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Perspectives

Anecdotal, Historical and Critical Commentaries on Genetics

One Hundred Years of Pleiotropy: A Retrospective

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ABSTRACT

Pleiotropy is defined as the phenomenon in which a single locus affects two or more distinct phenotypic traits. The term was formally introduced into the literature by the German geneticist Ludwig Plate in 1910, 100 years ago. Pleiotropy has had an important influence on the fields of physiological and medical genetics as well as on evolutionary biology. Different approaches to the study of pleiotropy have led to incongruence in the way that it is perceived and discussed among researchers in these fields. Furthermore, our understanding of the term has changed quite a bit since 1910, particularly in light of modern molecular data. This review traces the history of the term "pleiotropy" and reevaluates its current place in the field of genetics.

"PLEIOTROPY" refers to the phenomenon in which a single locus affects two or more apparently unrelated phenotypic traits and is often identified as a single mutation that affects two or more wild-type traits. The study of pleiotropic genes has typically involved evaluation of segregation patterns or, more recently, the mapping of mutant phenotypic traits to a single mutant locus; when two or more traits consistently segregate with a particular mutation, that mutation is classified as pleiotropic. The concept of pleiotropy has played a prominent role in theories of aging (Williams 1957; Zwaan 1999; Moorad and Promislow 2009), facilitation and constraints of the direction of selection (Hawthorne and Via 2001; Reusch and Wood 2007; Latta and Gardner 2009), models of adaptation (Fisher 1930; Orr 2000), speciation (Maynard Smith 1966; Tauber and Tauber 1989), and human diseases (Pyeritz 1989; Mackay and Anholt 2006; Wilkins 2010). Although at times obvious, pleiotropy can sometimes be difficult to demonstrate. It is often challenging to distinguish between close physical linkage of two distinct genes and actual pleiotropy (Flint and Mackay 2009). This can be further complicated in cases where traits are not well defined. A major goal in genetics is to determine when pleiotropy is caused by a single locus with multiple primary products and when a single gene product is incorporated in many different ways (He and Zhang 2006).

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Mendel described an early case of pleiotropy in his classic 1866 paper (Mendel 1866). His character number 3 for Pisum displays either a brown seed coat, violet flowers, and axial spots or a white seed coat, white flowers, and lack of spots. Mendel states that the three characters that are attributed to each strain are always found together, and he considers them to be correlated and under the control of a single factor. Whether the three characters (seed coat color, flower color, and axial spots) are due to a single gene or not is unknown, but the fact that Mendel believed them to be shows that he considered this sort of inheritance, albeit in a rather cursory manner.

The recognition of pleiotropic traits goes back even further than Mendel, as many medical syndromes were known to have multiple distinct symptoms and a simple "familial" component (Eckman 1788; Weil 1981; Pyeritz 1989). However, "pleiotropy" as a term was not formally described and defined until 1910 by the German geneticist Ludwig Plate. Consequently, this is the 100th year since pleiotropy was formally introduced into the scientific literature. In this article, I intend to provide an historical perspective on the progression of pleiotropy as well as establish some of the more important considerations related to its study.

THE BEGINNING: LUDWIG PLATE (1910)

The term "pleiotropie" was coined by the geneticist Ludwig Plate in a Festschrift (a book in honor of a respected person) to Richard Hertwigs, which was published in 1910. Plate was a prominent German

developmental geneticist in the early part of the century. He began his career as a student under Ernst Haeckel, taking over his position as the director of the Institute of Zoology at the University of Jena. As soon as he took over, Plate removed Haeckel from the museum, beginning a very public feud that resulted in legal proceedings. This was just one of many professional conflicts in which Plate was involved. He also was a member of the Nazi party and a misogynist, openly opposing the advancement of several Jewish and female colleagues (Levit and Hossfeld 2006).

Plate's main interest in genetics was as a means to understand evolution. Like many German geneticists of his time, he attempted to resolve Lamarckian ideas with Darwinian natural selection (Levit and Hossfeld 2006). He synthesized what he considered the important components of evolution and genetics into a program he called "Old Darwinism." The main structure of Old Darwinism was a combination of Lamarkian evolution, orthogenesis, and natural selection, studied in light of genetic heredity. Although Plate ascribed primary importance to natural selection, he felt that some adaptations could be explained only by his particular interpretation of Lamarkian evolution. He clung to these ideas throughout his life. These ideas, combined with his personal conflicts, severely damaged his reputation as a scientist. It can be argued that the concept of pleiotropy is his major legacy.

To support his concept of Old Darwinism, Plate studied the genetics of a variety of organisms. During the course of his own studies and through the results of others, Plate noted that some distinct phenotypes were explicable only through the mechanism of a single gene. His original definition of pleiotropy is as follows: "I call a unit of inheritance pleiotropic if several characteristics are dependent upon it; these characteristics will then always appear together and may thus appear correlated" (Plate 1910, quoted from McKusick 1976, p. 301). This original definition is still used today to describe the basic mode of action of pleiotropy. The same mechanism was described under the name "polyphean" in 1925 by Haecker, but by then pleiotropy had received enough attention to be established in the literature (Caspari 1952).

Plate further commented on the ubiquity of pleiotropy, stating that, "The more research into Mendelian factors advances, the more examples become known which can be explained only under the assumption of pleiotropy" (Plate 1910, quoted from McKusick 1976, pp. 301–302). His assertion of the extent and importance of pleiotropy has been a central theme that has been challenged and strengthened throughout the past 100 years as the way in which we study pleiotropy has changed.

DEVELOPMENT OF PLEIOTROPIC RESEARCH

One of the first experimental studies of the mechanism of pleiotropy (Gruneberg 1938) came during the Modern Synthesis period of evolutionary research. Hans Gruneberg was a young German biologist who captured the attention of J. B. S. Haldane. In 1933, he was invited to come to University College London by Haldane on recommendation of Hermann Muller and Richard Goldschmidt (Lewis and Hunt 1984). Haldane immediately suggested that Gruneberg begin studying rat developmental genetics. Gruneberg published an article on this topic in 1938. His major contribution was to divide pleiotropy into "genuine" and "spurious" pleiotropy. He asserted that genuine pleiotropy was characterized by two distinct primary products arising from a single locus whereas spurious pleiotropy involved a single primary product that was utilized in different ways. Gruneberg also considered a second form of spurious pleiotropy, when one primary product initiated a cascade of events with different consequences for the phenotype. He approached this distinction through the study of a particular genetic skeletal abnormality in rats. The pathology was the result of a new mutation, discovered in laboratory colonies of Marthe Vogt, that had multitudinous effects on skeletal development. By careful study of the anatomy of afflicted rats, Gruneberg was able to create a chart depicting the relationships of the various aspects of the phenotype. He concluded that, while both types of spurious pleiotropy were represented, this mutation did not constitute an illustration of genuine pleiotropy. Gruneberg's support for the reality of "genuine" pleiotropy (one locus specifying two or more different products) was further weakened by growing support for the "one gene/one enzyme" hypothesis of Beadle and Tatum (1941), 1945) published only a few years later (see below). In this respect, Gruneberg's use of the word "spurious" could be seen as a bad choice of terms because the majority of investigations into pleiotropy that followed focused on different mechanisms whereby a single gene product could be used in multiple ways. The term "spurious pleiotropy" subsequently fell into disuse. Although "genuine pleiotropy" continued to appear, it was used only to suggest that the mode was unlikely. Despite Gruneberg's feeling that mechanisms involving a single gene product were not true pleiotropy, he was to spend the rest of his career studying these genetic correlations in rats (Pyeritz 1989).

In 1941, Beadle and Tatum published an article providing support for the "one gene/one enzyme" hypothesis, an idea originally introduced (but not pursued) by Cuenot (1903). The essence of this hypothesis was that a single gene codes for a single protein. The developmental and physiological action of this single protein may be complex as it is incorporated into metabolic pathways, but the genetics was not. Beadle and Tatum's study on Neurospora fungus was fundamental to understanding how genes influenced phenotypic traits and proved to be widely influential in physiological genetics. However, it provided a limited view of gene action that was later expanded by molecular biology. The one gene/one

enzyme hypothesis left no room for mechanisms of genuine pleiotropy. Subsequently, emphasis shifted away from the distinction between genuine and spurious pleiotropy and focused more on different mechanisms by which a single gene product could produce multiple phenotypic traits. More about the history of this line of research can be found in Horowitz (1995) and Hickman and Cairns (2003).

A surge of interest in defining the developmental mechanisms of pleiotropy occurred in the mid-1950s. Although this was shortly after the discovery of the structure of DNA, molecular techniques did not advance enough to shed light on pleiotropic action until the early 1980s. In particular, two German geneticists played a prominent role in the renewed interest in pleiotropic mechanisms. Richard Goldschmidt (1955) and Ernst Hadorn (1945, 1961) more or less simultaneously used their knowledge of developmental physiology and genetics to elaborate on the various ways by which a single gene product could have multiple uses. Although they addressed the old mechanism of genuine pleiotropy, both authors perpetuated the belief that it was nonexistent and that a single gene was capable of producing only a single primary gene product. Hadorn (1961) made a particularly useful distinction between two types of pleiotropy that he referred to as "mosaic" and "relational." Mosaic pleiotropy describes instances where a single locus directly affects two phenotypic traits. Relational pleiotropy, in contrast, denotes the action of a single locus that initiates a cascade of events that impact multiple independent traits. (This form corresponds to Gruneberg's second form of spurious pleiotropy.) Although these terms are no longer in use, this distinction remains a useful one in the study of pleiotropy (Wilkins 1993).

At the same time that the physiological geneticists were struggling with the mechanisms of pleiotropy, population geneticists and ecological geneticists were running productive research programs around largely ignoring the details of pleiotropic gene action. As Sewall Wright stated in the first volume of his four-volume treatise on evolutionary genetics, "Pleiotropy has a broader meaning in population genetics than in physiological genetics" (Wright 1968, p.60). Although population geneticists acknowledged the physiological genetic assertion that genes produced only a single primary product (one gene/one enzyme), they felt that the important factor was how traits were correlated and what the effects of recombination would be on uncoupling phenotypic traits. This viewpoint led to a broader view of pleiotropy in ecology and evolution. This view was so broad that Wright and others asserted that there was "universal pleiotropy." That is, a mutation at any locus had the potential to affect almost all traits through direct and indirect influence. Universal pleiotropy was central to Ernst Mayr's (1963) emphasis on coadapted gene complexes and implicit in Fisher's geometric model of adaptation (Fisher 1930; Orr 2000). A contrasting view that has emerged more recently is the idea that organisms can be broken up into modules and that pleiotropy is restricted to action within these modules (Welch and Waxman 2003). Although pleiotropy is prevalent under the modular hypothesis, it is considerably more restricted than universal pleiotropy.

The continued study of pleiotropy in ecology and evolution proved very fruitful and led to some major research programs. In particular, G. C. Williams's hypothesis for senescence through antagonistic pleiotropy has proved to be one of the most well-known applications of pleiotropy in evolution and medicine. Following a suggestion by Medawar (1952), Williams (1957) suggested that genes with antagonistic effects at different life stages could contribute to aging in a way that natural selection could not alter. That is, genes with beneficial effects prior to reproduction but negative effects after reproduction would be favored by natural selection over those that increased longevity but were less favorable to reproduction and survival to reproductive age. Although Medawar suggested that this effect could occur if the genes were pleiotropic or closely linked, Williams emphasized that close linkage would not be sufficient. If natural selection could separate the effects before and after reproduction, then effects that were beneficial early in life and longevity could be maintained. However, if the genes were truly pleiotropic, then longevity would never be favored and senescence would be inevitable. This hypothesis has given rise to numerous research programs on aging from a human health perspective as well as on senescence as a component of evolution and ecological biology.

THE MOLECULAR AGE

It was not until the advent of sequencing in the late 1970s that molecular techniques became refined enough to shed light on genuine pleiotropic mechanisms. It was quickly discovered that a single locus could produce different primary products. These different primary products were found to occur at all levels of gene expression and protein processing. Good reviews of molecular mechanisms of pleiotropy can be found in Pyeritz (1989) and Hodgkin (1998).

Shortly after the first sequencing, it was found that a locus could have multiple or overlapping reading frames (Barrell *et al.* 1976; Sanger *et al.* 1977). That is, a strand could be read starting at different points such that a single locus could produce different mRNAs and different proteins. This finding has proved to be fairly common in bacterial genomes. Although the alternate reading frames are sometimes referred to as different genes, the fact that the information for two primary products is contained in one locus and the two products cannot be separated through recombination arguably fits the criteria for pleiotropy.

There are two ways in which alternate transcripts can be produced from a single locus: alternative splicing and alternate start/stop codons. These mechanisms were discovered a brief time after multiple reading frames and provided a mechanism for pleiotropy at the mRNA-processing level. Alternate start/stop codons exist within a locus, and transcription of these can result in truncated forms of proteins with altered function. Alternative splicing allows for different exons to be selected from a single locus (Weber *et al.* 1977; Denoto *et al.* 1981). It is known that mRNA strands must go through a processing stage before they can produce a protein. Introns must be spliced out, leaving only the exons (Berget *et al.* 1977). The whole strand is then given a cap and a tail (Furuichi *et al.* 1975). However, the splicing stage for mRNA from a single locus could be spliced in different ways to produce different processed mRNA strands. Each of these alternative splicing routes would lead to a different protein. Through RNA processing, a cell can produce multiple proteins from one DNA locus. Alternative splicing plays a role in many aspects of cell maintenance and development and is ubiquitous in higher eukaryotes (Black 2003; Reddy 2007).

The transcribed RNA can be further modified through mRNA editing, first described in 1986 (Benne *et al.* 1986). Through this process the cell is able to make actual nucleotide substitutions in the mRNA, leading to amino acid differences that can affect protein function. Although these changes can be slight, the effect on the function of the protein can be significant. Even a single substitution can impact amino acid composition, RNA secondary structure, or other forms of transcript processing (Maydanovych and Beal 2006). Editing occurs in different tissues or during differential expression and may play an overlooked role in adaptation (Gommans *et al.* 2009).

Multifunctional proteins are a final example of the molecular mechanisms of pleiotropy. In these cases, a single gene product is used for two or more functions or has different functions in different tissues. These mechanisms were reviewed and classified by Hodgkin (1998). One mechanism, in particular, involves a special class of multifunctional proteins ("moonlighting" proteins) that has recently received much attention. The classic example of "moonlighting" proteins is lens crystallins, which not only serve a structural function in eye lenses but also have enzymatic properties. This example is found under Hodgkin's "adoptive pleiotropy." However, in a recent review (Huberts and Van Der Klei 2010), the authors state that moonlighting proteins should not be considered pleiotropic as they are defined as multifunctional proteins with independent functions such that a mutation in the coding region for the protein need not affect more than one function. Given this as the current definition, I would not include protein moonlighting with other multifunctional proteins as a mechanism of pleiotropy.

CURRENT RESEARCH

More recent work has continued to explore the two major questions of pleiotropy: How extensive is pleiotropy in the genome (universal pleiotropy *vs.* modular pleiotropy) and how do common mechanisms of pleiotropy work? The genomic age and the accessibility of more advanced molecular techniques have provided insight into these questions from a variety of different angles.

Many of the early architects of the Modern Synthesis implicitly (Fisher 1930; Mayr 1963) or explicity (Wright 1968) invoked universal pleiotropy. That is, the assertion that any gene in a genome has the potential to affect all traits in some way. This assumption was included in many models of evolutionary process. Although not all of these models have been formally tested, those that have provide useful and biologically relevant results for evolutionary studies. However, experiments in gene manipulation conducted by the early physiological geneticists and more recently by molecular geneticists have suggested something quite different. In their studies, disruption of a single locus has limited and measureable phenotypic effects. To rationalize the utility of extensive universal pleiotropy with the experimental findings of limited pleiotropy, models have been constructed suggesting that the genome is modular (Welch and Waxman 2003). Genes may have extensive pleiotropic effects on phenotypes within their module but are limited with regard to the organism as a whole. This is a more restrictive view than that of universal pleiotropy. Several recent approaches have been taken to evaluate the extent of pleiotropy as more universal in nature or more modular.

It has been suggested that network theory may be a useful way to study the extent of pleiotropy through computation (Featherstone and Broadie 2002). Early research has suggested that gene expression networks follow small world and scale-free dynamics (Featherstone and Broadie 2002). That is, a few genes have extensive pleiotropic effects, but most genes are more limited in their effects on the phenotype. However, nearly all genes have some degree of pleiotropy. To test the extent of pleiotropy in a genome, Li *et al.* (2006) analyzed the protein interaction networks of *Saccharomyces cerevisiae*, *Drosophila melanogaster*, and *Caenorhabditis elegans*. They addressed several aspects of network properties including the "diameter" of the network. This is the mean shortest path length, or how many traits a given gene will affect on average. They determined that the diameter was ~4–5 edges. In other words, each gene in the three genomes affects on average four or five proteins. This supports the assertion that pleiotropic effects are more modular than universal.

Another study addressed this same question by using comparative techniques (Su *et al.* 2009). Using 321 genes from eight vertebrate species, the researchers were able to estimate the number of traits affected by each gene in their sample using comparative data from

protein sequence and microarray analysis in conjunction with mathematical modeling. They found that the number of traits affected per gene was about six to seven. This closely approximates the results from network analysis and further supports the modular pleiotropy hypothesis.

A more direct study was conducted by Wagner *et al.* (2008). This research used quantitative trait analysis to further expand upon Grunberg's work on rodent skeletal genetics. The study aimed to identify the magnitude of gene effects as well as the extent of pleiotropy through genotype–phenotype associations in mice. Interestingly, the results closely approximated those from computational and comparative approaches. The number of phenotypic traits per locus was found to be 7.8. This was a somewhat higher figure than previous studies but still far short of universal pleiotropy. Therefore, the conclusion from current studies is that pleiotropic effects of genes involve a small number of traits. Although there are no direct experimental results, the strong agreement among these studies is compelling.

A second line of study has been to dissect the action of a single gene. This approach is much like that of Gruneberg with his rats but with the added data from the actual gene sequence. In some cases, changes in multiple phenotypic traits can be traced to a change in a single nucleotide of a gene. Such mechanistic studies are informative in determining how often a single gene product is used for multiple purposes and how often multiple products arise from a single gene.

A particular mutant strain of yeast is characterized by a change from brown colonies to rust-colored colonies when grown in the presence of copper as well as by sensitivity to a range of drug compounds. These two traits segregate together and have been traced to a single amino acid polymorphism in the protein cystathione β-synthase (CYS4) (Kim and Fay 2007). CYS4 plays a role in the pathway converting hemicysteine to cysteine. Disruption of this pathway was biologically likely to affect both drug sensitivity and colony color changes. Although further investigation indicated that the gene network involved may be far more complicated, this is an excellent example of pleiotropy being investigated at the nucleotide level.

Knight *et al.* (2006) looked at a single-nucleotide change that allows *Pseudomonas fluorescens* to occupy a novel niche at the air broth interface in laboratory colonies. Previous work has shown that this nucleotide change produces a large number of pleiotropic effects (Maclean *et al.* 2004) and that it is necessary and sufficient for the habitat shift. The investigators in this study were able to show that the mutation affected the regulation of an entire gene network (involving >50 protein species) by "rewiring" it. Some of the genes in the network were upregulated, and some were downregulated. Both synergistic and antagonistic interactions were discovered. Further, changes involved several modules, indicating a more universal pleiotropy. This is one of the most compelling examples of pleiotropy associated with a single-nucleotide change. A separate study of a gene in the dopamine synthesis pathway (*Catsup*) associated individual traits with separate nucleotides (Carbone *et al.* 2006). The authors of this study concluded that *Catsup* is pleiotropic at the gene level but not at the nucleotide level. This raises interesting questions as to the unit of pleiotropic action that is relevant.

Whole-genome data have also proven useful in studying mechanisms of pleiotropy. He and Zhang (2006) took advantage of the genomic sequence data of *S. cerevisiae* to evaluate general patterns of pleiotropic action. They estimated the level of pleiotropy for 4494 genes under 21 different lab conditions. They compared the level of pleiotropy to the number of protein domains per gene, the number of molecular functions, the number of biological processes in which each gene was involved, and the number of protein–protein interactions. High pleiotropy was correlated with a high degree of protein interactions and biological processes but not with the number of molecular functions or the number of proteins per gene. The authors interpreted this to suggest that pleiotropic genes more often produced single multifunctional products.

An additional area that has generated some recent interest concerns the maintenance of pleiotropy. In particular, when pleiotropic action is antagonistic with regard to fitness it would seem that gene duplication and subfunctionalization would allow for an escape from fitness constraints. Waxman and Peck (1998) used mathematical modeling to suggest that pleiotropic traits under stabilizing selection are more likely to reach an optimum genetic sequence. This is in contrast to earlier models that did not allow for pleiotropy. In these earlier models slightly suboptimal sequences tended to predominate. This suggests that pleiotropic traits are more likely to be favored by natural selection. However, two more recent studies have found evidence for subdivision of pleiotropic traits through gene duplication. In one, QTL analysis of two paralogous regulatory genes in maize (*zfl1* and *zfl2*) indicated that both genes were associated with several disjunct traits (Bomblies and Doebley 2006). Although both genes were associated with the same suite of traits, the data further indicated that each gene was more strongly associated with some traits than others and that the traits with which they were most strongly associated were different for each paralog. The authors cautioned that further studies were necessary, but they also suggested that this may be a case of subfunctionalization that allows escape from pleiotropic effects that are antagonistic under agricultural conditions. More recently, Des Marais and Rausher (2008) used a combination of comparative methods, sequencing, and enzyme assays to examine a pleiotropic

gene that had duplicated in some lineages from the Convolvulaceae but not in others. These analyses indicated that duplication in the gene (*dihydroflavonol-4-reductase*) was more consistent with an adaptive escape from pleiotropic constraints than with a case of neofunctionalization. Taken together, these latter two examples suggest that it may be possible for gene duplication to provide and escape from constraints imposed by pleiotropic action, but more work in this area is surely needed.

CONCLUSIONS

The concept of pleiotropy has developed since its introduction into the literature 100 years ago, yet it still has the potential to develop further in the current genomic age. Major questions that were raised during the Modern Synthesis have yet to be settled. How universal is pleiotropy? How often do genes produce multiple products with disparate functions? Both of these questions have significant implications for evolutionary theory.

The ubiquity of pleiotropy as well as the interaction among affected traits impacts the tempo of adaptation to novel environmental input. Extensive pleiotropy, particularly when antagonistic, will often constrain adaptation, whereas synergistic pleiotropy confined to single phenotypic modules may allow populations to rapidly evolve phenotypic novelties that produce new solutions to environmental puzzles. General trends in the extent of pleiotropy and the effects on adaptation are particularly important in light of rapid anthropogenic environmental impacts (Reusch and Wood 2007).

Similarly, the mechanism of pleiotropy may respond to evolutionary dynamics in different ways. A single gene product with multiple effects will be strengthened or weakened by processes different from those that will impact a single gene that can produce multiple products. Multiple reading frames and alternative transcripts may be more difficult for evolution to disrupt than a single product incorporated into different pathways. Regulatory genes and their far-reaching pleiotropic effects can be considered a special case of pleiotropy that may have extensive consequences (Knight *et al.* 2006).

The evolutionary outcomes of pleiotropy are only half the story. What is the evolutionary origin of pleiotropic systems? Is pleiotropy an evolved trait, or is it simply a by-product of biochemical and genetic constraints? Answers to these questions will increase our understanding of how organisms can adapt and what generates the wide range of biodiversity that we observe around us. In addition, insight into the origin of genetic diseases and disorders will in some cases facilitate their treatment (Cheverud 1996).

In the history of physiological genetics, pleiotropy has often been overlooked and even discounted as an artifact of incomplete understanding of developmental processes. However, evolution and ecology studies of pleiotropy have provided rich interpretations of the evolutionary process. The molecular age has produced evidence that single genes are able to produce multiple products with pervasive effects on the phenotype. Even after 100 years, studies of pleiotropy have a great deal to tell us both in ecology and evolution and in physiology and medicine.

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