Int. J. Dev. Biol. 42: 501-505 (1998)

Seven types of pleiotropy

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ABSTRACT Pleiotropy, a situation in which a single gene influences multiple phenotypic traits, can arise in a variety of ways. This paper discusses possible underlying mechanisms and proposes a classification of the various phenomena involved.

KEY WORDS: *pleiotropy, redundancy, mutation, gene family, evolution*

One of the themes that runs through the work of Antonio García-Bellido, a theme which has been an important lesson to the present writer and to many other developmental biologists, is that the main aim of any experimental geneticist should be to understand the *normal* function of genes (García-Bellido, 1979). Mutations, the raw material of most genetic analyses, should be studied not for themselves, but for what they tell us about the wild type situation. It is all too easy to become beguiled by the subtle differences in phenotype that result from different mutations in an allelic series, or to expend effort in detailing the minutiae of a particular mutant phenotype, so it should never be forgotten that our main purpose is to understand and explain the normal situation.

Nevertheless, there are exceptions to this general rule. For example, studying the pathological consequences of transforming oncogenes is central to understanding cancer, and it is also clinically important to investigate the aberrant patterns of development caused by neomorphic developmental mutations in humans. Also, mutations provide the raw material for evolution, as well as for geneticists, so the detailed features of a mutant phenotype may be highly relevant to how natural selection has acted or will act on the underlying variant allele.

In addition, sometimes mutant phenotypes are so dramatic and complex that they challenge explanation. A single genetic alteration may lead to a host of alterations in the adult organism, sometimes in very unexpected ways. Pleiotropy, the condition in which a single gene affects multiple traits, may well be the rule rather than the exception in higher organisms. In the past, geneticists have usually preferred to focus on genes with a single well-defined function, for both conceptual and operational reasons. Most "housekeeping" genes (ubiquitously expressed), and many "luxury" genes (expressed in only one tissue) fall into this category, but most of the genes in animal genomes are expressed in some but not all tissues, and probably act differently in each situation.

As genetic methods are used to attack problems of increasing complexity, in organisms containing tens of thousands of genes and hundreds of tissue types, the problems raised by pleiotropic effects become ever more severe. It has become increasingly easy to define primary mutational lesions, or, in the case of reverse genetics, to create a defined mutation *de novo*. However, the connection between primary mutation and observed phenotype may still remain largely or completely obscure.

A further consideration is that pleiotropy must often have affected the course of evolution. Indeed, it is conceivable that in complex eukaryotes, pleiotropy may lead to major constraints on possible mutational avenues, as a result of the interwoven web of genetic and physiological interactions that are involved in development and function.

This article discusses pleiotropy from several angles: as a methodological problem, as a feature of past evolution, and as a significant factor in future evolution or genetic modification. More than one function may end up connected together at a single chromosomal location, as a result of chance, physiology or active selection, and consequently mutations at this locus will affect multiple phenotypic aspects, with obviously more complicated consequences for the experimenter (in the case of genetic analysis), for natural selection (in the case of evolution), or for artificial selection (in the case of breeding or genetic engineering).

There are multiple ways in which pleiotropy can come about, and the purpose of this article is to suggest a possible classification of the underlying phenomena. The title has been chosen in conscious reference to a classic work of English literary criticism, "Seven Types of Ambiguity", by William Empson (1930). Empson was one of the very few literary critics to have had any kind of scientific training, and his approach to textual analysis has components that reveal this background. According to Empson, ambiguity, whereby one word, phrase or sentence is made to carry multiple meanings, is one of the ways in which literary texts acquire their concentration and power. Ambiguity leads to some loss of certainty about precisely what a writer means, which is anathema to rigid linear logical thinking, but on the other hand it creates great benefits in broadening horizons, revealing new connections, and setting up resonances in the mind of the reader. In a very loose sense, pleiotropy is for the geneticist what ambiguity is for the critic: multiple functions embedded in the same object.

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0214-6282/98/$10.00

Printed in Spain

In this spirit, pleiotropic phenomena can be classified into a number of types, which have different levels of significance and importance. The types are illustrated with a series of examples, taken mostly from standard genetic systems such as *Saccharomyces cerevisiae, Drosophila melanogaster* and *Caenorhabditis elegans.* The basic classification is summarized in Table 1, and the seven types are discussed in the following sections.

Type 1. Artefactual pleiotropy

Sometimes a single mutation can affect more than one process, simply because two genes happen to be located next door to each other in the genome. A classic example is provided by the *Drosophila claret-nondisjunction* alleles, which affect both eye-color (the claret phenotype) and meiosis (increased nondisjunction). Most *claret* alleles do not have any effect on meiosis, however. The explanation for this behavior became clear when the *claret* gene was cloned: there is an adjacent gene which encodes a kinesin molecule, required for normal chromosome disjunction at meiosis, and the pleiotropic alleles are small deletions that affect both transcription units (Yamamoto *et al.,* 1989). A very comparable situation occurred with the *C. elegans* gene *unc-86*, which encodes a POU-domain transcription factor required for many terminal cell divisions and differentiation events (further discussed below, under Type 6). In addition, some alleles of *unc-86* also have a meiotic nondisjunction phenotype, although most do not (Hodgkin *et al.,* 1979; Finney *et al.,* 1988). Again, when the locus was cloned it became clear that the nondisjunction alleles were all small deletions affecting not only *unc-86* but also two adjacent transcription units, one of which is presumably required for normal meiosis (Finney *et al.,* 1989).

The multiple effects of such mutations are therefore artefacts of the process of mutagenesis, hence the name. Most commonly, such artefacts will be associated with small deletions rather than point mutations, but obviously situations may exist where two genes share a regulatory region, and therefore even a single base change might affect both genes. Also, position effects can potentially lead to artefactual pleiotropies, because changes at one localized point in the genome might affect the expression of a whole chromosomal region containing many genes, as a result of altered chromatin organization.

Organisms with compact, gene-dense genomes will be especially susceptible to artefactual pleiotropies. Both *Drosophila* and *C. elegans* are animals of this type, and may have descended from ancestors with larger genomes which have shrunk in size during evolution, for unknown reasons.

Many genes in *C. elegans* are organized in operons, transcribed as polycistronic units but then broken up into monocistronic messenger RNAs by a process of trans-splicing (Zorio *et al.,* 1994). In contrast to bacterial operons, the linked genes in these operons often lack any discernible functional connection, so the suspicion arises that many of the operons are simply a secondary consequence of genome compression. Perhaps, adjacent genes that were initially independently transcribed became closer together over evolutionary time, and eventually came to be co-transcribed, if there was no countervailing selection pressure against their co-expression. However, the arrangement of genes in operons does mean that the likelihood of artefactual pleiotropies is increased, and it is conceivable that this has created additional constraints on the evolutionary options available to organisms such as *C. elegans.* Alternatively, but less plausibly, one could argue that the operon arrangement provides opportunities for saltatory evolution, in that simultaneous changes in the expression of several different genes could be achieved with a single mutation in the operon promoter.

Type 2. Secondary pleiotropy

This is roughly equivalent to "relational pleiotropy", as defined by Hadorn (1961), and describes situations where a simple biochemical abnormality has multiple phenotypic consequences, sometimes with little superficial connection to the initiating mutation. Complex, long-lived organisms, especially those with advanced abilities to compensate for physiological dysfunction, are especially likely to exhibit secondary pleiotropies.

An old example, indeed one of the first human mutants to be understood at a biochemical level, is provided by the disease PKU (phenylketonurea). Here, a defect in the liver enzyme phenylalanine hydroxylase leads to excess plasma levels of phenylalanine. This in turn affects myelination of axons in the brain, and ultimately to mental retardation. Similar or more complex chains of physiological and developmental consequence can be seen in many human genetic disorders, such as the complex syndromes arising from thalassaemias. In *Drosophila*, a familiar example is provided by *rudimentary* mutations, affecting pyrimidine biosynthesis. Some of these lead to defective development of the wing, but not of the rest of the fly, because pyrimidine levels become rate-limiting for cell division in the rapidly proliferating tissues of the wing disc, but not elsewhere.

Sometimes, the connection between a biochemical defect and a distinctive phenotype is far from obvious, and the possibility of

TABLE 1

A CLASSIFICATION OF DIFFERENT TYPES OF PLEIOTROPY

Type	Situation	Example
1. Artefactual	Adjacent but functionally unrelated genes affected by the same mutation	*claret*
2. Secondary	Simple primary biochemical disorder leading to complex final phenotype	Phenylketonurea
3. Adoptive	One gene product used for quite different chemical purposes in different tissues	e-Crystallin
4. Parsimonious	One gene product used for identical chemical purposes in multiple pathways	*gpb-1*
5. Opportunistic	One gene product playing a secondary role in addition to its main function	*sisB/AS-C*
6. Combinatorial	One gene product employed in various ways, and with distinct properties, depending on its different protein partners	*unc-86*
7. Unifying	One gene, or cluster of adjacent genes, encoding multiple chemical activities that support a common biological function	*cha-1 unc-17*

other kinds of pleiotropy should be considered (Types 4-7 below). For example, the human Lesch-Nyhan syndrome, arising from loss of the enzyme HPRT, is associated with dramatic self-mutilating behavior. This has been ascribed to abnormal levels of purine in the brain, but exactly how or why this should lead to such a specific behavioral change is not yet known. More obscure yet is the connection between melanin synthesis and the formation of a normal optic nerve projection (reviewed by Guillery *et al.,* 1995). Albino mutants are known in many mammalian species, usually resulting from mutations of tyrosinase. These mutants have low levels of retinal melanin, but also exhibit incorrect projections from retina to brain, with abnormalities in the optic chiasm, as in cross-eyed Siamese cats (and cross-eyed tigers too!). As we understand more about developmental mechanisms, some of these pleiotropies may become explicable in terms of secondary effects, but some may not.

Type 3. Adoptive pleiotropy

One might also use the term "exaptational pleiotropy" for this category, because it describes cases where a pre-existing protein has clearly been co-opted, or "exapted", in evolutionary terminology (Gould and Vrba, 1981), to execute an additional function unrelated to its original biochemical role. The best example of this kind of effect is provided by crystallin proteins, which constitute the most abundant proteins in lens tissue, and play a structural and refractive role (reviewed by Tomarev and Piatigorsky, 1996). Remarkably, in many cases these are familiar metabolic enzymes such as LDH (ε-crystallin) and enolase (τ-crystallin).

It seems likely that cases like this, where the same gene product is used for totally different purposes in different tissues, are likely to be transient on an evolutionary timescale. If gene duplication occurs, there will be an opportunity for the two functions to be optimized independently, and the two copies will rapidly diverge in regulation, protein sequence, or both. In the example of δ-crystallin, which corresponds to the enzyme arginosuccinate lyase, the lens-specific version of the protein is encoded by a different gene from the major metabolic versions, so pleiotropy has been lost.

Many protein families provide possible examples of this effect - for example, α-lactalbumin and lysozyme, which are clearly related in sequence and structure, but have very different physiological functions. Sometimes gene duplication may have preceded the adoption of a new functional role, but the example of ε-crystallins demonstrates that genuinely bifunctional genes can arise without duplication, and can persist for significant periods of evolutionary time.

Type 4. Parsimonious pleiotropy

This type encompasses cases where the same enzyme catalyzes the same chemical reaction in multiple different pathways. Loss of this enzyme will then have complex metabolic consequences. For example, the same proteins are used at several steps in the biosynthesis of isoleucine and of valine (acting on different substrates), so knocking out any of these enzymes in bacteria leads to simultaneous prototrophy for both amino acids. In such cases, there has presumably been no pressure to evolve independent regulation of the two pathways, either at the gene level or at the enzyme level, so the organism employs the same protein in each pathway.

Some regulatory proteins also exhibit parsimonious pleiotropy, such as the β subunit of G proteins in *C. elegans.* There are many genes encoding G α subunits in the nematode, each with specific roles and patterns of expression, but apparently only one gene for the β subunit, *gpb-1.* A knockout of this gene, achieved by reverse genetics, leads to multiple phenotypic consequences, because of the involvement of G protein signaling in so many different processes (Zwaal *et al.,* 1996). All the specificity is achieved by use of different α subunits; as far as is known, the β subunit is executing an identical biochemical function in all these cases, so a single gene suffices.

Type 5. Opportunistic pleiotropy

This is related to the two preceding types, and describes cases where a regulatory protein appears to have been recruited to perform an additional role in a different tissue, distinct from its major and more ancient role. Illustrations are provided by the genes acting as "numerator elements" on the X chromosome of *Drosophila*. Sex in *Drosophila* is determined by the ratio of X chromosome dosage to autosomal dosage, and a small number of sites on the X chromosome act early in development as major numerators in setting this ratio (reviewed by Cline and Meyer, 1996). At least two of the numerator sites, *sisB* and *runt,* encode transcription factors that play important roles later in development, and one can assume that their action as numerators, acting as transcriptional activators of the target gene *Sxl,* is a relatively recent evolutionary acquisition.

The distinction from Type 3 is that the biochemistry is basically the same; the distinction from Type 4 is that the interacting partners are different. Also, as compared to Type 4, one role is secondary, and perhaps more subject to rapid evolutionary change.

Type 6. Combinatorial pleiotropy

This term applies to the large number of cases where a single protein product interacts with a variety of different partners in different cell types, and as a result has altered specificity and/or biochemical activity in each different situation. Mutations affecting this protein will therefore have multiple and potentially diverse effects on a variety of tissues.

Many, perhaps most, transcription factors in multicellular organisms fall into this category. Even in unicellular eukaryotes, the same combinatorial strategy is observed. For example, the proteins that control yeast mating type include the DNA binding protein *encoded by MATα2*, which has different functions in α cells and in diploids (reviewed by Herskowitz *et al.,* 1992). By itself, in haploid α cells, it acts to repress α-specific genes, but in diploid cells, it acts in combination with *MATα1* to repress an additional set of haploid-specific genes.

The nervous system seems to be a realm where combinatorial strategies are especially important, since there are a very large number of closely related cell types to be generated in nervous tissue, each with distinctive anatomy, connectivity and physiological properties. An illustration is provided by the simple and well-studied nervous system of *C. elegans,* with 302 neurons and about 115 definable cell types. The POU-domain transcription factor encoded by the gene *unc-86* is expressed in 57 out of the 302 neurons, and as a result affects the development of multiple cell

types and physiological functions: touch sensitivity, locomotion, egg-laying and so on (Finney *et al.,* 1988; Finney and Ruvkun, 1990). Its action is necessary but not sufficient to generate the various neuron types -for example, the *mec-3* gene, another transcription factor, contains UNC-86 binding sites in its promoter, but *mec-3* is only transcribed in ten cells, and only six of these ten differentiate into mechanosensory neurons. Other factors must act combinatorially with UNC-86 and MEC-3 to achieve the necessary specificity (Mitani *et al.,* 1993).

The distinction between Type 6 and Type 4 (Parsimonious pleiotropy) is that in the combinatorial situation, the biochemistry of the protein *changes* from context to context, sometimes radically (for example, from transcriptional activator to transcriptional repressor). The distinction from Type 5 (Opportunistic) is less clear-cut, and probably much combinatorial pleiotropy evolved from an initially opportunistic state. Opportunistic proteins can act in isolation, however, whereas the emphasis in Type 6 is on the diversity that can be generated by heteromeric combinations of different proteins in different cell types.

Other kinds of protein can also be deployed combinatorially, for example the subunits of membrane receptors. The mammalian receptors for the lymphokines IL-3 and GM-CSF have the same β subunit, but different α subunits, and as a result the β subunit probably has different properties in the two receptors (Kitamura *et al.,* 1991).

Type 7. Unifying pleiotropy

This final type is of a different nature from those preceding, because it describes cases where the multiple functions of a locus or gene are all related, in ultimate biological output, but the immediate chemical functions are diverse. The different enzyme activities, binding domains or structural components may all be included in the same polypeptide chain, or they may be encoded by adjacent cistrons under common regulatory control. In either case, mutation of the locus can have complex physiological consequences, which may be hard to explain if the underlying biology is not understood.

Operons in bacteria obviously reflect this kind of modular organization, and achieve common control by using a single promoter for all the components of an enzymatic pathway or structural assembly process. Operons are much less common in multicellular organisms, and unification is often achieved by using multifunctional proteins. There are many cases where two or more enzymes are encoded by separate cistrons in prokaryotes, but assembled into a single polyprotein in eukaryotes.

Such polyproteins offer an additional possible advantage, which is that metabolites may be more efficiently channeled from one catalytic site to the next, if all the sites are connected by the same polypeptide backbone. Even in bacteria, polyproteins occur, though they are less prevalent than in higher organisms. The fatty acid synthetase polypeptide of mammals contains seven enzymatic activities, and a corresponding multienzymatic protein is found in a few bacterial species, although it is broken up into separate monofunctional proteins in most bacteria. Argument continues as to whether channeling is significantly advantageous. Probably each biochemical situation has its own particular properties, favoring channeling to various degrees (for discussion, see Davidson *et al.,* 1993).

These are cases where the function of the locus is unified at the level of an overall biochemical function, such as the synthesis of a particular metabolite, and all the subfunctions correspond to simple enzymatic steps. More sophisticated assemblies may unite synthesis together with cellular organization. For example, the *cha-1* and *unc-17* genes of *C. elegans* encode, respectively, choline acetyltransferase (the enzyme responsible for acetylcholine synthesis), and the acetylcholine transporter protein, responsible for loading acetylcholine into synaptic vesicles. The two genes overlap, and have identical 5' non-coding exons, but generate otherwise completely different transcripts and proteins, as a result of alternative splicing (Alfonso *et al.,* 1994). A comparable cholinergic operon also occurs in mammals, indicating that this is a conserved and advantageous organization (Bejanin *et al.,* 1994).

Yet more complicated assemblies bring together genes or activities that affect a great variety of cellular or organismal functions. Loci controlling sexual phenotype in many organisms provide illustrations, ranging from simple cases like the yeast *MAT*α locus (encoding an activator for one set of genes, and a separate repressor for another set of genes), to "supergenes" with many clustered genes for different aspects of sexual phenotype. The evolutionary forces that result in tight linkage are obvious in these cases. For example, in primroses, the genes controlling flower structure and pollen incompatibility must remain linked, because recombination would lead to non-functional flower types. In other cases, the advantage of clustering is less obvious. Hox clusters appear ubiquitous in animals, but in both *Drosophila* and *C. elegans,* the Hox cluster is partly broken up, and does not seem to depend on linkage for its function -for discussion, see Mann (1997).

As with metabolic pathways, different activities can be built into the same polypeptide chain, or closely related polypeptides generated by alternative splicing. Examples are provided by sex determination genes in both flies and worms. The *Drosophila Sex-lethal* gene affects dosage compensation (probably in two different ways), somatic sexual phenotype, and germ line sexual phenotype (reviewed in Cline and Meyer, 1996). The *C. elegans* gene *sdc-3* similarly affects both sex and dosage compensation, and the two functions appear independently mutable, with the function-specific mutations mapping to different parts of the SDC-3 protein (Klein and Meyer, 1993). In many cases, however, the different functions presumably involve incompatible biochemistry, and therefore cannot be included in the same polyprotein.

Cases of unifying pleiotropy are usually easy to recognize, because the biology is interpretable, but there may be situations where it is not immediately apparent. A case in point is the *C. elegans* gene encoding cytochrome b, which forms an operon with the cell-death regulator *ced-9* (Hengartner and Horvitz, 1994). Initially, this association seemed accidental, but recent work has suggested a possible involvement of cytochrome with apoptosis.

Conclusion

This survey is intended to illustrate the variety of ways in which one locus can govern multiple phenotypic traits. No doubt, different and more sophisticated classifications of pleiotropy could be proposed, and the distinction between some of the seven types is

sometimes blurred, as already admitted. However, imposition of any conceptual framework on the biological world is rarely possible without some fudging.

Some of the phenomena discussed are simply accidents of the way that evolution and development occur, but others are more interesting. From the point of view of abstract design, Types 6 and 7 represent elegant and powerful strategies for the construction and propagation of complex organisms. For Type 6 (Combinatorial pleiotropy), using regulatory proteins in different combinations permits the generation of many more tissue types than would be possible with a simple one-to-one correspondence between regulator and tissue. For Type 7 (Unifying pleiotropy), assembling modules of function provides the advantage of robustness, so that advantageous combinations are not broken up by recombination, and such clusters may also permit more rapid co-evolution of different components.

However, all the types of pleiotropy carry a possible long-term penalty in terms of evolutionary constraints. A gene supporting more than one biological function is likely to be more limited in its envelope of possible variations than is a monofunctional gene. Moreover, alleles that are selectively advantageous with respect to function in one tissue may be disadvantageous with respect to function in another tissue, or at a different time. This effect, known as “antagonistic pleiotropy”, can apply to any of the seven types discussed here. Antagonistic pleiotropy has been invoked as an important factor in complex life history traits such as aging. Some genes that are beneficial in early life will lead to reduced lifespan, but may nevertheless be selected because of their advantageous properties during the main reproductive phase of an organism’s existence.

Ultimately, certain genes may acquire so many functions that some form of evolutionary stasis sets in. Of course, gene duplication and divergence should offer an opportunity to escape from this trap. According to recent theoretical work (Nowak *et al.,* 1997), the redundancy resulting from duplication may be less subject to rapid drift and disappearance than had been assumed, so there may be no real problem. If so, one wonders why redundancy is not even more prevalent. Those of us who work with non-redundant genes should be grateful!

In the future, as large-scale DNA sequencing projects generate immense amounts of information about the genomes of phylogenetically diverse organisms, it should become possible to reconstruct some of the evolutionary history of regulation in eukaryotes. From such history, wider rules and principles may become apparent, such as factors that have affected the distribution of pleiotropy and redundancy in different gene families, and the particular modes of biological regulation used in different developmental and physiological arenas. This kind of holistic analysis of genomes, functions and evolution seems likely to create a whole new intellectual continent for biologists to explore, in the decades to come.

Acknowledgments

I thank Patricia Kuwabara and Alison Woollard for useful suggestions and comments on the manuscript.

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