OPINION

The flexible genome

Ralph J. Greenspan

A principal assumption underlying contemporary genetic analysis is that the normal function of a gene can be inferred directly from its mutant phenotype. The interactivity among genes that is now being revealed calls this assumption into question and indicates that there might be considerable flexibility in the capacity of the genome to respond to diverse conditions. The reservoir for much of this flexibility resides in the nonspecificity and malleability of gene action.

Historians say that a key element in Thomas Hunt Morgan's success was his decision to separate the question of the transmission of heritable traits from that of the mechanism of their realization[1,2]. By concentrating exclusively on the transmission of traits, the original fly researchers were able to focus on the association of genes with chromosomes and on the position of loci relative to each other. The mechanism of realization of a trait — the connection between gene and phenotype — was left to later generations (that is, us).

The lion's share of effort in addressing the relationship between gene and phenotype has gone into isolating mutants that deviate drastically from normal, and then into studying their molecular characteristics and interactions. When relatively few genes were known (around the mid-1970s), it was difficult to imagine mechanisms through which phenotypes might be produced. There was simply not enough to go on. As more genes were identified, sensible and logical models could be constructed to represent their interactions and roles. The models were generally drawn as pathways, an analogy that originated in biochemical genetics and has since driven virtually all the genetic analysis of complex phenotypes. Now, however, as each new suppressor screen increases the number of constituents, and their interrelationships become more complicated and less exclusive, murkiness has again returned[3].

Difficulties arise because of the circuitousness of the path from gene to phenotype — a problem that is particularly acute in the study of behaviour. Not only are behavioural phenotypes very sensitive to non-genetic influences, but also the highly interconnected network of the nervous system sets up an additional layer of complexity between the gene and the realization of the phenotype. The quandary presented by all these issues calls for a fresh look at our assumptions about genetic analysis and a consideration of how well our current concepts serve us.

Ex uno plura

Specificity has been the shibboleth of modern biology. The concept of molecular specificity, for sequence and for macromolecular structure, formed the basis of the molecular biology revolution during the 1950s and 1960s (REF. 4). Together with concepts that emerged from studies of enzyme–substrate and ligand–receptor interactions, an important shift in thinking took place at that time towards a view of biological mechanisms as an assembly of pieces, each with its own specific and restricted part to play.

During the subsequent decades, even before the techniques of molecular biology flowed into field after field, the concept of specificity dominated much of the thinking. Especially in the study of mutations that affect development, neurophysiology and behaviour, it became standard to claim specificity of phenotype as a justification for biological importance, even if such specificity did not stand up to closer scrutiny (TABLE 1).

But it was not always so. What made the shift towards specificity a revolution was that it supplanted a previous world-view, one in which biological mechanisms were highly fluid and interactive processes[5–7]. To the extent that the components of this process were imagined — and imagine them was about all one could do back then — they had to be versatile. Early ideas of protein function

Table 1 | Examples of revealed pleiotropy in *Drosophila*

Mutant	Initial specific phenotype	Gene product	Ultimate extent of pleiotropy
dunce	Associative conditioning[45]	cAMP phosphodiesterase[46]	Embryonic patterning, female fertility[47]
latheo	Associative conditioning[48]	ORC homologue[49]	Imaginal disc formation, cell proliferation in CNS[49]
optomotor-blind	Optomotor response[50], development of motion-detecting neurons	T-box transcription factor[51]	General optic-lobe development, wing, leg and abdominal patterning[11,52]
no-action-potential	Nerve conduction[53]	RNA-helicase homologue[54]	Male viability, regulation of X-linked genes[55]
no-receptor-potential-A	Photoreceptor potential[56]	PI-phospholipase C[57]	Circadian rhythms[58], olfaction[59]

(cAMP, cyclic AMP; CNS, central nervous system; ORC, origin of replication; PI, phosphatidylinositol.)

postulated, albeit wrongly, that antibodies could adapt to the shape of an antigen or that enzymes were constantly changing in their substrate specificity. In the realm of gene action, there was the recognition that genes were versatile. Certain mutations clearly affected many different aspects of the phenotype of the organism and were thus categorized as pleiotropic[8,9]. Their existence did not run counter to the prevailing ideology.

All these ideas were quickly put aside with the advent of molecular biology and the realization that 'sequence is destiny'. The search then began for specifically dedicated molecules and genes. The expectation of finding specificity was so strong that pleiotropy was sufficient grounds for dismissing the importance of a gene[10]. The first signs of trouble came with the attempts to identify all the genetic steps in a developmental pathway. In analyses of the vulva in the nematode *Caenorhabditis elegans* and the compound eye in *Drosophila*, it became clear that whereas a few genes were indeed specific to a pathway, many other equally crucial ones were not. In some cases, these other genes, more ubiquitous in their action, were already known as mutants that affect different developmental events, such as the *Notch* gene in *Drosophila*, or were known as homologues of mammalian cellular oncogenes, such as *Ras*. The mutant phenotypes of these genes alone were not very informative, but their involvement was revealed through interactions with genes that were already implicated in vulva or eye development. Pleiotropy could not be ignored.

Mutations of neural development and of behaviour have always been particularly subject to downgrading if found to be pleiotropic[10]. But most of the interesting mutants initially described as specific have ultimately had their true pleiotropic nature revealed[10,11] (TABLE 1). As a result, we have been forced into treating the subject more seriously. It might well turn out that pleiotropy is intrinsically important to the genetic construction of behaviour and that it follows from the fundamental network nature of gene interactions and of the nervous system.

Too many genes

> "Too many notes." Joseph II's comment upon hearing Mozart's *The Abduction from the Seraglio*.
>
> "There are just as many notes, … neither more nor less, as are required." Mozart's reply.
>
> *Amadeus* by Peter Schaffer[12]

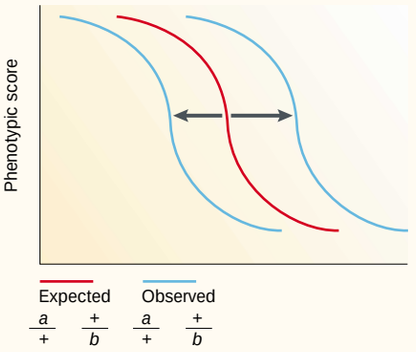

Figure 1 | **Genetic epistasis.** Interactions among a set of genes can be sensitively measured by constructing *trans*-heterozygotes between mutant alleles *a* and *b* (that is, by generating an individual of genotype *a* +/+ *b*) of different loci and then scoring them for quantitative phenotypes, such as behavioural scores. (Quantitative traits are influenced by a large number of genes, often of small individual effect.) An interaction is deemed to be significant if the observed score deviates statistically from the expected score. Expected scores are calculated on the basis of a measure of the average interactivity of each allele ('general combining activity'[29]) with all of the others.

The search for genes has been a chief concern of geneticists since variants first began to be mapped — the more, the better, for filling in spaces along chromosomes, regardless of phenotype. The quest has become more directed during the past several decades of developmental and behavioural genetics. The guiding assumption has been that mutants reveal the key constituents and that they will explain the underlying mechanisms. There is no doubt that this approach has been successful in expanding and shaping our view of when, where and how genes function[13].

Central to this world-view is the genetic pathway, originally modelled in conjunction with the enzymatic transformations of intermediary metabolism[14]. The extension of the analogy beyond metabolism made its debut in phage morphogenesis[15], in which it represented the sequential assembly of a phage particle. In more contemporary studies, it has been further adapted to represent several kinds of processes, including pattern formation[16] and cell-fate determination[17]. It has even been stretched to apply to morphogenetic events[18] and, in behavioural studies, to phases in memory consolidation[19].

The aspiration of this approach, based again on the phage morphogenesis work, is saturation mutagenesis for variants that 'specifically' affect the phenotype in question. Sadly, this might have been applicable only to phage morphogenesis. We now know that the search for early embryonic patterning mutants in *Drosophila* missed many crucial genes despite the fact that it met the criterion for saturation. This was due not only to the important role of maternal genes in the formation of the embryonic axes, but also, more importantly for this discussion, to the fact that many of the relevant loci did not happen to mutate to a phenotype that was restricted enough to be picked out. Eventually, mutants in this more refractory class of genes were found by using several strategies: screens for enhancer or suppressor mutations, in which a starting mutation sensitizes the system to further genetic perturbations[20]; screens of insertional mutations in which an inserted reporter gene shows tissue-specific expression (so called 'enhancer traps'[21]); and screens of insertional mutations that were designed to cause overexpression of the gene that neighbours the insertion site (so-called 'EP' lines[22]).

Many of the loci that have been identified using these approaches are pleiotropic genes, such as *Notch* and *Ras*, that have a significant role in many other processes besides those that underlie the phenotype under consideration and that cannot mutate so readily to a sufficiently 'specific' phenotype. But this does not account for all the missed genes. Some of the relevant mutants are silent by themselves and show an abnormal phenotype only in conjunction with a mutation at another locus; the sensitized background is a prerequisite for seeing the defect[20].

The ensuing proliferation of identified genes creates problems of interpretation, such as which genes are most important and how they all interconnect. (The consequences of these developments for scientific discourse were recently discussed in this journal[3].) Not the least of these problems is the diminishing value of the pathway analogy. Rather than running in linear paths, the increasing complexity of relationships among genes is better described as a distributed network. Some genes produce more damage than others when mutated, but this depends heavily on the context of other alleles that are present, and so it is difficult to arrange them in a simple order of importance. The interactions that have defined various pathways are not wrong, just not exhaustive. They are part of a much larger picture.

Behaviour: an extreme case

Pleiotropy has been the hallmark of hunts for behavioural mutants all along. As mentioned above, most behavioural mutants in the fly are the result of rare, phenotypically specific alleles of genes that act more widely[10,11]. Behavioural mutants are also extremely

sensitive to variations in the genetic background — the natural, genetic heterogeneity in laboratory stocks (also referred to as 'modifiers'). Not only do experiments need to be conducted such that mutants and controls are on the same background, but also mutant phenotypes will often fade over time as the result of unintended selection for such modifiers. The dependence of mutant phenotypes on the strain background has been well documented for learning mutants in the mouse[23]. The spontaneous disappearance of mutant phenotypes in long-term cultures, well known at the level of folklore, has been reported for mutations that affect learning and mushroom body development in the fly[24]. It is presumed to be the result of spontaneous selection for modifying alleles that are present in the population. The sensitivity to genetic background is a special case of the more general observation that mild genetic differences can produce large phenotypic effects on behaviour[25,26]. Such extreme sensitivity to subtle genetic variation, and the ubiquitous presence of the requisite variation, argues strongly against any sort of linear pathway model for the action of genes on behaviour. It is unlikely that the variation needed to cause 'background effects' would be found so often if only a narrow set of dedicated genes could interact with each other.

Apart from sensitivity to the background, gene interactivity in behaviour also shows up in conventional suppressor screens[27]: in tests of double mutants in which each one has a pronounced effect on its own[28]; and in more sensitive tests, between mutants that are too mild to exert much effect on their own (known as epistasis). The latter tests for epistatic interactions are perhaps the most important for the current discussion because they rely on quantitative phenotypes and so can reveal subtle interactions (FIG. 1).

Such gene interactivity has been shown for a set of recessive olfactory mutants[29]. Because the mutants were isolated independently of each other, and thus were not selected for interactions (as they would have been in a suppressor screen), there was no *a priori* expectation that they should show a high level of interactivity, but they did. These characteristics imply a highly interconnected, highly interactive system — a network rather than a pathway.

Is behaviour an extreme case of such interactivity? It is certainly not the only phenotype to show subtle effects in such tests. A set of randomly generated, transposable *P*-element insertions in *Drosophila* produced significant epistatic effects on metabolic enzyme activities[30]. In this case, the phenotype tested was not the criterion on which the strains were chosen (that is, at random). Nonetheless, most combinations affected the measured enzyme activities. The high degree of interactivity shown between genes that are chosen at random argues even more forcefully for a wide-ranging, highly interconnected system.

Where behaviour might be exceptional is in the sensitivity of the macroscopic phenotype to these subtle perturbations. As a result, even mild genetic variants might not be silent. This is nothing other than the same sensitivity to genetic background described earlier for behavioural mutations. The only difference is that here the experimenter is creating the background differences such that the modifiers are defined loci.

E pluribus pauca

Characterization of the gene system as a network helps to explain some of the phenomena discussed above: the non-pathway-like relationship between elements, the synergistic interactions between so many elements, and the great sensitivity to genetic background. This network of genes, in turn, must operate through the networks of cellular machinery and anatomy in the nervous system to influence behaviour[31]. Filtration through successive networks provides greater potential for interactivity and synergy.

Networks do not function in the same way as pathways. Network elements can take on new roles as conditions change. They are more versatile, less narrowly determined. In such a system, the same output can be produced in various ways. This property, particularly when discussed in the context of knockout mutations with no apparent effect, has often been called redundancy[32]. But the compensation that occurs in a network after removal of elements is not redundancy. Redundancy implies substitution of identical elements to preserve the same overall structure, as well as the same outcome. And there are certainly occasional cases of actual substitution that involve duplicated genes[33]. A more general mechanism, however, lies in the potential for biological networks to respond with broader adjustments. Sometimes this preserves the initial outcome (that is, 'no phenotype'[25]), other times it produces a new outcome. Either way, the system recruits available elements and makes changes. The pleiotropy of so many genes, and the access to the range of gene functions that it confers, contributes to this recruiting ability.

These systems characteristically have many non-identical elements (for example, genes) that are highly interconnected, but with non-uniform patterns of connectivity (FIG. 2a). This type of connectivity gives the elements the capacity to produce the same result by different strategies, in contrast to a redundant system in which the same result is produced by the same strategy. The technical term for this kind of system is 'degeneracy'[34], most familiar in reference to the genetic code, but more recently expanded to describe a fundamental property of biological systems in the context of nervous system function and developmental biology[34–36]. When an element is knocked out in such a system, adjustments are made, the system takes on a different configuration, and alternative solutions can be used (FIG. 2b).

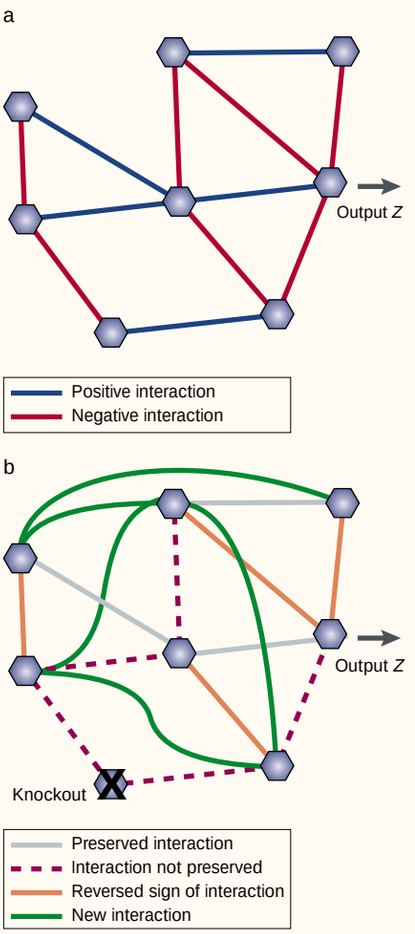

Figure 2 | **Gene network interactions.** Interactions in (**a**) a hypothetical gene network, and in (**b**) the same network with one gene knocked out. When one element of the system is changed ('knocked out'), the rest of the system changes in response. In this instance, the output (*Z*) is unchanged, illustrating the phenomenon of degeneracy. Under different conditions, the output might be different, producing a mutant phenotype in some cases, or a new emergent property in others. 'Positive' and 'negative' refer to whether a phenotype improves or degrades as a result of the interaction. 'Reversed sign' refers to a change in the direction of effect in that interaction.

"The science of genetics was founded on the premise that genes are stable and separable. This principle does not preclude the ability of the entire gene system — the genome — from showing flexibility and versatility as a fundamental aspect of its operation."

Network adjustments occur even after relatively mild perturbations. This has already been indicated by the sensitivity of genetic tests for epistasis[29,30] (FIG. 1), and has been reaffirmed in the global monitoring of gene and protein expression. Changes in nutritional state produce widespread changes in gene expression and protein populations in yeast[37,38], as do viable knockout mutations in the mouse[39] and even subtle, hypomorphic mutations in the fly (J. Minden and R.J.G., unpublished observations). A previous characterization of redundancy as "investigator inadequacy"[10] is consistent with the current discussion. If the system is not actually functioning in the same way, then a diligent investigator can reveal that fact through appropriate perturbations.

'Robust', 'buffered' and 'emergent' are epithets frequently applied to biological networks, which reflect the ability of these networks to survive perturbations[40,41]. Feedback loops and back-up pathways have been invoked to account for these properties. Feedback loops are generally quite local in their effects, unlike the more wide-ranging gene interactions that have been detected[29,30]. Back-up pathways are even more problematic, requiring selection both for the specific pathways and also for their back-up pathways. This, in turn, requires a degree of evolutionary directedness and specificity[32] that is hard to reconcile with what we know about the imprecision of evolution. A more flexible and fluid view of the relationships among these signalling and regulatory systems (for example, FIG. 2) allows for the same net result without invoking a predetermined mechanism for it. The malleability and versatility of gene networks and their ability to 'find new solutions' when constituents are changed, help to account for the properties of robustness, buffering and emergence.

The relationships that have been defined as pathways are no doubt real, but they need not be invariant. Their relationships are embedded in broader and more plastic networks that can be reconfigured depending on the immediate circumstances. Their ability to do this will be a function of the available components and their pleiotropy, as well as of the external conditions around the cell and its past history. Some parts of the system will no doubt show greater flexibility than others.

Multicellularity also increases plasticity in the responses of a system. If interactions are available between cells that are themselves non-identical, then the reservoir of possible adjustments and combinations increases exponentially. The degree of responsiveness to perturbation seen in a yeast cell is amplified many times over in a metazoan with many cell types and organ systems, each of which is dynamic in its own right. When the complexity of the nervous system is added into the equation, the potential emerges for new capabilities in response to genetic changes.

The science of genetics was founded on the premise that genes are stable and separable[42]. This principle does not preclude the ability of the entire gene system — the genome — from showing flexibility and versatility as a fundamental aspect of its operation.

Newton might not have liked Darwin

If the orderly steps of the pathway analogy no longer present a viable picture, and if instead we must come to grips with a more diverse, interconnected and non-exclusive view of biological mechanisms, then certain assumptions must be let go. One of these is that every gene (and, implicitly, every protein) has evolved to fulfil some particular function — sometimes referred to as the 'Panglossian paradigm'[43]. The widespread finding of pleiotropy militates against this view, as does the ability of biological networks to reconfigure and improvise many strategies towards the same end. Nor is such a view commensurate with the randomness and messiness of evolution. There are neither "too many notes" nor are there "just as many notes, neither more nor less, as are required." Evolution has found a third way.

An assumption borrowed from physics that also falls by the wayside is that we can treat subsets of any system in isolation and still preserve its essential, invariant properties. The high degree of interactivity as revealed, for example, in tests of epistasis and in global monitoring of gene and protein expression, indicates that even subtle changes to such a system can alter its properties. When these changes are not subtle, as in knockout mutants, the system-wide responses are likely to be great. The responses might be masked by the success of the system in using alternate strategies to preserve the output, but the mechanism used to achieve that outcome is nonetheless different. This creates interpretive difficulties in analysing null mutations, recalling those previously encountered in interpreting brain lesions[44]. When a system is highly interactive, functions that are missing after a lesion cannot be accurately assigned to the missing element in any restrictive or exclusive sense. Reference must also be made to how the rest of the system has changed in response. It might serve us better to treat network events as aggregate, system-wide phenomena (system states) rather than as individual events or isolable pathways.

Isaac Newton might have liked the neat view of biological systems made up of dedicated components, with causal roles that can be studied in isolation, and in which particular starting conditions give rise to uniquely predictable responses. Charles Darwin, by contrast, might have felt more at home with the idea of a complex, emergent system made up of many non-identical components, with non-exclusive roles, non-exclusive relationships, several ways of producing any given output, and a great deal of slop along the way. We have been Newtonians for the past several decades in our thinking about gene action. It is time to become Darwinians.

Ralph J. Greenspan is at The Neurosciences Institute, 10,640 John Jay Hopkins Drive, San Diego, California 92121, USA. e-mail: greenspan@nsi.edu

Links

DATABASE LINKS *Notch* | *Ras* | *dunce* | *latheo* | *optomotor-blind* | *no-action-potential* | *no-receptor-potential-A*

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OPINION

Predicting adaptive evolution

Robin M. Bush

Phylogenetic trees reconstruct past evolution and can provide evidence of past evolutionary pressure on genes and on individual codons. In addition to tracing past evolutionary events, molecular phylogenetics might also be used to predict future evolution. Our ability to verify adaptive hypotheses using phylogenetics has broad implications for vaccine design, genomics and structural biology.

It is well documented that some genes evolve more quickly than others; for instance, in the human species, certain histone genes are highly conserved, whereas immunoglobulin loci are extremely polymorphic[1]. A lack of genetic variation might indicate the occurrence of purifying selection — a force that preserves the adapted condition and that is therefore typically observed in functionally important genes. By contrast, extensive variation in genes indicates that the encoded protein might benefit from undergoing amino-acid replacements. Such positive selection has been recently observed in genes that have an adaptive function. Until now, it has been difficult to link the patterns of molecular variation to the selective pressures responsible for them. However, in some systems, notably in viral species, sufficient sequence data now exist to test adaptive hypotheses directly using phylogenetic analysis.

Phylogenetic trees are a graphic means of reconstructing evolution on the basis of similarity between the characters of the individuals under study; the length of a horizontal branch on the tree reflects the amount of change between an individual and its nearest ancestor (BOX 1). Evolutionary pressure on a gene or codon can be detected by comparing the rates of synonymous (silent) and non-synonymous (amino-acid changing, or non-silent) nucleotide substitutions across the branches of a tree. In the absence of selection, the synonymous and non-synonymous substitution rates should be equal (FIG. 1a). Most coding genes show an