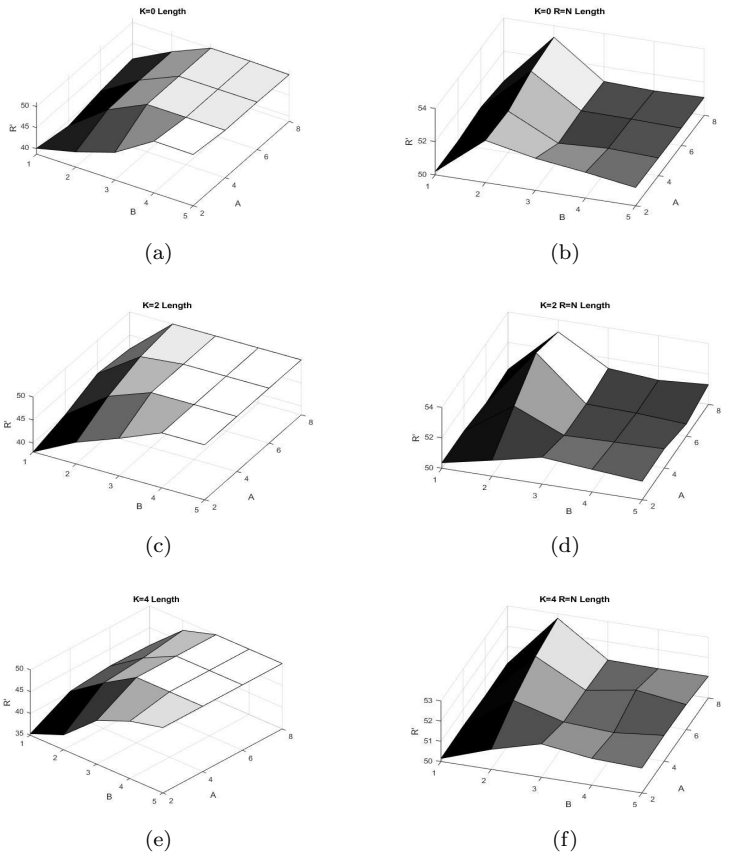
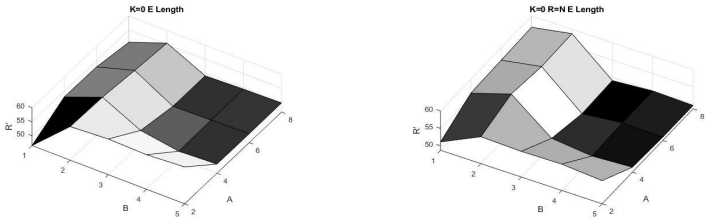


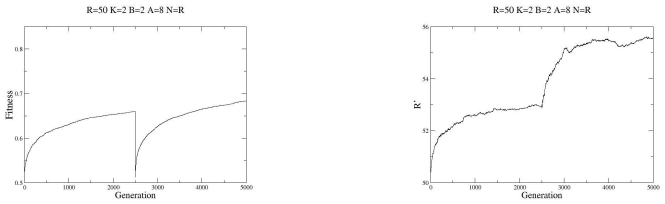
Figure 9. Showing network sizes (R') reached after 5000 generations for combinations of network connectivity (B), different alphabets (A), for various degrees of fitness landscape ruggedness (K).

ness as it re-adapts. Figure 11 shows an example case of the effect on fitness and network size when the whole fitness landscape is randomly recreated for the given K, i.e., each of the entries in the lookup table of each of the N genes is assigned a new value in the range 0.0 to 1.0, after 2,500 generations. Here $N = R$, as in Figure 9 (right column) where growth was seen for all B, particularly B=2, for all A. As can be seen, there is a significant drop in the fitness level at the point of change before it recovers to a similar level achieved before the change. The effect on network size R' is to cause a similar level of growth as from the original length before the change. The same behaviour is seen in all cases above where network size increased (not shown). Growth in response to an alternating change in the fitness landscape has pre-



(a) (b)

Figure 10. Showing example network sizes (R') reached after 5000 generations for combinations of network connectivity (B), different alphabets (A) and number of explicit fitness calculation nodes (N=10 left, N=50 right), where the fitness is calculated as the average over each update cycle.



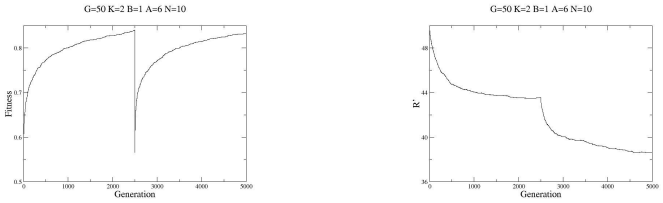
(a) (b)

Figure 11. Typical behaviour when the fitness landscape changes randomly under conditions where growth is seen to emerge during evolution.

viously been noted in a Boolean GRN model [26]. Conversely, under the conditions where networks decreased in size significantly (Figure 9, left column), further decreases in size are seen in the non-stationary fitness case (Figure 12).

6. Conclusions

Whilst binary discrete dynamical system models of GRN have proven useful both theoretically and practically, they clearly represent a simplification of the biology, e.g., in the face of noise [27]. This paper has explored the effects of increasing the size of the alphabet of gene states within such models, finding that the general properties are seemingly preserved, under different updating and fitness sampling schemes. That is, GRN become increasingly chaotic with increasing connectivity (B), an effect which increases with the number of states (A), and evolution



(a) (b)

Figure 12. Typical behaviour when the fitness landscape changes randomly under conditions where networks decrease in size during evolution.

is better able to manipulate low B networks - since their attractors typically contain one or a few states - to find high fitness solutions. This general result is supported by data from biological GRN which appear to be relatively sparsely connected: on average it seems $1.5 \leq B \leq 2$ (e.g., see [28]).

It has previously been suggested that increases in genome length are an inherent property of evolution on rugged fitness landscapes [25]. Despite a selective pressure against growth, results here show it is a common event in low connectivity networks (B), regardless of the size of the space of possible gene states (A), when the effects of the underlying ruggedness of the landscape ($N = R$) or the attractor space (U fitness evaluations) are most prominent. The most significant growth was seen for $A > 2$.

As noted above, in traditional RBN, B=2 has been formally identified as a critical regime where similar states lie on trajectories that tend to neither diverge nor converge. Formal analysis of increasing the number of gene states A suggests the critical regime of connectivity tends towards B=1 [7], somewhat contrasting with biology. Such analysis assumes all multi-valued states and logic functions are equally likely which has been suggested as potentially unrealistic [29]. However, with simulated evolution able to shape the logic functions (and node connections) here, the fitness difference between B=1 and B=2 is typically significant for $A > 2$. Results here indicate the largest increases in complexity typically occur at B=2, regardless of A. The interaction between these two processes may account for the variation from the data from biology and the theoretical prediction for B=1 with increasing A. Note the model is unable to capture how increasing complexity may open new niches where competition is reduced; the lower fitness for B=2 may be less significant with speciation.

Current work is exploring the effects of increasing the number of

gene states on the potentially related aspect of the evolution of gene expression times (after [30]), as well as other mechanisms such as epigenetic control (e.g., after [31][32]).

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