Complexification of Gene Networks by Co-evolution of Genomes and Genomic Parasites

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Keywords: Evolution in Silico, Evolutionary Design, Genomes, Genomic Parasites, Artificial Transposons, Co-evolution, Gene Networks, Gene Co-option, Complexification, Genetic Algorithms.

Abstract: The co-evolution of species with their genomic parasites (transposons) is thought to be one of the primary ways of rewiring gene regulatory networks (GRNs). In this communication, we computationally explore some of the essential co-evolution aspects of hosts (GRNs) with their transposons. We implemented an evolutionary search of an appropriate GRN model design on the example of the *Drosophila* gap-gene network. Simple artificial transposons capable of spreading and transposition were implemented. With the model, we explored the hypothesis that targeting destruction of some of the regulatory connections in the GRN via the action of transposons can produce negative selection pressure. Functionally external genes can be recruited (co-opted) into the GRN under this selection pressure following transposon rewiring of the GRN. Over evolutionary time, transposition events are able to disrupt these new regulatory connections, leading to repeated cycles of recruitment, rewiring and optimization. This process can produce increasingly large GRNs with the same basic functions.

1 INTRODUCTION

The extensive area of modern evolutionary computation (EC) was inspired by ideas from biological evolution. Contemporary biological evolution theories inspire computer science fields to work out and implement novel evolutionary algorithms. In turn, biology, especially in the area of systems biology, has been and is currently influenced by contemporary EC ideas and approaches. One product from the cross-dissemination of ideas between systems biology and EC (in particular, genetic algorithms, GA) is a set of modern techniques to design real genetic network models, termed the *evolution in silico* approach.

The evolutionary design of GRNs in nature involves the establishment of gene-gene connectivity (wiring) and tuning of the connection strengths, followed by multiple rounds of rewiring and retuning to optimize the GRN for a particular function. These wiring and rewiring events involve specific molecular mechanisms. One such mechanism is the co-evolution of gene networks and their parasites, termed transposons. Co-evolution is proposed to be one of the key mechanisms of gene network evolution. The co-evolutionary relationships of the hosts (gene networks) and parasites (transposons) can be roughly subdivided as competitive or mutualistic. The 'arms race' nature of competitive relationships can lead to high levels of complexity, a manifestation of which is gene network outgrowth.

Gene Network outgrowth by Recruitment of Novel Members: In early metazoan evolution, gene networks specifying developmental events in embryos may have consisted of no more than two or three interacting genes. Over time, new genes were incorporated into the primitive networks (Wilkins, 2002). While an initial hypothesis might be that new functions require novel genes, whole genome sequencing has shown that the apparent increases in developmental complexity do not correlate with increasing total numbers of genes. Therefore, the evolution of developmental pathways may most commonly proceed by recruiting pre-existing external genes into pre-existing networks to create novel functions (True and Carroll, 2002). Developmental evolution may act primarily at the

M. Holloway D., B. Kazansky A. and V. Spirov A..
Complexification of Gene Networks by Co-evolution of Genomes and Genomic Parasites .
DOI: 10.5220/0004170802380244
In *Proceedings of the 4th International Joint Conference on Computational Intelligence* (ECTA-2012), pages 238-244
ISBN: 978-989-8565-33-4

level of genetic regulation (Carroll et al., 2001).

The invertebrate segmentation network is among the best studied gene ensembles, with a wealth of diverse experimental data providing a unique opportunity for investigating known and hypothetical evolutionary scenarios in detail. In particular, the level of understanding of the segmentation gene network for the fruit fly (*Drosophila melanogaster*) has made it, for many years, extremely popular for functional and evolutionary computer simulations (Reinitz and Sharp, 1995; Jaeger et al., 2004; Sánchez and Thieffry, 2001; Manu et al., 2009ab). Modelling this gene ensemble has become a benchmark test in modern systems biology (e.g. Azevedo et al., 2006; Umulis et al., 2008; Bieler et al., 2011; Gursky et al., 2011). This network has also been used in the development of evolution *in silico* approaches (François et al., 2007; Spirov and Holloway, 2009; 2010; 2012).

Comparisons of segmentation networks between primitive and higher insects indicate that evolution proceeds in the direction of network complexification (Patel, 1994; Sommer and Tautz, 1993). The transition from grasshoppers to flies, with an (at least) doubling of the number of genes in the network, appears to have been solved by evolution over a short geological time span. Increase in the segmentation network size often involved recruitment of genes from other networks.

Transposons, Genomic Parasites: A universal property of life is that all successful systems attract parasites. Parasites are so common that hosts eventually co-evolve immunity. The parasites then co-evolve strategies to circumvent the new immunity. And the hosts respond by co-evolving defences to repel the parasites again.

Transposons are a unique type of parasite residing in the host genome. Co-evolution of transposons and host genomes are thought to produce arms races within the DNA of organisms. Transposons jump between different parts of a genome to propagate themselves, and these events are usually to the detriment of the host (Makalowski, 1995). Many transposons have a unique DNA sequence that acts as a forwarding address and directs the transposon to a complementary DNA sequence in the host genome (Makalowski, 1995).

Transposons are a major source of genetic change (Lozovskaya et al., 1995; King, 1992). It has been estimated that 80% of spontaneous mutations are caused by transposons (Makalowski, 1995). Transposons have co-evolved with their hosts through selection. The activities of transposons are likely to participate in the rewiring of pre-established regulatory networks (for example, Wallace et al., 1991; Girard and Freeling, 1999).

While the problem of growth and complexification of GRNs has received much attention in recent years, the mechanism remains unknown. How does recruitment happen? What exactly forces the GRNs to co-opt genes? Some studies assume that recruitment occurs by chance (at a very low level) and is then subject to evolution. Other researchers believe that there are special evolutionary mechanisms to perform this task. These could involve complicated systems to re-arrange the genetic material. Transposons might participate in evolutionary events at this level. Even assuming that transposons can drive complexification of GRNs, it is unknown exactly how this could occur. An initial hypothesis might be that transposons force GRNs towards more complexity by forced evolution (negative selection). In this communication, we computationally explore some of the essential co-evolutionary aspects of hosts (GRNs) and transposons in regard to the problem of GRN evolutionary growth. We show that particular transposon-GRN interactions are capable of producing GRN enlargement.

2 METHODS AND APPROACHES

The idea of implementing artificial transposons to facilitate the evolutionary search in EC has been of great interest since the mid-1990s (Spirov, 1996; Spirov and Samsonova, 1997; Spirov and Kadyrov, 1998; Spirov and Kazansky, 2002ab; Spirov et al., 2009). This approach has been developed by several teams (Nawaz Ripon et al., 2007; Tang et al., 2008; Chan et al., 2008; Simões and Costa, 1999ab; 2000; 2001; Liu et al., 2009). Our main interest in this area is to use artificial transposons to facilitate gene network model design.

2.1 Modelling the Segmentation Network

We use an *in silico* approach to simulate evolution of the gap gene network which is central to fly segmentation. Our model for the gap gene network (adapted from Reinitz and Sharp, 1995; Manu et al., 2009a) is a system of differential equations describing the regulatory interactions of 4 gap genes (*giant*, *gt*; *hunchback*, *hb*; *Krüppel*, *Kr*; *knirps*, *kni*) under the control of a maternal Bicoid (Bcd) protein gradient. Real spatial patterns of gene expression

were used to fit the model (see Spirov and Holloway, 2009, Fig. 1A-C). The model parameters for the gene interaction strengths are varied, and the solutions are selected by GA (details below) based on how well they fit the gap gene data. This selection produces networks that describe the particular interactions (and quantitative strengths) between the component genes (e.g., Spirov and Holloway, 2009, Fig. 1D). In this way, we can use a model of our current understanding of fly segmentation to study the evolutionary dynamics of how the segmentation network may have arisen and how this might reflect on its current characteristics.

Gene-gene regulatory matrix W: The gap gene proteins (Kr, Gt, Kni and Hb) are variables in the model, with the rates of their concentration changes dv_i^a/dt (for each gene product a in each nucleus i), defining a system of number of proteins times number of nuclei ODEs given by

$$dv_i^a/dt = R_a g(u^a) + D_a \Delta v_i^a - \lambda_a v_i^a \qquad (1)$$

where R_a represents protein synthesis, D^a represents diffusion, and λ_a represents decay. $g(u^a)$ is a sigmoid regulation-expression function.

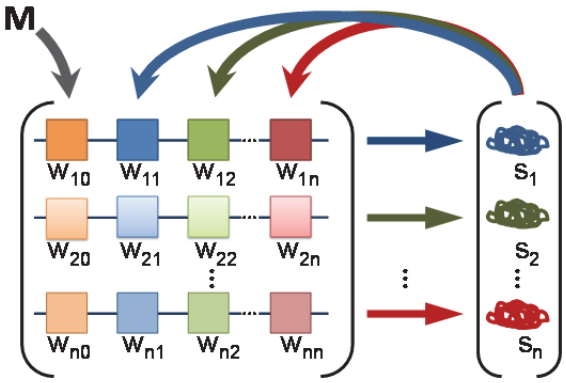

Figure 1: The gene-gene interaction matrix, a core element of modelling GRNs. Each gene (horizontal arrow) is regulated by the products of other genes via upstream enhancer elements (boxes). The strength and direction of regulation (depicted as differently coloured saturation levels) are a function of both the regulatory element and the abundance of its corresponding gene product. The left-most column W^{a0} corresponds to the regulatory elements for the action of the spatially-graded morphogen, M = Bicoid (external factor for the GRN). The genotype is represented as the matrix, W, of the regulatory interactions, and the phenotype is the vector, Ŝ, of the gene product levels at equilibrium. Modified from Siegal and Bergman (2002).

u^a is given by $u^a = \sum_b W^{ab} v_i^b + W^{a0} v_i^{Bcd} + h^a$. Parameters W^{ab} constitute a gene interconnectivity matrix, v_i^{Bcd} represents the concentration of Bcd in nucleus i, which is constant in time. W^{a0} describes the regulatory input of Bcd to each gene. Bcd is a general activator for all four considered gap genes. h^a represents the regulatory input from ubiquitous factors.

The gene interconnectivity matrix, W^{ab}, is the key component for describing the GRN. Fig. 1 depicts this matrix in detail. The W^{ab} elements represent the activation of gene a by the product of gene b (with concentration v_i^b) if positive, repression if negative, and no interaction if close to zero.

2.2 Evolution *in Silico* to Design the Gene Network Model

We simulate population dynamics by repeated cycles of mutation, selection and reproduction (the general GA approach). The program generates a population of double string chromosomes (described in more detail in 2.3). The genome of each host consists of N chromosomes represented in the W^{ab} matrix (Fig. 1). The main chromosome string consists of N+1 floating-point values (Fig. 1).

The initial floating-point strings of the chromosomes are generated at random. All of the chromosome sets are consecutively evaluated (according to their fit to the data, see eqn. 2), an average score is calculated and the winners' offspring substitutes for the losers in the process of reproduction. Next, a predetermined proportion of the chromosome population undergoes mutation (small changes are made to selected chromosomes' coefficients). The cycle is then repeated for each generation.

The set of ODEs (1) was solved numerically by Euler's method (Press et al., 1988). We minimized the cost function, *E*, by adjusting parameters W^{ab} in equation (1):

$$E = \sum_b (v_{i\ model}^a + v_{i\ data}^a)^2 \qquad (2)$$

The remaining parameters were found in preliminary runs and then used as fixed parameters.

We extended this standard scheme with our own procedures to simulate the interaction between hosts

and transposons.

2.2.1 Introduction and Withdrawal of New Genes

We used a Gene Introduction operator to add a new gene to the network (at a rate of 5 – 10% per generation, depending on the computation). Specifically, this added a new row and column to the W^{ab} matrix, which could be then be operated on by mutation and crossover (discussed below). For simplicity, new rows are always added above existing rows (cf. Fig. 1). To evaluate the importance of this one-way process in forcing networks to recruit new genes, we also introduced a Gene Withdrawal operator which removes a row and column from the W^{ab} matrix (at a rate of 2 - 10% per generation, depending on computation). Gene Withdrawal does not operate if the network is minimal (N = 4 genes).

2.3 Artificial Transposons for GA

We define an artificial transposon as a marked block of the host's code. This mark is transmissible from host to host. We use double-string chromosomes: the main string (floating-point) is used for the host code and a second string (binary) is used for the transposon marks. Secondary string values are 1 for a mark and 0 otherwise. Chromosomes are therefore in the following format:

the mark string: 1 0 0 … 0
the main string: α_1 α_2 α_3 … α_n

where the α_k are floating-point values (only the α_1 element is transposon-marked in this example). (Each element α_k is the value of W^{ab}, Fig. 1.)

Artificial Transposons as Mutators: As with biological transposition, action of an artificial transposon is deleterious to the host in our model. For an example of how this is implemented within the $W^{b\leftarrow a}$ gene interaction matrix, consider a transposon infection in the upper left element, $W^{A\leftarrow M}$ (highlighted):

$W_{A\leftarrow M}$	$W_{A\leftarrow A}$	$W_{A\leftarrow B}$	$W_{A\leftarrow C}$	…
$W_{B\leftarrow M}$	$W_{B\leftarrow A}$	$W_{B\leftarrow B}$	$W_{B\leftarrow C}$	…
$W_{C\leftarrow M}$	$W_{C\leftarrow A}$	$W_{C\leftarrow B}$	$W_{C\leftarrow C}$	…
…	…	…	…	…

The transposon's deleterious action is then implemented by decreasing the value of the infected host element $W^{b\leftarrow a}$. Specifically, we halve the $W^{b\leftarrow a}$ value in each generation. This quickly drops the element value to near zero. In this manner, the transposon effectively cuts the $b\leftarrow a$ regulatory connection.

Spread of Artificial Transposons: Transposons tend to form clusters in host chromosomes. We simulated this feature by spreading transposon infection by at most one element per generation. In this operation a transposon can mark the $W^{b\leftarrow a}$ element above it as a new transposon. The following represents transposon infection spreading from the 2nd row (1st column) to the 1st row:

$W_{A\leftarrow M}$	$W_{A\leftarrow A}$	$W_{A\leftarrow B}$	…
$W_{B\leftarrow M}$	$W_{B\leftarrow A}$	$W_{B\leftarrow B}$	…
$W_{C\leftarrow M}$	$W_{C\leftarrow A}$	$W_{C\leftarrow B}$	…
…	…	…	…

$W_{A\leftarrow M}$	$W_{A\leftarrow A}$	$W_{A\leftarrow B}$	…
$W_{B\leftarrow M}$	$W_{B\leftarrow A}$	$W_{B\leftarrow B}$	…
$W_{C\leftarrow M}$	$W_{C\leftarrow A}$	$W_{C\leftarrow B}$	…
…	…	…	…

Transmission of Transposons: We used fixed transposon coordinates to transmit transposons from host to host. (Whole transposons were never moved along the chromosomes.) The two-place transmission operator was implemented as follows: First, a pair of hosts was chosen at random; then a chromosome from either host was scanned for transposon marks. If a transposon was found, it was replicated in the partner chromosome, regardless of the original string character in the target chromosome. Copying only occurred if the secondary strings had transposon marks.

3 RESULTS AND DISCUSSION

In all of the computational experiments described, we begin from a four-gene ensemble of obligate gene founders (the initial network). We fit this 4-gene ensemble to experimental data for expression of four *Drosophila* gap genes (section 2.1; for further details see Spirov and Holloway, 2009). The spatial patterns of these four genes are the only selection criteria in the evolutionary computations; they are a stabilizing selection. Starting from the initial 4-member GRN (GRN_{ini} = GRN_4), up to 3 extra genes can be recruited to the network.

Transposons invade all hosts of the initial population. Transposon length can vary from 1 to 4 elements ($TE_{ini} = TE_{1\text{-}4}$).

For typical runs, the size of the host (and transposon) population is 4000 chromosomes; the mutation rate is 15% per generation; the 15% of individuals with the highest scores are marked for reproduction (truncation strategy); the rate of transposon horizontal transmission is 25% per generation; the rate of new gene recruitment is 25% per generation; the transposon action occurs to 25% of the infected elements per generation; and the rate of transposon spread is 25% per generation.

We also ran computations for evolutionary growth of the host's GRN with lower transposition pressure: with a mutation rate of 1.5% per generation; 1.5% of the highest scoring individuals marked for reproduction; a rate of transposon horizontal transmission of 5.0% per generation; rate of new gene recruitment 5.0% per generation; rate of transposon action 5.0% per generation; and rate of transposon spread 5.0% per generation.

3.1 GRN Outgrowth

Our computational experiments demonstrate that artificial transposons, as defined above, are able to force their host's evolution towards greater GRN complexity. This complexification is due to recruitment of novel genes into the GRN to escape the selection pressure of the transposons. Analysis of the computational solutions shows that a substantial proportion of the recruited genes participate significantly in a network's activity.

Our simulations show that this effect of transposons on gene recruitment is robust over a wide range of parameter values (at least a ten-fold variation). It is a sustainable and robust characteristic of the host-transposon system.

In earlier experiments, we observed gene recruitment and GRN outgrowth in the absence of transposon operators (Spirov and Holloway, 2009). Genes can become co-opted simply by chance; the co-option of genes into existing GRNs is a very general feature of the evolution of regulatory networks. However, GRN outgrowth by random recruitment can be very slow. With transposon operators, selective pressure is greatly increased, speeding up recruitment and GRN outgrowth. In addition, as implemented here, the transposon pressure results in very specific GRN architectures.

3.2 Co-evolution of GRNs and Transposons

The GRNs in this study rely on a spatial gradient of a morphogen, M (external to GRN feedback), to establish appropriate spatial expression patterns. In *Drosophila* segmentation, M corresponds to the maternally supplied Bcd protein gradient. In our simulations, we have focused on transposon infections in the 1st column of the W matrix, representing the effects of M on the network genes. Infections in this column isolate the GRN from its external input (M is the only activator of the network genes; see Fig. 1).

Consider a transposon infection on the initial GRN (of length, say, TE_4) at the element of the first gene (A) representing activation by M (Fig. 2). Once

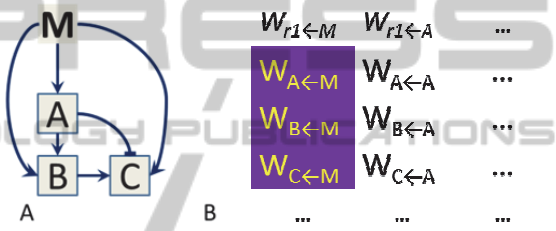

Figure 2: Transposon infection affecting M activation of the GRN. A) Representation of the gene interactions in the network. B) Infection in the W column representing M activation.

placed, the transposon systematically reduces the strength of the element. In this way, the transposon interferes with the reading of the M gradient by the GRN. Because reading the spatial gradient is critical to the function of the GRN, the transposon drastically reduces the fitness score of the "infected" GRN.

As evolutionary time continues, a novel gene, e.g. R1, can be recruited to the GRN. If R1 becomes activated by M, and gene A acquires the ability to be activated by R1, the newly expanded GRN (R1-GRN) can again appropriately form spatial pattern. R1-GRN requires both a rewiring of connections and retuning of parameter values from GRN_{ini}. R1-GRN can achieve a good score and is insensitive to the initial length TE_4. R1-GRN quickly becomes abundant (even dominant) in the population.

However, as time proceeds, the transposon will spread upwards in the matrix: the original TE_4 will become TE_5 and infect the M element of R1 (Fig. 3).

With additional evolutionary time, this process can reoccur with recruitment of a new gene, R2. I.e., R2 can become a target of the M gradient and be recruited as an activator for A, R1 or other genes.

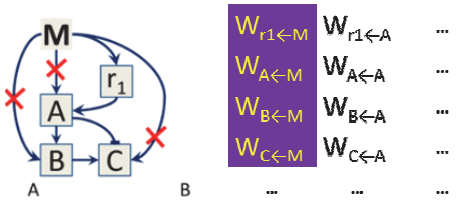

Figure 3: Infection of a recruited gene, R1. A) Representation of the network; B) matrix representation.

The new R2-GRN then escapes the transposon pressure and becomes abundant in the population. These co-evolutionary cycles can occur repeatedly, in the process forming increasingly large GRNs, but all with the same basic function of creating the appropriate spatial gene expression patterns.

4 CONCLUSIONS

1) Targeted destruction of key regulatory connections in a GRN by transposons can produce negative selection pressure on the GRN.

2) Initially non-functional genes can be co-opted into the GRN to respond to this selective pressure. Co-opted genes substitute the regulatory connections under transposon attack (i.e., they rewire the GRN).

3) We have focused on the co-option of genes to restore disrupted connections to external morphogen signalling. This is relevant to the maintenance of gradient-reading GRNs (critical in biological development) despite transposon attacks.

4) For gradient-reading GRNs, we have observed outgrowth and complexification of the networks under the selection pressure of targeted transposon activity.

5) The co-option of novel genes by gradient-reading GRNs to overcome transposon effects can repeat over many cycles of co-evolution between the GRN and the transposon. The increasingly large GRNs solve the same basic function of gradient reading.

ACKNOWLEDGEMENTS

This work was supported by Joint NSF/NIGMS BioMath Program, 1-R01-GM072022 and the National Institutes of Health, 2R56GM072022-06, 2-R01-GM072022.

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