Review

CellPress

The origins of mutational robustness

Mario A. Fares[1,2]

[1] Instituto de Biología Molecular y Celular de Plantas (CSIC-UPV), Valencia, Spain
[2] Department of Genetics, Smurfit Institute of Genetics, University of Dublin, Trinity College Dublin, Dublin, Ireland

Biological systems are resistant to genetic changes; a property known as mutational robustness, the origin of which remains an open question. In recent years, researchers have explored emergent properties of biological systems and mechanisms of genetic redundancy to reveal how mutational robustness emerges and persists. Several mechanisms have been proposed to explain the origin of mutational robustness, including molecular chaperones and gene duplication. The latter has received much attention, but its role in robustness remains controversial. Here, I examine recent findings linking genetic redundancy through gene duplication and mutational robustness. Experimental evolution and genome resequencing have made it possible to test the role of gene duplication in tolerating mutations at both the coding and regulatory levels. This evidence as well as previous findings on regulatory reprogramming of duplicates support the role of gene duplication in the origin of robustness.

Robustness to mutations and its role in evolution

All biological systems are resistant to genetic variation and environmental changes; a property known as robustness [1,2]. That is, despite significant changes in the inputs to a system (e.g., genetic or environmental variation), the outputs (e.g., phenotypes) after the perturbation are equivalent to those before the perturbation of the system. Waddington was amongst the first to realize that developmental programs, such as the development of wings in *Drosophila*, are generally resilient to minor perturbations caused by environmental changes, including heat stress or osmotic stress, and he called this property canalization [3–5]. Since the work of Waddington, robustness has become synonymous with canalization. Subsequent work has shown that robustness is not restricted to development but is a universal property of many levels of complexity.

At the molecular level, systematically mutating bacteriophage T4 lysozyme showed that the enzyme continued to function more than half the time after testing 2015 single amino acid mutations. This robustness likely accounts for the persistence of large protein variability observed in T4 populations [6,7]. In metabolic networks, studies based on flux balance analyses have been performed focusing on gene products from essential metabolic networks of the bacterium *Escherichia coli*, including glycolysis, pentose phosphate, and tricarboxylic acid pathways. Such studies have revealed that the flux could be brought down to levels as low as 15% for enzymes of the pentose phosphate pathway, and 19% in the tricarboxylic cycle acid reactions without compromising optimal cellular growth, suggesting that metabolic networks are largely robust to fluctuations in the reactions substrates or efficiencies [8]. Gene expression is often subject to noise but the level of expression noise depends on the location of the gene in the genome [9,10]. Indeed, the expression of essential genes is robust (i.e., they maintain low noise levels in their expression) in the face of internal cellular factors that often alter gene expression, including the switching of chromatin between open and closed states [9,10]. The robustness of essential genes to factors that introduce expression noise may have a selection basis because essential genes are often clustered and located in genome regions with open chromatin organization [11]. Cells are also robust to single gene deletions [12,13]. Knockout strains of the yeast *Saccharomyces cerevisiae* exist for 96% of open reading frames, indicating a remarkable tolerance to single gene deletions, although many of these genes are required under certain growth conditions [12].

Glossary

Cryptic genetic variation: genetic variants that differ from the most abundant genotypes in the population that are phenotypically silent causing no effects on the fitness of individuals carrying such variation.
Evolvability: capacity to generate heritable phenotypic variation that may be adaptive in a particular context [90].
Exaptation: a trait that serves a particular function in the current context but that may serve another function in another context [91].
Genetic interactions: also known as epistasis and refers to the dependent effect of deleting one gene based on the presence of one or more modifier genes. Deletion of two interacting genes from an organism would lead to effects that are significantly more compensatory or aggravating than the multiplicative effects of single gene deletions.
Mutational robustness: is the extent to which the phenotype of an organism (i.e., morphology or functional performance) remains constant in spite of mutations to its genotype.
Neofunctionalization: another of the fates proposed by the classic view of gene duplication is that, while one copy preserves the ancestral function, the other, devoid of selective pressure, can explore alternative functions and innovate.
Paralogs: genes from the same species that share the same ancestry.
Partitioning of ancestral functions: when a multifunctional gene (e.g., a gene with a catalytic and regulatory functions) duplicates, both of the copies can mutate asymmetrically in two different functional domains, such that one copy keeps one of the ancestral functions (e.g., regulatory), and the other keeps the complementary ancestral function (e.g., catalytic).
Phenotypic plasticity: ability of organisms to change their phenotype, maintaining the same genotype, with changes in the environment [92].
Subfunctionalization: Ohno [46,49] proposed that after gene duplication, asymmetric mutations in the resulting gene copies could lead to a partitioning of ancestral functions.

Corresponding author: Fares, M.A. (faresm@tcd.ie, mfares@ibmcp.upv.es).
Keywords: mutational robustness; gene duplication; evolutionary capacitors; experimental evolution; evolvability; regulatory plasticity.

0168-9525/
 http://dx.doi.org/10.1016/j.tig.2015.04.008

At the level of development, there is mounting evidence of robustness to perturbations in the signals that tissues receive extrinsically. For example, during *Drosophila* oogenesis, follicle development is guided in part by the coordinated growth rate of both the germline and somatic cell lines as well as by extrinsic signals. Despite this tight coordination and communication, a recent study found that mature eggs with the normal size and shape can be produced in the context of aberrant extrinsic signaling, highlighting the overlapping layers of regulation that make process robust [14]. Despite the ubiquity of robustness, the mechanisms that give rise to robustness, specifically mutational robustness (see Glossary), and the question of whether mutational robustness is a selectable, and hence evolvable, trait remain under intense debate [15,16].

Of the various manifestations of robustness, the ability to withstand mutations is of particular interest because it has implications for evolution. Mutational robustness and the origin of innovations are linked through phenotypically neutral variants of a gene in the population. In robust systems, many variants of a gene can be tolerated while maintaining the same phenotype (i.e., these genetic variants are neutral). When these genotypic variants are connected through single mutational steps (i.e., two neighboring genotypes are separated by a single mutation) they form a network called genotypic network [2]. In a robust genotypic network, the transition between genotypes is phenotypically silent (i.e., the two connected genotypes encode the same phenotype). However, subsequent mutations in a particular genotypic background may cause new phenotypic manifestations. Within a given population, there will be many genotypic backgrounds, in some of which a new mutation will have a phenotypic effect and in others it will not. The number of different phenotypes emerging from new mutations will increase with the number of neutral genotypes (i.e., possible backgrounds) in the population. Consequently, if there are a large number of neutral genotypes, the population can access more new phenotypes [2,17,18]. Thus, the larger the genotypic network (i.e., the larger the mutational robustness of a population), the higher the potential is to produce novel adaptations [19]. The details however matter: increasing robustness up to intermediate levels increases evolvability, but when a set of possible accessible phenotypes has reached its maximum, adding more genotypes to the genotypic network does not increase evolvability (Box 1). Several studies have shown a relation between the size of the genotypic network and the potential access to different phenotypes. For example, analyses of genotypic networks for protein structures reveal that different regions of the network provide access to different neighboring structures

Box 1. Robustness and evolvability

The link between robustness and evolvability is subject to certain limitations, with intermediate levels of robustness yielding higher evolvability, as a trade-off exists between increasing the diameter of the genotypic network and the overlap between phenotypes accessible by all the genotypes in the network. As this overlap increases, the correlation between robustness and evolvability declines (Figure I) [19]. Take for instance two genotypic networks generated by a number of genotypic backgrounds (Figure IA). Genotypic backgrounds within the same network are connected through single mutations, and mutations in a specific genotypic background that lead to a new genotype from the same genotypic network has no phenotypic effect. Conversely, a mutation that leads to a genotypic background from a different network produces a different phenotype. If we measured evolvability as the ratio between the number of accessible phenotypes (in this case two, Figure IA, green and black) and the number of genotypes in the black network (three genoytpes that lead to black phenotype), this would yield a value of 0.66 (2 phenotypes/3 genotypes = 0.66) in our example. Increasing the robustness of the black network is equivalent to increasing the number of genotypic backgrounds that yield to the same phenotype (Figure IB). New mutations in this wider genotypic network are more likely to provide new phenotypes, each of which is encoded in a different genotype network (Figure IB). For example, in Figure IB, the black phenotype is encoded by two additional genotypes compared to Figure IA, but this increase allows accessing three phenotypes (green, brown, and red) through subsequent mutations. In this case, the evolvability of the black network has increased compared to that in Figure IA (4 phenotypes/5 genotypes = 0.8). Increasing the genotypic network by two additional genotypes (Figure IC) allows certain nodes access to more than one phenotype as a result of exhausting the full phenotypic space, which leads to the overlap between the set of accessible phenotypes for genotypes of the same network. This phenotypic overlap between genotypes means that while the robustness of the system increases its evolvability does not (4 phenotypes/7 genotypes = 0.57). In conclusion, increasing robustness increases evolvability [90–92] as long as the genotypes of the same genotypic network cannot access all possible phenotypes.

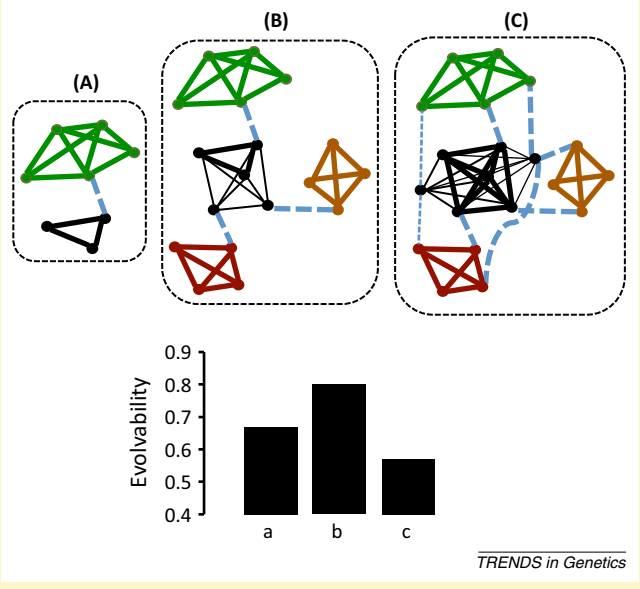

Figure I. Intermediate levels of mutational robustness increases evolvability. Neutral genotypic networks are those in which several genotypes (circles) are connected through single mutations and lead to the same phenotype (phenotype is symbolized with the color of the network). A small genotypic network **(A)** has a low potential to evolve novel phenotypes (e.g., network a presents an evolvability of 0.67). As the network increases in size **(B)**, the number of accessible phenotypes through subsequent single mutations increases disproportionally more (e.g., evolvability of network B has increased 13% by adding two additional genotypes to the network). Large genotypic networks (e.g., high robustness, such as in genotype **C**), decreases evolvability (network C has decreased its evolvability to 0.50) because genotypes overlap in the space of their accessible phenotypes.