The road to modularity

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Abstract | A network of interactions is called modular if it is subdivided into relatively autonomous, internally highly connected components. Modularity has emerged as a rallying point for research in developmental and evolutionary biology (and specifically evo–devo), as well as in molecular systems biology. Here we review the evidence for modularity and models about its origin. Although there is an emerging agreement that organisms have a modular organization, the main open problem is the question of whether modules arise through the action of natural selection or because of biased mutational mechanisms.

Variational module
A set of covarying traits that vary relatively independently of other such sets of traits. Variational modules are recognized by higher than average correlations among traits.

Functional module
Features that act together in performing some discrete physiological function.

Developmental module
Either a part of an embryo that is quasi-autonomous with respect to pattern formation and differentiation, or an autonomous developmental signalling cascade.

Quasi-autonomy
A lower than average grade of connectedness: the elements of modules are highly interconnected, but to an increased extent are unconnected to other modules. Also called quasi-independence.

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All organisms are highly integrated and cohesive systems that interact with their environment in coherent and often predictable ways. But in spite of the integration of particular parts, many organisms display obvious signs of structural and functional heterogeneity among these parts. Higher organisms possess different cell types for specialized functions, such as nerve cells for the transmission of information, muscle cells for locomotion and glandular cells for endocrine and exocrine functions. And cell types tend to be packaged in spatially cohesive parts or organs such as the brain, liver and muscles. Even single-celled organisms have discrete and separable functional systems such as the flagellum and the mitotic spindle. In the past 10 years this functional and structural heterogeneity has been considered under a new conceptual umbrella, that of 'modularity', although the ideas that support this concept are much older. Modularity is an abstract concept that seeks to capture the various levels and kinds of heterogeneity found in organisms, and it is considered a fundamental aspect of biological organization[1]. Various kinds of modules have been distinguished. A variational module is composed of features that vary together and are relatively independent of other such sets of features[2]. A functional module is composed of features that act together in performing some discrete physiological function that is semi-autonomous in relation to other functional modules[1]. A developmental module is either a part of an embryo that is quasi-autonomous with respect to pattern formation and differentiation[3], or an autonomous signalling cascade[4]. A module, therefore, is a part of an organism that is integrated with respect to a certain kind of process (natural variation, function, development and so on) and relatively autonomous with respect to other parts of the organisms.

The modularity concept has gained popularity more-or-less simultaneously in molecular biology and systems biology[5,6], developmental biology[3] and evolutionary biology[2], and in cognitive psychology[7]. We will not attempt to cover modularity in the cognitive sciences, which have generated a huge literature with little overlap or relevance for developmental evolution (but see REF. 8). In this Review, we look at how the consideration of modularity has contributed to the study of developmental evolution, in particular through its application in molecular, developmental and evolutionary biology. We will focus on two topics: first, the empirical evidence for modularity, and second, the various ideas about the origin of modularity.

Evidence for modularity

All ideas of modularity refer to a pattern of connectedness in which elements are grouped into highly connected subsets — that is, modules — which are more loosely connected to other such groups. In this context, elements can be nucleotides in an RNA molecule, proteins in a cell, cells or morphological characters. The connections can be physical, for instance, protein–protein interactions or amino-acid contacts within a protein, or they can be dynamical, as in the case of gene regulatory networks, or statistical, such as the pleiotropic effects of genes causing correlations among phenotypic traits (variational modularity). Modularity thus refers to very different kinds of connections and elements; however, it can still be considered a uniting principle. Molecular, physical interactions lead to dynamical connections (for example, gene regulation, or development) that in turn lead to variational connections among macroscopic phenotypic traits. On all these levels, however, modularity is important in its own right, not only because the molecular and dynamical interactions cause macroscopic patterns of covariation, but also because variational modularity influences the evolutionary dynamics of species, which in turn affects the evolution of molecular networks.

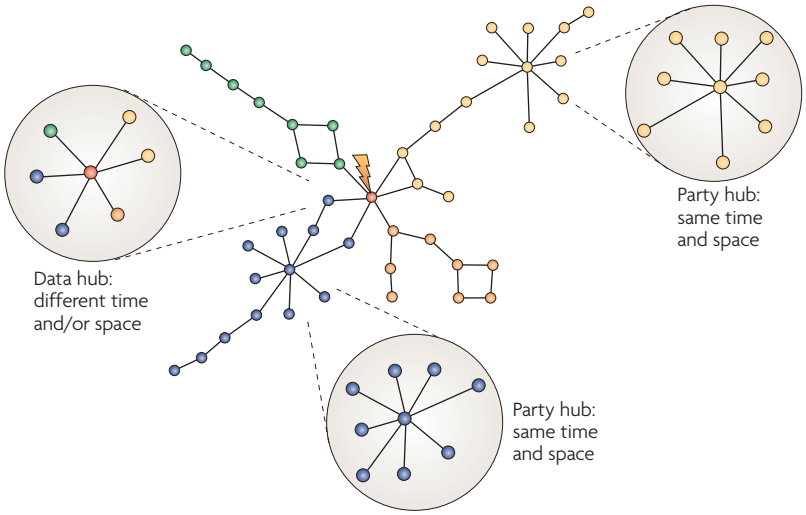


Figure 1 | **A protein network with two types of highly connected nodes (protein).** 'Party' hubs interact simultaneously with many partners, whereas 'date' hubs interact with their partners at different times and/or locations. The interactions are inferred from mRNA coexpression patterns. The coloration reflects the mRNA expression similarity. The figure is reproduced from *Nature* REF. 13 © (2004) Macmillan Publishers Ltd.

Evidence for modularity of course varies depending on the level at which it is studied[9], and we provide examples from protein–protein interactions, gene regulation and variational modularity.

Protein–protein interactions

Many functions of proteins are mediated through their physical interaction with other proteins. For instance, the cytoskeleton consists of various proteins that form supramolecular structures such as microtubules, and transcription factors affect gene expression through their physical interactions with other transcription factors and the proteins of the transcriptional complex. Research into the structure of molecular networks was greatly stimulated by the publication of large-scale protein–protein interaction studies[10–12]. Analytical methods to identify and study modular network structure vary from simply setting an arbitrary threshold number of interactions per node, which must be surpassed to consider a node a part of the module[13], to the various cluster analytical methods[14,15]. Although there are still considerable problems in using these data because of high false-discovery rates and other statistical issues, results have stimulated intense interest in the large-scale structure of biological interaction networks.

The approach that is often used is to first identify the central nodes (hubs), which are characterized by a higher than average number of interactions. For instance, Han *et al.*[13] detected two types of hubs in the protein–protein interaction network of yeast (FIG. 1): hubs with interactions that are both spatially dense and simultaneous ('party' hubs), and hubs with interactions that are widely distributed in space and time ('date' hubs; (but see REFS 16,17). Fraser provided evidence that party hubs mediate within-module interactions, whereas date hubs integrate between modules[18]. In support of this model, Fraser showed that mutational effects at party hubs are less widespread (limited pleiotropy), indicating that effects tend to be limited to a module. On the other hand, mutations of date hubs have larger, more widespread effects (more extensive pleiotropy), owing to their interactions across many modules[18,19]. There is also a difference in the rate of evolution of hub genes within modules and those between modules. Surprisingly, the rate of evolution in terms of amino-acid substitutions is higher for date hubs (inter-module), even though they have more deleterious effects when deleted. Date hubs are also found in fewer and less distantly related species. Both facts suggest that inter-module interactions are more often subject to evolutionary modification than intra-module interactions.

Modularity of protein–protein interactions has also been assessed by their evolutionary cohesion[20,21]. Chen and Dokholyan[21] compared the evolutionary rates of proteins within and between modules and found that genes in the same module have more similar rates of evolution than genes from different modules. This result applies both in terms of sequence evolution and gene expression evolution. At the interspecific level, Campillos and colleagues[20] showed that modules can be evolutionarily cohesive, that is, they can be conserved in several taxonomic groups. If there is variation among groups, the components of an evolutionary module are frequently gained or lost together[20,22]. Genes in evolutionarily stable modules have lower gene-duplication rates and tend to be involved in environmental interactions[20]. However, one has to be careful in applying this criterion for assessing the biological significance of a candidate module. For example, comparing yeast with human and *Drosophila melanogaster* networks might reveal variation in the presence of modules[23], but the criterion may be too severe for assessing the biological relevance of a putative model. There are biologically important interactions that have arisen more recently than the most recent common ancestor of animals and yeast; for instance, the transcription factor Ultrabithorax (UBX) has protein–protein interactions in fruitflies that are relevant for insect development, but which are absent in the UBX of shrimp and velvet worms[24,25]. Interactions that are less widely represented among species can still be biologically important.

Gene regulatory networks

Genes participating in gene regulatory modules are expected to be coexpressed[26–29]. Methods used to reveal co-regulated target genes involve clustering genes on the basis of the similarities in gene expression changes[30,31]. As demonstrated by Thieffry and Sanchez[32], preliminary identification of quasi-independent subunits of gene regulatory networks can be made through a qualitative representation of gene regulatory interactions in the form of logical networks. The activity of each gene is represented as a logical variable (for instance, the logical value 'true' corresponds to 'the gene is active') and regulatory interactions are represented as logical functions (for example, AND or OR). In this way, the authors were able to decompose complex regulatory networks

Cluster analytical methods
A family of computational methods used to classify a set of objects according to some measure of similarity or dissimilarity. Most frequently, hierarchical clustering methods are used, in which objects are put into a hierarchical scheme of classification.

Hub
A node of a network that is involved in a higher than average number of interactions with other nodes.

into a set of intertwined circuit modules. However, to reliably establish the modular effects of regulatory genes requires extensive gene expression data, preferably combined with results from targeted manipulation of regulatory genes. Segal and colleagues[33] developed a tool for the identification of such master regulatory genes and their corresponding suites of target genes. By partitioning gene expression as a function of sets of regulatory genes, this method enables detection of target, as well as regulatory, genes.

An interesting question is whether coexpressed sets of genes correspond to conserved sets of regulatory gene interactions. Tanay and colleagues[34] have shown that coexpression modules can be maintained in evolution despite changes in the regulatory genes that activate them. A phylogenetic analysis of regulatory elements in the ribosomal protein expression module in fungi revealed that regulatory genes can substitute for each other through the intermediate evolution of redundant *cis*-regulatory elements.

Variational modularity

Variational modules were first recognized as sets of correlated traits, that is, through the analysis of correlation matrices of quantitative traits[35]. Character variation was found to be highly structured, and apparently influenced by both developmental pathways and functional factors (FIG. 2). The influence of common developmental pathways is suggested by higher than average correlations among serially homologous traits[36], the fact that traits with the same developmental origin are more highly correlated than average[37], and the similarity of covariation and gene expression territories[38,39]. In addition, correlated traits also tend to be dedicated to the same function, and thus represent both variational and functional modules[40]. This duality is relevant, as some models for the origin of variational modularity predict that functional modularity selects for variational modularity (see below). On the genetic level, morphological modularity arises from modular pleiotropy, with pleiotropic effects often being restricted to subsets of functionally and/or developmentally related traits[40–47]. These modular effects are hierarchically structured; for example, Kenney-Hunt[48] found a nested hierarchy of skeletal modules starting with the body axes versus limb skeleton, cranial versus postcranial modules within the axial skeleton, skull versus mandibular modules within the cranium and tooth-bearing parts versus the muscle attachments within the mandible. In addition to the pleiotropic effects of single loci, Wolf and colleagues[49,50] have shown that epistatic interactions can also affect multiple traits simultaneously ('epistatic pleiotropy') and contribute to the covariation among phenotypic traits.

Most recently, independent developmental mechanisms for two adaptively independent traits have been demonstrated with respect to beak shape in Darwin's finches[51,52]. Evolution of beak shape and size is a major mode of adaptation in these birds, leading to distinct beak shapes that are adapted to different food items[53,54]. The evolution of these beak shapes requires the independent adjustment of two traits, beak length and depth. Abzhanov and colleagues have shown that these two traits are regulated by two non-overlapping gene regulatory cascades: beak length is regulated by the calmodulin-dependent pathway[52], whereas beak depth is regulated by a bone morphogenetic protein 4 (BMP4)-dependent pathway[51] (FIG. 3). Hence, length and depth might therefore be evolvable with little interference between traits. It will be interesting to further determine whether and how these results relate to the genetic correlation among those traits, because independent evolvability is determined by the pattern of genetic covariation[55].

In the finch *Geospiza conirostris*[56], beak length and depth are only moderately correlated (r_G = 0.199+/–0.096 s.e., where r_G is the genetic correlation) even though each of them is highly heritable and strongly correlated with body size. This is consistent with the idea that beak length and depth are largely independent traits. But this pattern is not found consistently across *Geospiza* species. In *Geospiza fortis* and *Geospiza scandens*, Grant and Grant[57] found quite strong correlations between beak length and depth (r_G = 0.798 and 0.482, respectively). It is unclear what causes this discrepancy, but hybridization and gene flow could contribute to increased genetic correlations because genes from different species will remain partially linked, causing genetic covariation.

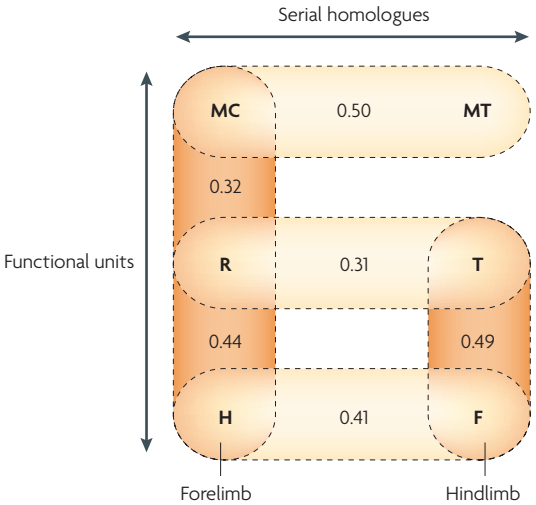


Figure 2 | **Correlations among the limb bones lead to variational modularity.** Variational modularity among quantitative traits is influenced by functional and developmental factors. Forelimb and hindlimb lengths are serially homologous and correlated owing to common development. As a consequence, corresponding elements in the forelimb and hindlimb form variational modules, for example, the femur and the humerus or the tibia and the radius. Upper and lower limbs are correlated because of their physical proximity and common function, and form two additional variational modules — a forelimb and a hindlimb module. The numbers represent the phenotypic correlations between the respective elements in the macaque. F, femur; H, humerus; MC, metacarpals; MT, metatarsals; R, radius; T, tibia. Modified with permission from REF. 36 © (2005) Society for the Study of Evolution.

Correlation matrix
A table of the correlation coefficients among quantitative traits, which summarizes the degree to which different traits covary as a result of genetic and environmental influences. Sets of strongly covarying traits are called variational modules.

Serially homologous traits
Traits that are repeated within the organism, such as vertebrae in the body axis, teeth in the jaw or segments of repeated limbs.

Modular pleiotropy
A genetic architecture in which a set of genes tends to have pleiotropic effects on the same set of traits, but few and weaker effects on other traits.

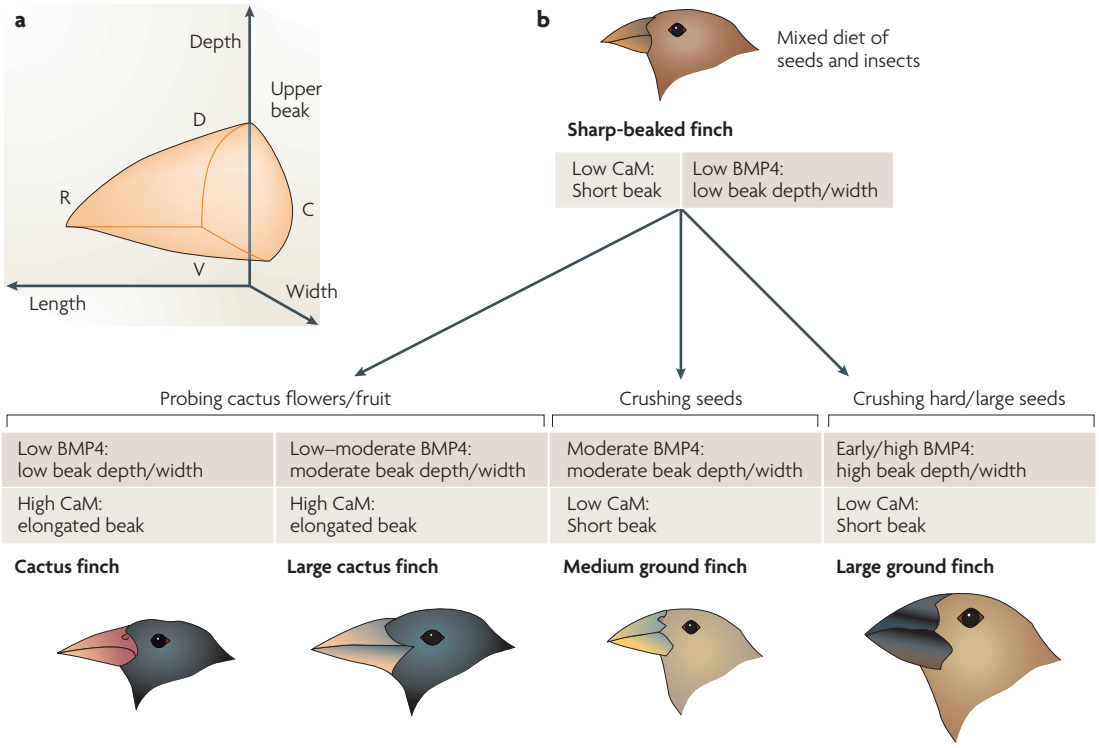


Figure 3 | **The developmental basis for variational independence among beak traits in Darwin finches.** Beak morphology along length and depth axes (shown in part **a**) is regulated by the seemingly independent effects of two major developmental genes — bone morphogenetic protein 4 (*BMP4*) and calmodulin (*CaM*)) — the differential expression of which results in a range of adult morphologies that correspond with their specialized diets. The basal morphology is illustrated in part **b** by a generalist morph. C, caudal; D, dorsal; R, rostral; V, ventral. Modified with permission from *Nature* REF. 52 © (2006) Macmillan Publishers Ltd.

This shows that the evolutionary independence of two traits is by no means guaranteed by the existence of independent developmental pathways.

Variational modularity in RNA secondary structure. Modularity in RNA molecules is "the partitioning of RNA molecules into subunits that are simultaneously independent with respect to their thermodynamic environment, genetic context and folding kinetics"[58]. Assessment of modularity in RNA secondary structure is aided by the availability of realistic folding models that allow evaluation of large numbers of sequences by computational methods.

As the temperature increases, a non-modular RNA will go through several secondary structures with little if any similarity to the original secondary structure as it melts (FIG. 4). In the case of a modular RNA, each of its substructures melts individually. So, the modularity of the overall structure is reflected in the fact that the substructures dissolve without perturbing the other elements. Another method that is used to assess modularity in secondary-structure elements is to 'graft' a nucleotide sequence that folds into a secondary-structure element into a random sequence and see whether the folded chimeric RNA maintains the original secondary-structure elements. It was shown that secondary-structure elements of evolved RNA sequences are less context-sensitive than random RNA sequences that fold into the same structure. This is the case for RNAs from a computational evolution experiment[59], and for the secondary structure of RNA virus genomes[60].

The origin of modularity

In contrast to other organismal traits, modularity is an abstract concept that refers either to patterns of functional and molecular interactions or to the distribution of mutational effects on the phenotype. As such, modularity does not interact with the environment and so does not directly contribute to the fitness of an organism. Consequently, the evolutionary explanation of modularity is not as straightforward as the explanation of the evolution of a claw or wing shape. But even abstract features of organisms have an evolutionary history and thus require an evolutionary explanation. Here we will classify the various models according to the role that they assign to natural selection: 'neutral' models, in which natural selection is at most a secondary force; or models in which natural selection drives the origin of modularity by direct or indirect effects. A crucial goal for ongoing work on these models is to devise testable predictions that could falsify some or all of these models. As discussed below, it is easy to make such predictions for some of these models, but not yet for all.

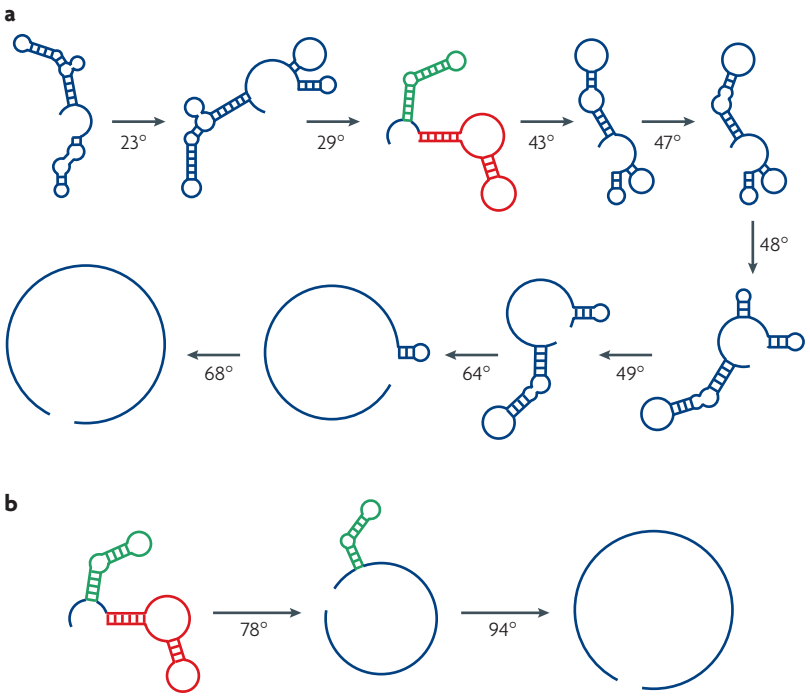


Figure 4 | **Modularity of RNA secondary structure.** In RNA, modularity is reflected in the melting of individual substructures with increasing temperature without perturbing the other elements. As the temperature increases, a non-modular RNA (shown in part **a**) will go through several secondary structures. By contrast, each substructure of a modular RNA (shown in part **b**) melts individually. Green and red secondary-structure elements are shown in both the modular and the non-modular RNAs, but in the modular RNA the red element can be seen to melt without affecting the green element. Figure modified with permission from REF. 95 © (2002) Wiley Periodicals.

Neutral models

We classify models as neutral if the guiding hand of natural selection is not necessary to bring about modularity. That does not necessarily mean that natural selection is not involved in some of these models but, if it is, the role of natural selection is secondary rather than formative. Here we discuss two types of model that have been proposed to explain the origin of modularity.

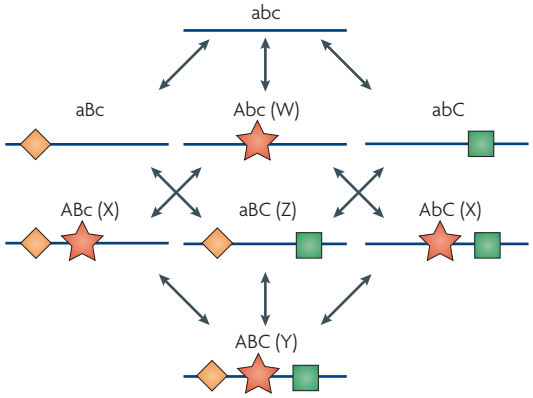
Duplication–differentiation. It has been shown that the highly non-random structure of protein–protein interaction networks can be explained by simple models of gene duplication without the intervention of natural selection. Here we will focus on a specific model[61,62] that has been shown to lead to a modular network structure as an example of the idea of 'modularity for free', which refers to a mutational process that produces a modular structure without the intervention of selection.

The duplication–differentiation (DD) model considers network diagrams in which the nodes represent proteins and the edges that connect the nodes represent protein–protein interactions. The network grows by randomly selecting a node (that is, a gene that codes for a protein) and duplicating it. The new protein inherits all the interactions from its 'parent'. This assumption is plausible given that the ability of a protein to interact with other proteins is determined by its structure, which is not changed by gene duplication. Following the duplication is a phase in which the various interactions of the new protein are deleted with the probability δ, or a new interaction is added with the probability α. This assumption that only the interactions of the new protein are modified, and not the old, is motivated by the fact that new genes duplicate the functionalities of the parental gene and are thus vulnerable to loss and modification immediately after duplication, whereas the interactions of the older proteins are maintained by stabilizing selection. Initially, it was shown that this model can reproduce the most salient features of protein–protein interaction networks, such as sparse connections and the distribution of the number of interactions at each node. This result requires that the rates of deletion and addition are tuned to what has been estimated for the yeast interaction network. In a later paper, the same authors showed that this model also leads to modularity[63,64].

The mathematical analysis of the DD model reveals an interesting problem in explaining real interaction networks. The model does not specify the rate at which interactions of new proteins are removed or added. Hence, the model has two free parameters, the rate of losing an interaction δ and the rate of acquiring a new interaction α. Modularity and the other network characteristics only emerge in a relatively narrow range of these parameters. The model itself does not explain why real protein–protein interaction networks evolve at the rates that lead to the topological features of real networks. This point was duly noted by the authors and they suggested two possibilities. One is that sparse networks — networks that have a small average number of interactions per protein — are favoured by natural selection because networks that are too densely connected may lead to dynamical instabilities[65,66], although a certain level of connectivity is necessary to maintain functional integrity. The other possibility is that natural selection directly favours the existence of the emergent modules to subscribe cellular functions. In either case, the modularity is neither adequately explained by the mutational process nor by natural selection alone, but by an interaction between the two. The DD process favours the origin of modular network structures by mutation, but natural selection is needed to tune the process to actually realize this potential.

Neutral modular restructuring. Transcriptional regulation of a gene with multiple functions is often achieved by modular molecular mechanisms, sometimes called subfunctions. For instance, the gene *even skipped* (*eve*) is expressed in seven stripes along the body axis of the *D. melanogaster* embryo, but the expression of stripe 2 is governed by an enhancer that lies close to the transcriptional initiation site, whereas the other stripes are regulated by elements further upstream. This modular structure of gene regulation in conjunction with gene duplication can lead to paralogous genes, each of which is dedicated to expression in different body parts. This is the case for engrailed 1 (*En1*), which in mice is expressed both in the paired appendage as well as in the

Box 1 | **The subfunction-fission model**



This model, described in REF. 68, assumes a total of eight potential allelic states [a/A,b/B,c/C] at a locus coding for an enhancer. The small letter stands for the allele that does not have the binding site for the corresponding transcription factor. For instance, [aBC] has binding sites B and C for transcription factors β and γ, but no binding site for α. Transcription factor α is globally functional over both tissues, whereas β and γ are specific for different tissues. Furthermore, the model assumes that redundancy in the transcription factor binding site is neutral, so that, for instance, the allele [ABC] has the same fitness as [Abc], because transcription in the latter allele is driven by the global transcription factor α in both tissues, whereas in the allele [ABC] either A or B and C are redundant, and do not affect the phenotype. However, the alleles [abc], [aBc] and [abC] are all deleterious because they lack expression in at least one tissue. Hence, there are five neutral alleles in this model, called W, X, Y and Z in the figure.

Force *et al.*[68] calculate the time it would take, by mutation and genetic drift, to convert the ancestral allele [Abc], with only one function, into the allele Z = [aBC], which has two non-overlapping subfunctions. The model is based on the fact that enhancers undergo extensive remodelling in the kind and number of transcription factor binding sites, often without apparent phenotypic effects[92]. The model envisions a two-phase process. The first phase involves accretion, degeneration and replacement of the enhancer that functions in both tissues. In this process, the transcription factor binding site A is replaced with two distinct binding sites B and C for the tissue-specific transcription factors β and γ. In the second phase, the new enhancer is duplicated and undergoes a degeneration–complementation process that creates two independent enhancers, each maintaining a different tissue-specific transcription factor binding site. The second step mirrors the process that has been proposed for the subfunctionalization of duplicated genes[93].

hindbrain and spinal cord, whereas the two paralogues in zebrafish, *engl* and *engl b*, are differentially expressed in the hindbrain and the pectoral fin bud, respectively. Hence, the modularity of gene regulation can lead, in the wake of gene duplication, to the evolution of developmental modularity. The key question then is, why do modular mechanisms of gene regulation, or subfunctions, evolve?

Alan Force and colleagues have proposed a neutral scenario for the origin of two distinct subfunctions from a more global ancestral subfunction[67,68] (BOX 1). The authors show that in small populations a transition from an enhancer with non-redundant transcription factors to one with redundant factors is possible in a reasonable amount of time. This is consistent with the results obtained for the evolution of transcriptional control of coexpression modules[34], where redundant transcription factor binding sites evolve. However, it is also clear that the origin of subfunctions is not a necessary outcome of their model. In fact, it is not even the most likely outcome. At most, the model suggests that subfunction fission is possible by non-adaptive mechanisms, but is not a necessary result.

This model can, however, be viewed from a different angle, namely, as a process that creates variation that selection can act on. For large populations, Force and colleagues calculate the equilibrium frequencies of the five viable alleles (described in BOX 1) and show that the redundant allele [aBC] is about the third most frequent allele, but is still at only 10–20% frequency in the population. This frequency is not impressive if we allow only neutral processes, but it is highly significant if we consider this process to provide the genetic polymorphism for selection. If natural selection favours independent changes in gene regulation in the two tissues, then the availability of an allele at ≥10% in the population makes the evolution of modularity a quasi-deterministic outcome. Hence, the model could be seen either as a stand-alone non-adaptive mechanism that occasionally produces modular gene regulation, or as a variational mechanism that supplies natural selection with genetic variation that can be selected for its modular effects. More detailed population genetic studies of enhancer variation will be necessary to distinguish between these two interpretations of the model. For instance, it will be important to test whether the evolution of a modular *cis*-regulatory element is driven by natural selection. This could be done using methods that compare the rate of acquisition of transcription factor binding sites with the neutral rate of nucleotide substitution[69–71]. If the acquisition of modular *cis*-regulatory elements is driven by selection, then the neutral model will be falsified; if the rate is consistent with neutral evolution, the involvement of directed selection will be falsified.

Models involving natural selection

Here we consider three kinds of scenarios. In the first, modularity directly contributes to higher fitness and can be selected more or less directly. The second could be called 'variational adaptation', by which traits that often need to change together, owing to environmental pressures, are integrated into a module, and traits that rarely need to be changed at the same time are packed into different modules. The third scenario could be called 'differential erosion of pleiotropic effects', by which selection for robustness preferentially removes some pleiotropic effects, leading to modularity. A more comprehensive discussion of other models can be found in REFS 72,73.

Direct selection for modularity. As mentioned above, modularity is an abstract property; to affect fitness, modularity must interact with other traits. One proposal under which modularity could be considered as a direct target of selection is the constraints to adaptation model[74]. In this proposal, modularity is selected if it breaks a developmental constraint and thereby makes adaptive phenotypes accessible that would be genetically unattainable otherwise. Another possible scenario in which modularity would directly benefit fitness is when ontogenetic development of the optimal phenotype itself is aided by a modular organization of development. This has been shown in two artificial life studies, one of which uses network learning as a model

Box 2 | **Modularity aids the development of an adapted phenotype**

It has long been known that an artificial neural network can learn certain tasks by implementing simple rules that modify the strength of neural connections. However, this ability to learn is impaired if the same neural network is asked to learn two different tasks, a phenomenon called neural interference or cross-talk. A paradigm for this problem is learning to recognize an object on an array of receptors (for example, a 'retina') and to determine where the object is located on the retina, the so-called 'what' and 'where' problem[94]. Neural interference can be eliminated if the two tasks are learned by two non-overlapping subnetworks. DiFerdinando and colleagues[75] have shown that a genetic algorithm will evolve a modular network that is dedicated to the two functions spontaneously. In this simulation, the genetic algorithm was limited to evolving the general architecture of the network, namely, which neuron is connected with which. A learning algorithm was then used to determine the strength of the connections, that is, the actual function is learned or developed during the ontogeny of the individual, whereas the architecture is evolved by selection. Modularity evolved because it aided the learning of the task during the lifetime of the individual, and thus directly improved the fitness of the phenotype.

of ontogeny[75] (BOX 2), whereas the other simulates artificial ontogenies of robots under the control of gene regulatory networks[76]. These results are interesting as they point towards possible direct fitness benefits for modularity, but it is not yet possible to decide what exactly led to the evolution of the modular architecture in these models as the evolutionary process was not analysed in any detail. It could be that these examples are in fact better understood as consequences of selection for developmental robustness, and could thus fall under the category of indirect selection, which is discussed below.

Variational adaptation. Under the term variational adaptation, we summarize several proposals that consider modularity as an adaptation in the broad sense to the pattern of adaptive pressures from the environment. The basic idea is that environmental changes do not always require changes in all aspects of the organism. There might be a need to adapt to dry conditions, requiring changes in the excretory system and the body surface to limit water loss, but in other environments the population might need to improve the visual sense organs, as, for instance, in dense forests. Hence, adaptive pressures affect functional systems differently, and selection might favour a genetic encoding of characters that makes these functional systems independently variable[5,77,78].

There is good empirical evidence that variational modules often correspond to functional subsystems (see above). The hypothesis that this match is caused by natural selection is suggested by the finding that the origin of functional specialization among serially homologous traits is accompanied by a decrease in the correlation among these traits. For instance, Berg[79] has shown that flower parts that are adapted to specialized pollination functions are less correlated with the vegetative parts of the plant than less specialized flowers[80]. This has recently been confirmed with respect to the bracts of *Dalechampia scandens*, which have been recruited into pollination function relatively recently[81]. A study of mammalian limb bone variation showed that the lower correlation of specialized parts is also evolutionarily derived[36]. These authors compared the covariation structure of limb bones of six species showing a broad correlation among serially homologous traits, such as the lengths of the lower forelimb and hindlimb. The only species in their sample that showed no significant correlation between forelimb and hindlimb lengths was the bat species, which supports the hypothesis that functional specialization, for example, flight in bats, leads to variationally independent modules.

Initial attempts to simulate the evolution of variational modularity with quantitative genetic models failed (summarized in REF. 72), suggesting that, if selection can produce modularity at all, it must do so through interaction with constraints from the mutational and developmental processes rather than by selection acting on an unstructured continuum of possibilities. In fact, the first simulation study that succeeded in evolving modularity in a modularly fluctuating environment used a highly structured 'syntax' to represent genetic change[82]. Kashtan and Alon simulated the evolution of two kinds of artificial systems — a network of logical circuit gates and an artificial feedforward neural network. The results were similar in both models, so only the first model is discussed here. In these experiments, the 'organism' was a network of NAND (not-and) gates that were connected to six input lines and a single output. The organisms were evolved through random mutations that changed connections between a fixed number of NAND gates, and the fitness of a genotype depended on the network's ability to compute the input–output relationship of a given logical function with four variables. For such a function, there are 2^4 possible inputs (0/1 values for the variables X, Y, Z and W). First, Kashtan and Alon evolved circuits to calculate the goal function:

$$G1: = (X \text{ XOR } Y) \text{ AND } (Z \text{ XOR } Y) \quad (1)$$

It took on average 9,000 iterations to evolve a circuit that computed the goal function. Note that this goal function is hierarchically modular, consisting of two XOR (exclusive either-or) functions and an AND function. However, the circuits that evolved to solve this problem were not modular at all. Next, they simulated the evolution of circuits with two goal functions that switched every 500 iterations:

$$\begin{aligned} G1: &= (X \text{ XOR } Y) \text{ AND } (Z \text{ XOR } Y) \\ G2: &= (X \text{ XOR } Y) \text{ OR } (Z \text{ XOR } Y) \end{aligned} \quad (2)$$

In this case, the evolved networks were highly modular (FIG. 5). Note that the two goal functions differ from each other in a modular way; that is, of the three logical components, they differed in only one, namely, the function that connects the two XOR functions (either AND or OR). In fact, the evolved network was able to adapt to the different goal functions with few changes. In this model, the modular structure of the 'genotype' matches the modular structure of the variable 'environments' (represented by the two goal functions). Furthermore,

Neural network
Neural networks are a class of mathematical and computational models that aim at simulating the activity of networks of nerve cells.

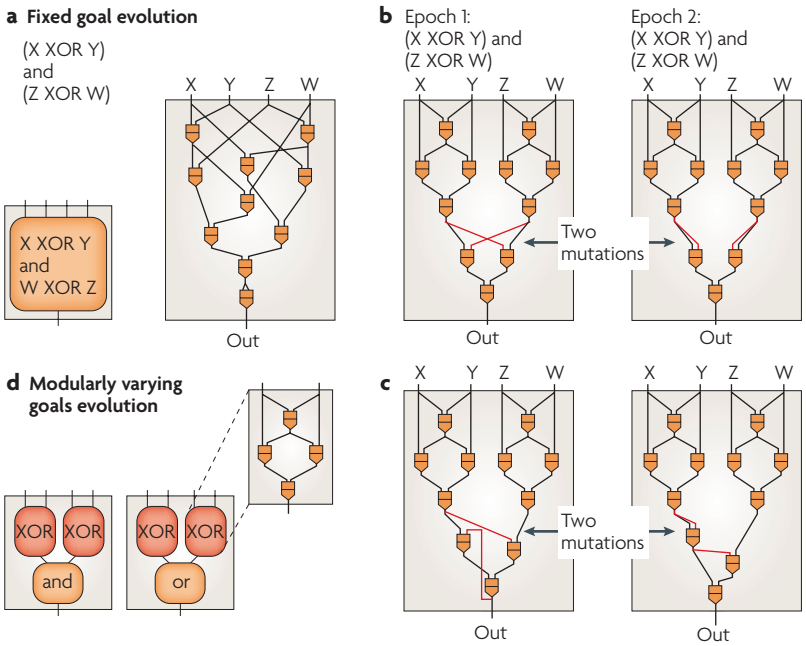


Figure 5 | **Evolution of modular circuits due to modular variation in the environment.** The simulation study by Kashtan and Alon[82] has shown that modular circuits can evolve by random change and selection if the environment varies in a modular way. The circuits are composed of NAND (not-and) gates, shown here as the end product of simulated evolution. Circuits that evolved under a constant environment (part **a**) were not modular, whereas those that evolved under a modularly varying environment (part **d**) were modular (shown in parts **b** and **c**). The circuits in parts **b** and **c** resulted from different simulation runs. Red lines show the connections that are rewired when the environments are switched. The substructure of the modular circuit is shown in part **d**. Modified with permission from REF. 82 © (2005) US National Academy of Sciences.

the modular genotype had high evolvability with respect to adapting to the variable environment, because of its modularity.

Of course, the question remains as to whether this or a similar process can also occur in real organisms. So far, only a partial answer can be provided. The strength of the pleiotropic effects of genes on various traits have been shown to be genetically variable as a result of differences in epistatic interactions among loci for different traits — so-called differential epistasis[83] (BOX 3). We propose a simple model that shows that natural selection can act on this variation to either increase the correlation among traits or decrease it depending on whether the traits are simultaneously under directional selection or not (BOX 3). Modular selection, according to which sets of traits are more often selected together than others, can lead to a reinforcement of pleiotropic effects among co-selected traits and suppression of pleiotropic effects that are not selected together.

Differential erosion of interactions. Another way that selection can indirectly drive the evolution of a trait is by correlated selection response. In this scenario, selection acts on a character that directly affects fitness, but any character that is genetically correlated with the adaptive character will also evolve[55]. For instance, selection on body size can lead to changes in skull shape if there is a genetic correlation between body size and skull shape. Some theoretical results suggest that something similar may occur with modularity.

In a seminal paper, Ancel and Fontana[59] investigated the evolution of RNA secondary structure in a computational model of RNA folding. In their model, they allowed for environmental variation in the form of suboptimally folded secondary structures, and showed that three properties increased during evolution: environmental and genetic robustness, and modularity of secondary structure elements. In short, environmental robustness was shown to be the directly selected property, and genetic robustness and modularity evolved as correlated selection responses.

It makes sense that modularity and robustness are correlated in RNA secondary structure. Modularity, as assessed here, limits the consequences of genetic and environmental influences to subsets of the phenotype. In general, the presence of a secondary-structure element, that is, a stem–loop region, does not depend only on the existence of complementary base pairs to form the stem, but is also influenced by nucleotides outside the stem–loop region, which influence whether there are alternative secondary structures that compete with the formation of these elements. Ancel and Fontana have shown that this dependency on the sequence context decreases with increasing robustness of the overall secondary structure. Translated into the language of genetics, this says that the evolution of robustness led to weaker pleiotropic effects on the structure elements of nucleotide substitutions outside the element. Because these results are based on a biophysical model of RNA folding, it is likely that this property is generic at least for RNA secondary structure. Similar correlations between thermodynamic stability, genetic robustness (designability) and the existence of protein domains have been suggested by Li and collaborators[84].

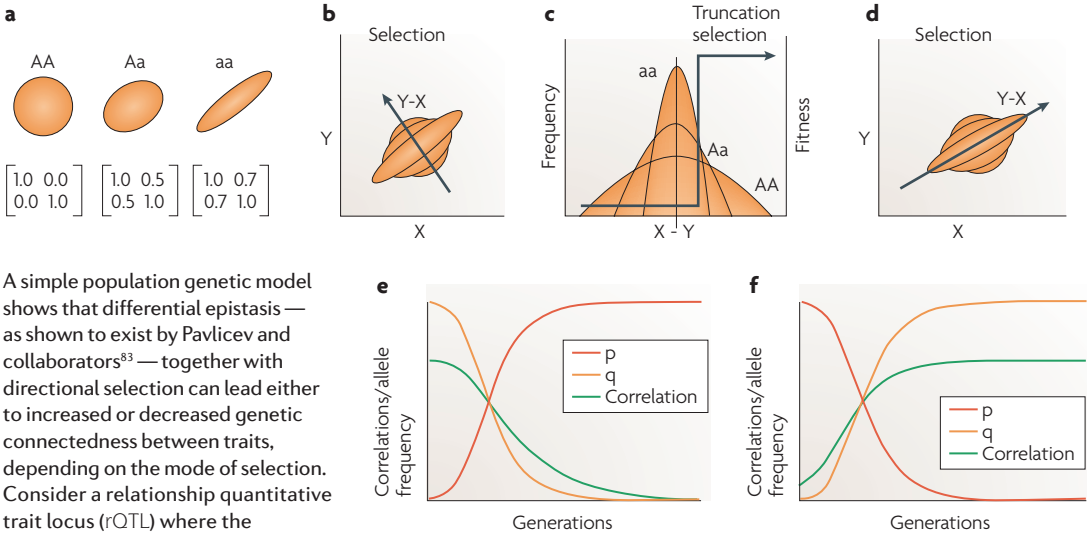
The evolution of modularity as a consequence of selection for robustness can be understood as a case of differential elimination of pleiotropic effects[85]. The effect of a mutation increases with the number of characters it affects (pleiotropy). For example, Cooper and colleagues have shown that reduced pleiotropy correlates to fitness canalization in yeast[86]. Hence, robustness can increase by reducing the number of pleiotropic effects per mutation, for instance, by limiting mutational effects to a specific module.

This model can be generalized to a 'differential erosion model' for the evolution of modularity. If indirect effects of mutations can be more easily suppressed (that is, are 'soft') by selection for robustness than direct effects, then modularity can evolve. This would imply that genes that are involved in a functional module should have more limited effects on fitness than genes that are hierarchically above the modules and that regulate and control whole modules (evidence for this was reviewed in the section on protein–protein interactions[19]). Furthermore one would predict that functional interactions within a module, that is, those mechanisms that underlie 'hard' genetic effects, are phylogenetically more stable than effects that transcend individual modules

Differential epistasis
Describes a situation in which pleiotropic effects of a locus are affected differently by the genetic background.

Canalization
Coined by Conrad Waddington to describe the tendency of wild-type phenotypes to be more stable than mutant phenotypes. In recent literature, the same phenomenon is often called robustness and is intensely studied at all levels of organization from macromolecular structure to ecosystems.

Box 3 | **A simple model for the evolutionary modification of pleiotropic effects**



A simple population genetic model shows that differential epistasis — as shown to exist by Pavlicev and collaborators[83] — together with directional selection can lead either to increased or decreased genetic connectedness between traits, depending on the mode of selection. Consider a relationship quantitative trait locus (rQTL) where the phenotypic correlation between traits X and Y, but not the means, vary with the genotype (part **a**), so that the three genotype-specific distributions are superimposed one on the other (part **b**). Phenotypic correlations vary from 0.0 for the AA homozygotes, 0.5 for the heterozygote, and 0.7 for the aa homozygotes. Consider the situation in which the A allele first appears in a population that is fixed for the a allele. This population will have a high positive correlation between X and Y of 0.70. Threshold directional selection in the Y–X direction with greater than 50% of the population on the low side of the threshold (part **c**) favours the A allele and a lower phenotypic correlation. The A allele will increase in frequency towards 1 and the correlation will decrease towards 0 (part **e**), dismantling the original XY module. Likewise, if the a allele invades a population that is fixed for the A allele and there is selection in the X+Y direction (part **d**), the a allele will increase in frequency and the correlation will increase from 0.0 to 0.7, creating a module of coordinated, co-selected, traits (part **f**). Thus, directional selection can either integrate previously independent traits or dismantle existing modules, depending on the direction of selection relative to the existing pattern of integration by acting on the genetic variation in intertrait relationships at relationship loci. This re-organization of modules will also lead to an increase in genetic variation in the direction of selection owing to epistatic interaction between the rQTL and other loci that affect trait means, thus accelerating the rate of evolution in the population. Selection can lead to a modular configuration that fits the patterns of correlated selection among traits so that traits that are selected together will become integrated with each other, losing their connections with other non-selected or contrarily selected traits.

(again, the evidence has been reviewed above). Hence, the pattern of evolution found for interaction hubs in the yeast protein–protein interaction network are consistent with the erosion model for the origin of modularity[85], but we note that the same pattern can also be the consequence of evolution after modules have already originated; namely, a mode of evolution by which modules remain conserved and change only in their context, regulation and deployment. In either case, the data only confirm the presence of the genetic architectural constraints that are assumed by the erosion model for the origin of modularity.

Conclusions

The study of modularity has greatly advanced during the past 10 years in terms of the quality and amount of data available. There is a broad agreement that modularity is real and biologically significant. Research describing various forms of modularity will proceed to include more comparative studies as genomic data is expanding, and will give important information about the evolutionary dynamics of modules. However, there are two areas that still require more attention. One is the effect of modularity on evolution, for example, its possible effects on evolvability and adaptive radiations[87–91]. This aspect could not be covered in this Review. The second is the origin of modularity. From our reading of the literature, this branch of research is still mostly based on model analysis rather than data. It is likely that we have not yet fully explored the range of theoretical possibilities to explain modularity, and more theoretical work will still be valuable. The models reviewed here, however, suggest an emerging theme. It seems that the origin of modularity requires both a mutational process that favours the origin of modularity and selection pressures that can take advantage of and reinforce the mutational bias. Hence, an understanding the molecular details of the mutational processes affecting the molecular networks and the genotype–phenotype relationship will be as crucial as the ecological pressures and opportunities that favour modular structure of the organisms. Effective attempts to falsify some of these models will depend on the identification of model systems in which the origin of modularity can be investigated at the molecular level, with both comparative as well as experimental methods.

rQTL
A region of the genome that influences the correlation (that is, the relationship) among two or more quantitative phenotypic traits, for example, the correlation between body size and limb length. These genomic regions thus affect the genetic architecture of the phenotype and potentially provide the genetic variation that is necessary for the evolution of modularity.

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DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
En1 | *eve*
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UBX

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