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Patterns and Evolutionary Consequences of Pleiotropy

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Abstract

Pleiotropy refers to the phenomenon of one gene or one mutation affecting multiple phenotypic traits. While the concept of pleiotropy is as old as Mendelian genetics, functional genomics has finally allowed the first glimpses of the extent of pleiotropy for a large fraction of genes in a genome. After describing conceptual and operational difficulties in quantifying pleiotropy and the pros and cons of various methods for measuring pleiotropy, I review empirical data on pleiotropy, which generally show an L-shaped distribution of the degree of pleiotropy (i.e., the number of traits affected), with most genes having low pleiotropy. I then review the current understanding of the molecular basis of pleiotropy. In the rest of the review, I discuss evolutionary consequences of pleiotropy, focusing on advances in topics including the cost of complexity, regulatory versus coding evolution, environmental pleiotropy and adaptation, evolution of ageing and other seemingly harmful traits, and evolutionary resolution of pleiotropy.

1. INTRODUCTION

Pleiotropy refers to the phenomenon of one gene or one mutation affecting multiple traits. For example, mutations causing cystic fibrosis, primarily a lung disease, also cause male infertility (Sokol 2001). The phenomenon of pleiotropy had been recognized long before its formal definition in 1910 by the German geneticist Ludwig Plate (Plate 1910). For instance, some medical syndromes were known to have a suite of symptoms and a simple familial component (Pyeritz 1989, Stearns 2010). Another example is in the foundational paper of genetics in which Gregor Mendel reported the cosegregation of three traits in garden peas—brown seed coat, violet flowers, and axial spots versus white seed coat, white flowers, and no spots—and considered them to be controlled by a single locus (Mendel 1866). For two reasons, pleiotropy is commonly thought to be widespread. First, any organism has many more phenotypic traits than genes, so at least some genes are pleiotropic. Second, given the high prevalence of interactions among various parts of an organism and interactions among genes/mutations (Tyler et al. 2009, Zhang 2017), it is extremely unlikely that a mutation would affect only one trait.

Pleiotropy has significant implications for many areas of biology and is frequently invoked in theories and explanations of a broad spectrum of phenomena, including genetic disease (Carter & Nguyen 2011, Byars & Voskarides 2020), cancer (Rodier et al. 2007, Bien & Peters 2019), senescence (Williams 1957, Promislow 2004, Flatt & Partridge 2018), between-trait trade-offs (Guillaume & Otto 2012, Yang et al. 2014, Burmeister et al. 2020, Mauro & Ghalambor 2020), sexual conflict (Rice 1992, Bonduriansky & Chenoweth 2009, Innocenti & Morrow 2010), generalism versus specialism (Jerison et al. 2020, Bakerlee et al. 2021), cooperation (Foster et al. 2004, Bentley et al. 2022), evolutionary constraints on genes or traits (Barton 1990, Waxman & Peck 1998, Carroll 2005, He & Zhang 2006, Jiang & Zhang 2020), adaptation (Fisher 1930, Orr 2000, Otto 2004), neofunctionalization (Hughes 1994, Francino 2005, Bergthorsson et al. 2007, Hittinger & Carroll 2007), and speciation (Berlocher & Feder 2002, Kirkpatrick & Ravigne 2002, Rundle & Nosil 2002). For example, a mutation may improve one trait but worsen another, causing compromises among adaptations of different traits. It has been proposed that the above situation creates a cost for complexity—complex organisms are less adaptable than simple organisms because mutations are inherently more pleiotropic in the former than the latter (Fisher 1930, Orr 2000). Pleiotropy is the main theoretical reason behind the hypothesis that morphological evolution occurs more frequently via *cis*-regulatory sequence changes than protein sequence changes, because the former are thought to be less pleiotropic than the latter (Carroll 2005). It has been suggested that a number of disease mutations are maintained in the population because of their benefits to other aspects of life that are often hidden (Carter & Nguyen 2011).

For a long time, theoreticians have assumed universal pleiotropy, a notion that every gene/mutation affects every trait, despite the availability of only limited data on pleiotropy. Thanks to the development of high-throughput biology, the last two decades have witnessed a rapid accumulation of empirical data that allow the assessment of general patterns and various evolutionary consequences of pleiotropy. Here, I first review classifications of pleiotropy, conceptual and operational difficulties in measuring pleiotropy, methods for quantifying pleiotropy, empirical patterns of pleiotropy, the molecular basis of pleiotropy, and causal relationships among multiple effects of a mutation. I then review our understanding of some important evolutionary consequences of pleiotropy and discuss outstanding questions.

2. CLASSIFICATION OF PLEIOTROPY

Strictly speaking, pleiotropy is a characteristic of mutations. However, when the mutation of interest is a null mutation of a gene, which abolishes all functions of the gene, the mutational pleiotropy is also known as gene pleiotropy, a property of genes.

German biologist Hans Grüneberg was among the first to study the mechanistic basis of pleiotropy when researching a genetic skeletal abnormality in rats in the 1930s. He divided pleiotropy into genuine and spurious; genuine pleiotropy results from multiple products of a single gene locus, whereas spurious pleiotropy is caused by a single product that is used in multiple ways or initiates a series of events with multiple phenotypic effects (Grüneberg 1938). While Grüneberg's genuine and spurious categories are both considered true biological pleiotropy today, attempts to distinguish types of pleiotropy by different underlying causes have not stopped.

He & Zhang (2006) classified gene pleiotropy based on whether it is conferred by multiple molecular functions of the gene product (type I pleiotropy) or by multiple phenotypic consequences of a single molecular function (type II pleiotropy). A typical example of type I pleiotropy involves the mammalian serum albumin, which binds to fatty acids and toxic metabolites but is also involved in the oxidation of nitric oxide (Rafikova et al. 2002), a clear indication of multiple molecular functions. By contrast, the yeast gene HIS7 exhibits type II pleiotropy, because its product glutamine amidotransferase uses a single catalytic activity in both histidine biosynthesis and purine nucleotide monophosphate biosynthesis. Type I and type II pleiotropy loosely correspond to genuine and spurious pleiotropy, respectively, although He and Zhang emphasized the number of molecular functions of the gene product, while Grüneberg emphasized the number of gene products.

Hadorn (1961) distinguished mosaic from relational pleiotropy. Mosaic pleiotropy arises when a single locus separately affects multiple phenotypic traits, while relational pleiotropy arises when a single locus starts off a cascade of events influencing multiple traits, similar to Grüneberg's second form of spurious pleiotropy. Mosaic and relational pleiotropy were subsequently renamed horizontal and vertical pleiotropy, respectively (Tyler et al. 2009), two terms that are now commonly used in human genetics. For example, mutations truncating the human αB-crystallin (*CryAB*) gene are associated with both cataracts (Liu et al. 2006) and dilated cardiomyopathy (Inagaki et al. 2006) because the gene is expressed in both the eye and heart and performs apparently unrelated functions in the two organs; these mutations are said to exhibit horizontal pleiotropy. By contrast, the sickle cell mutation in the β-globin gene causes hemoglobin to polymerize when deoxygenated, deforming red blood cells into the sickle shape, which hinders red blood cells from flowing in capillaries and causes ischemia in organs and peripheral tissues (Tyler et al. 2009). This series of phenotypic effects follow directly from one to the next, so the sickle cell mutation is said to exhibit vertical pleiotropy.

A mutation exhibits synergistic or concordant pleiotropy if its phenotypic effects on two traits are in the same direction (typically in terms of fitness or its proxies) and antagonistic pleiotropy if they are in opposite directions. For example, a mutation that accelerates the sexual maturation of an animal but reduces its fecundity is antagonistically pleiotropic.

The same phenotypic trait measured in different environments is often regarded as different traits, especially in microbial studies (Dudley et al. 2005). Hence, a mutation with an effect on a trait in multiple environments can be considered pleiotropic, and such pleiotropy is referred to as environmental pleiotropy. Environmental pleiotropy is related to but does not equal gene by environment interaction (G×E), which refers to the phenomenon of a mutation exerting different effects on a trait in different environments (Wei & Zhang 2017, Li & Zhang 2018). Antagonistic environmental pleiotropy means that the mutational effects are in opposite directions in two environments, so antagonistic environmental pleiotropy is by definition G×E. By contrast, with concordant environmental pleiotropy, the mutational effects in two environments may or may not differ so may or may not exhibit G×E. Similarly, G×E may or may not imply environmental pleiotropy because the mutational effect could be nonzero in both environments or nonzero in only one environment.

When the phenotypic variations of two traits among genotypes are genetically mapped to the same genomic region, it is usually unknown (due to limited mapping resolutions) whether these variations are both caused by the same genetic polymorphism. In such cases, the genomic region is considered to exhibit statistical pleiotropy, which may or may not represent biological pleiotropy, depending on whether the multiple phenotypic effects are caused by the same polymorphism or not (Wagner & Zhang 2011, Hackinger & Zeggini 2017, Watanabe et al. 2019). In this review, unless otherwise mentioned, pleiotropy refers to biological pleiotropy.

In addition, because mutational effects may be environment dependent, mutational pleiotropy (excluding environmental pleiotropy) can also be environment dependent. For example, whether a mutation has concordant or antagonistic pleiotropic effects on the maximum growth rate and carrying capacity of yeast's logistic population growth is influenced by the growth medium (Wei & Zhang 2019).

3. QUANTIFYING PLEIOTROPY

3.1. Conceptual Difficulties

A phenotypic trait is an observable characteristic of an organism such as the height, weight, eye color, blood pressure, ABO blood type, oxygen saturation, mRNA concentration of a particular gene in a particular tissue, educational attainment, or personality of a human. An organism has almost an infinite number of traits, although many traits are correlated with one another, such as the lengths of the left and right arms of a human. In the most extreme case, two traits may be perfectly correlated (e.g., the height and twice the height of a tree). To quantify the pleiotropy of a mutation, we must measure the phenotypic effects of the mutation on multiple traits. What traits should be measured and counted when quantifying pleiotropy? Obviously, the height and twice the height of a tree should not be regarded as two traits in quantifying pleiotropy, but how about other traits that are highly correlated with one another without the correlation being as obvious as in the above example (e.g., the tree height and a complex mathematical function of the tree height)? Because of the above difficulty, pleiotropy is often said to be the phenomenon of a mutation affecting multiple seemingly unrelated traits, but what exactly are seemingly unrelated traits? Are the lengths of the left and right arms of a human seemingly related? What about the lengths of the left arm and left leg (or right leg)—are they seemingly related or not? Different people would have different answers. Because the correlation between traits can be anywhere from -1 to 1, it is difficult to objectively define whether two traits are seemingly unrelated. In practice, when quantifying pleiotropy, researchers generally include all traits measured, excluding those with a correlation of 1 or -1 (Wagner & Zhang 2011).

3.2. Operational Difficulties

Two operational difficulties in quantifying pleiotropy exist. First, there is no way to phenotype all traits of an organism, so it is impossible to quantify the pleiotropy of any mutation comprehensively. As a result, all estimates of pleiotropy are based on a limited set of traits. Furthermore, the traits examined in a mutant are often related to the trait initially found to be impacted by the mutation. For example, a heart-disease-causing mutation tends to be examined for potential effects on the heart or cardiovascular system. Pleiotropy estimated from such data is typically not comparable across mutations. Alternatively, a fixed set of traits may be investigated in many mutants, usually because these traits are relatively easy to phenotype. As a result, the estimated pleiotropy is commonly considered comparable for the involved mutations. However, a mutation may have detectable effects only on a group of related traits, for example, pertaining to a specific

organ, so inclusion/exclusion of this group of traits in phenotyping drastically alters the estimated pleiotropy of the mutation. Because the traits phenotyped are usually such a small fraction of all traits, caution is needed in comparing pleiotropy among mutations.

Second, the phenotypic effect of a mutation on a trait may be too small to detect. Measuring the mutational effect on a trait means comparing the phenotypic trait between two genotypes that differ only by the mutation of interest. All measurements have errors; if the magnitude of a mutational effect is smaller than that of the measurement error, the mutational effect cannot be detected, causing an underestimation of pleiotropy. How sensitive need phenotyping methods be? Ideally, if we are concerned with the evolutionary fate of a mutation, a sensitive method should be able to detect a phenotypic effect on a trait (E, measured by the fractional change in trait value) that has a fitness effect (s) whose absolute value exceeds the magnitude of genetic drift, because the fate of the mutation would be influenced by natural selection arising from this phenotypic effect. The magnitude of genetic drift is one divided by the effective population size (N_e), which is $\sim 10^5$ for vertebrates, $\sim 10^6$ for invertebrates and land plants, $\sim 10^7$ for unicellular eukaryotes, and 10^8 or greater for free-living prokaryotes (Lynch et al. 2011). Let us assume that, when $|E|$ is small, $|s| = k|E|$, where k is a trait-specific parameter that may be referred to as trait importance (Ho et al. 2017); k is 100 times the fitness effect caused by a 1% change in trait value. Therefore, a sensitive phenotyping method should be able to detect a phenotypic effect size of $1/(kN_e)$. The greater the product of trait importance and effective population size, the higher the demand for phenotyping sensitivity. Phenotyping sensitivity often cannot reach $1/(kN_e)$, causing an underestimation of pleiotropy. For example, when $k = 0.1$ and $N_e = 10^6$, $1/(kN_e) = 0.001\%$, but almost no phenotyping can detect an effect equal to 0.001% of the wild-type trait value. However, when k is very small (e.g., 10^{-4}), a detected phenotypic effect (e.g., $E = 10^{-2}$) may not have a fitness effect exceeding $1/N_e$, especially in species with relatively small N_e such as large mammals. Although pleiotropy is defined according to the number of phenotypic traits affected regardless of whether each phenotypic effect has an associated fitness effect, a phenotypic effect without a fitness effect or with a fitness effect whose size is smaller than $1/N_e$ is of minimal evolutionary importance. Hence, a statistically significant phenotypic effect may or may not be evolutionarily significant.

3.3. Forward Genetic Approaches

Pleiotropy is quantified by either forward or reverse genetic approaches. Forward genetic approaches determine the genetic loci—genes or mutations—responsible for the phenotypic variation of a trait of interest. The most common forward genetic approaches are quantitative trait locus (QTL) mapping and genome-wide association (GWA) mapping. QTL mapping analyzes the genotypes and phenotypes of relatives in a known pedigree or from a designed cross, while GWA mapping analyzes the genotypes and phenotypes of nonrelatives. In both cases, however, a locus identified to be responsible for a phenotypic variation may be causally responsible for the phenotypic variation or linked with a locus causally responsible for the phenotypic variation. When a locus is found by forward genetics to be responsible for the variations of multiple phenotypic traits, the locus is said to exhibit statistical pleiotropy. Depending on the resolution of the QTL or GWA mapping, which is determined by multiple factors such as the density of genetic markers used, recombination rate in the relevant genomic region of the species, and sample size, the distance between a causal locus and the locus identified from forward genetics may be quite large (i.e., low mapping resolution). Increasing the mapping resolution in forward genetics lowers the statistical pleiotropy of a locus, because the locus would represent fewer linked causal loci. Therefore, when comparing the level of statistical pleiotropy across loci, it is important that all loci compared are subject to the same set of forward genetic analysis with similar mapping resolutions.

3.4. Reverse Genetic Approaches

Reverse genetic approaches determine the phenotypic consequences of known mutations and involve generating mutations and measuring their effects on a set of traits. A mutation is said to be pleiotropic when it is found to affect multiple traits. Because the phenotypic effects determined in reverse genetics are causal, the estimated pleiotropy is biological. Therefore, reverse genetics is preferred over forward genetics for estimating (biological) pleiotropy. One caution is that, because the sensitivity of phenotyping varies among traits, if different mutants are phenotyped for different traits, the estimated pleiotropy may not be directly comparable among mutations. The type of mutations generated in reverse genetics is another variable to consider when comparing pleiotropy. Certain types of mutations are expected to be more pleiotropic than other types. For example, null mutations of a gene presumably abolish all functions of a gene whereas missense mutations may impact the gene function only partially. By convention, gene pleiotropy is measured by phenotyping null mutants.

4. PATTERNS OF PLEIOTROPY

4.1. Fisher's Geometric Model and Universal Pleiotropy

In an influential model of phenotypic adaptation known as the geometric model, Fisher (1930) imagined a high-dimensional space in which each dimension represents a phenotypic trait and each position in this space represents a genotype and corresponding phenotype. The relative fitness of a phenotype is determined by its distance in the space from the optimal phenotype; the smaller the distance, the higher the fitness. A mutation moves an organism from one position to another in this space. Just as a random line in a two-dimensional space is extremely unlikely to be parallel to a predefined axis, any random mutation in the high-dimensional space is expected to affect all phenotypic traits, albeit by different amounts. Therefore, the geometric model suggests universal pleiotropy—every mutation affects every trait. However, it is important to recognize that the geometric model is a toy model not expected to reflect biological realities. Furthermore, it appears that Fisher assumed that different genotypes must have different phenotypes, although at least in principle, different genotypes could have the same phenotype. Under the latter scenario, a mutation may not affect any phenotypic trait.

Nonetheless, the notion that every gene or every mutation affects every trait is often assumed in theoretical population genetics. Philosophically, this notation seems to make sense, because all traits of an organism are related to some extent as they together make up the organism; consequently, a mutation would affect all traits when it affects one trait. In the most extreme case, one could argue that a mutation from one nucleotide to another at a genomic position influences the cellular concentrations of the two nucleotides, which could have ripple effects on all traits of the organism even though the effect size is presumably exceedingly small for almost all traits. The notion of universal pleiotropy makes the statement that a particular gene or mutation affects a trait meaningless and both forward and reverse genetics futile, because every gene or mutation is already assumed to affect every trait. More fundamentally, universal pleiotropy is unfalsifiable and therefore not a scientific hypothesis, because one can always argue that the statistical power is insufficient whenever the mutational effect on a trait is not found to be statistically significant (Wagner & Zhang 2012).

4.2. Patterns Revealed by Forward Genetics

Statistical pleiotropy has been estimated by a number of forward genetic analyses in the last 15 years. For instance, Kenney-Hunt et al. (2008) and Wagner et al. (2008) mapped 120 QTLs

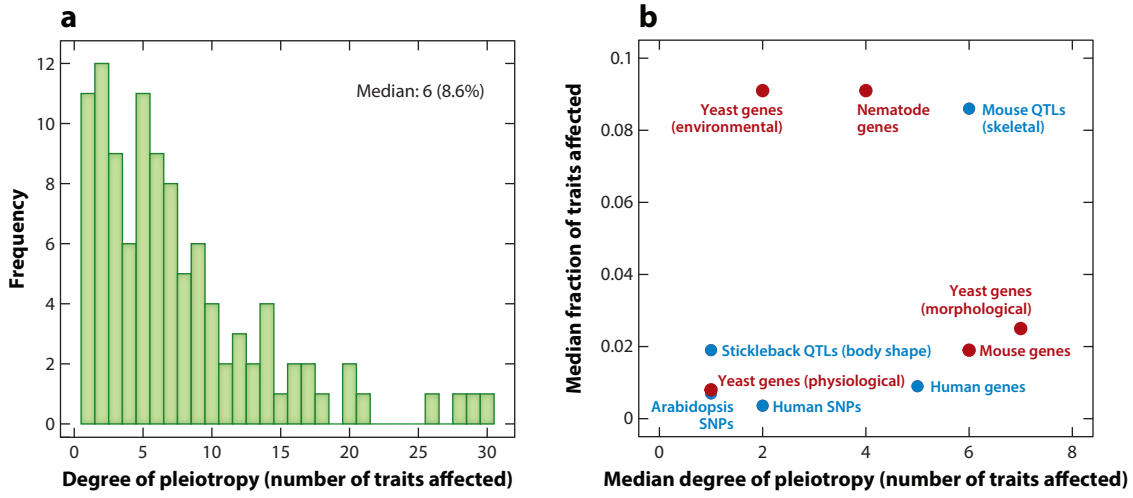

Figure 1

Frequency distribution of the degree of pleiotropy (i.e., the number of traits affected). (*a*) Distribution of pleiotropy of 120 mouse QTLs underlying 70 skeletal traits. The median degree is 6 traits, corresponding to 8.6% of all traits examined. (*b*) Median degrees of pleiotropy and median fractions of traits affected from forward and reverse genetic surveys of pleiotropy in human, mouse, stickleback, nematode, Arabidopsis, and yeast. Blue dots represent forward genetic surveys; red dots represent reverse genetic surveys. Abbreviations: QTL, quantitative trait locus; SNP, single nucleotide polymorphism. Panel *a* is based on data from Kenney-Hunt et al. (2008, table 3). Panel *b* is based on results from Albert et al. (2008), Wagner et al. (2008), Wang et al. (2010), Frachon et al. (2017), and Watanabe et al. (2019).

underlying the variations in 70 traits of the bony skeleton between inbred mice selected for either small or large body sizes. They reported that the distribution of pleiotropy is L shaped, with most QTLs affecting a few traits and a few QTLs affecting many traits (**Figure 1*a***). The median pleiotropy is six traits, or 8.6% of the traits examined. Similarly, Albert et al. (2008) mapped 26 QTLs underlying the variations in 54 position traits of the body shape between two closely related three-spined stickleback species: a Pacific marine species and a benthic lake species. Again, the distribution of pleiotropy is L shaped, with the median pleiotropy being only 1 trait, or 1.9% of the traits examined (**Figure 1*b***). By today's standard, the mapping resolution and sample size were both moderate in these two studies: The mouse study genotyped 1,040 individuals for 471 genetic markers, while the fish study genotyped 372 individuals for 248 genetic markers. A low mapping resolution due to the use of relatively few genetic markers can lead to an overestimation of (statistical) pleiotropy while a small sample size makes small effects undetectable and so leads to an underestimation of pleiotropy.

Using GWA mapping, Frachon et al. (2017) mapped single nucleotide polymorphisms (SNPs) underlying 144 eco-phenotypes in the model plant *Arabidopsis thaliana*, again reporting an L-shaped distribution of pleiotropy with a median of 1 trait (**Figure 1*b***).

GWA mapping has been extensively performed in humans for many diseases and other phenotypic variations (Welter et al. 2014, Chesmore et al. 2018). A meta-analysis of 558 traits from well-powered GWA studies (with sample sizes exceeding 50,000 individuals) offered a global view of statistical pleiotropy in humans (Watanabe et al. 2019). At the gene level, the distribution of pleiotropy is L shaped (when including only genes with at least one significant association), with a median pleiotropy of 5 traits or 0.90% of the traits examined (**Figure 1*b***). Two genes, *TNXB* (encoding tenascin-X) and *ATF6B* (encoding activating transcription factor 6 beta), are the most pleiotropic genes, each affecting 123 traits. At the SNP level, the distribution of pleiotropy is

also L shaped (when including only SNPs with at least one significant association), with a median pleiotropy of 2 traits or 0.36% of the traits examined (**Figure 1*b***). The most pleiotropic SNP, located in an intron of *ATXN2* (encoding ataxin 2), affects 79 traits; despite this observation, exonic SNPs are generally more pleiotropic than intronic SNPs (Watanabe et al. 2019).

4.3. Patterns Revealed by Reverse Genetics

Wang et al. (2010) performed an analysis of gene pleiotropy using reverse genetic data from yeast, nematodes, and mice. They analyzed three data sets in the yeast *Saccharomyces cerevisiae*: (*a*) measures of 279 cellular morphological traits of single-gene-deletion strains (Ohya et al. 2005), (*b*) growth rates of single-gene-deletion strains in 22 different environments (Dudley et al. 2005), and (*c*) 120 literature-curated physiological functions of genes recorded in the Comprehensive Yeast Genome Database. In all three cases, the distribution of pleiotropy is L shaped, with the median pleiotropy equal to 7 (2% of the traits examined), 2 (9%), and 1 (1%) for the morphological, environmental, and physiological pleiotropy data sets, respectively (**Figure 1*b***). The nematode data set was based on the phenotypes of 44 early embryogenesis traits in *Caenorhabditis elegans* treated with genome-wide RNA-mediated interference (Sonnichsen et al. 2005). The mouse data set was based on the phenotypes of 308 morphological and physiological traits in gene-knockout mice recorded in Mouse Genome Informatics. In both species, the distribution of pleiotropy is approximately L shaped, with the median equal to 4 (9% of traits examined) and 6 traits (2%), respectively (**Figure 1*b***). Note that all of the above pleiotropy estimates could be underestimates as a result of a limited statistical power for detecting mutational effects (Hill & Zhang 2012, Paaby & Rockman 2013a).

The relationships between a set of genes and a set of traits can be described by a bipartite network of genes and traits, in which a link between a gene node and a trait node indicates that the gene affects the trait. A question of interest about pleiotropy is its potential modular structure (Wagner et al. 2007), where links within modules are significantly more frequent than those across modules. Indeed, the modularity is dozens of standard deviations (SDs) greater for each of the observed pleiotropy networks than for the mean of the corresponding rewired networks where gene–trait links are randomly drawn under the condition that the number of links for each gene and the number of links for each trait are unchanged (Wang et al. 2010).

4.4. Size Distribution of Mutational Effects on Multiple Traits

Although mutational pleiotropy is commonly measured by the number or fraction of traits affected by the mutation, the effect sizes also matter. Wang et al. (2010) measured the mutational effect size by Z score, which is the absolute effect size divided by the SD of the trait value among wild-type individuals. They found that the size distribution of the effects of a yeast gene deletion on 279 morphological traits is approximately normal, with the mean close to 0, for most gene deletions examined. However, the SD of the normal distribution varies substantially across different gene deletions; the greater the SD, the greater the number of traits significantly impacted by the deletion (Wang et al. 2010). This phenomenon suggests the possibility of quantifying pleiotropy by the SD, which is presumably less influenced by phenotyping sensitivity than is the degree of pleiotropy.

5. MOLECULAR BASIS OF PLEIOTROPY

First, a mutation can be pleiotropic because it affects multiple genes that overlap in their regulatory or coding sequences. Overlapping genes, commonly defined as genes that overlap in

their mRNA sequences, have been extensively documented in viral, prokaryotic, and eukaryotic genomes (Wright et al. 2022). More common than overlapping genes is the scenario where multiple genes share regulatory nucleotides that are untranscribed, such as enhancers controlling multiple linked genes, bidirectional promoters, and promoters of operons. For example, the opsin locus control region (LCR) regulates the expression of red and green opsin genes on the human X chromosome; a dysfunctional LCR can cause the loss of both red and green opsins, leading to blue cone monochromacy (Carroll et al. 2010).

Second, a mutation can be pleiotropic because it affects multiple RNA or protein products of a gene. It is common for a gene to have multiple products, for example, due to alternative splicing, RNA editing, alternative transcriptional initiations, and alternative translational initiations, although not all products necessarily have distinct functions (Zhang & Xu 2022). For instance, human *LEF1* encodes lymphoid enhancer binding factor 1, which regulates the transcription of Wingless/Integrated (Wnt)/β-catenin genes. Two different protein isoforms are produced as a result of alternative transcriptional initiations: The longer isoform recruits β-catenin to Wnt target genes, whereas the shorter isoform cannot interact with β-catenin and instead suppresses the Wnt regulation of target genes (Arce et al. 2006). Thus, a mutation occurring in the region shared by the two isoforms potentially affects both isoforms and their respective functions.

Third, a mutation can be pleiotropic because it affects one protein product that has multiple molecular functions such as the mammalian serum albumin mentioned in Section 2 (He & Zhang 2006).

Fourth, a mutation can be pleiotropic simply because a single molecular function of a protein is directly or indirectly involved in multiple biological processes. For example, a protein may have only one molecular function as a transcription factor, but this transcription factor may directly regulate many target genes. Hence, a mutation reducing the DNA-binding affinity of the transcription factor could affect the expression of multiple target genes. In addition, the resulting expression changes of the target genes could impact the expression of their target genes, causing indirect effects of the mutation concerned. For example, a natural polymorphism in an intron of the gene encoding the transcription factor FLM influences *Arabidopsis thaliana* growth and color by affecting *FLM* splicing (Hanemian et al. 2020).

He & Zhang (2006) asked whether pleiotropy is more likely to be caused by the third or fourth mechanisms listed above. They compared yeast genes with different levels of environmental pleiotropy but found them to have similar mean numbers of molecular functions. By contrast, yeast genes with higher degrees of environmental pleiotropy tend to participate in more biological processes. Further, their protein products are found in more cellular components and have more protein-interaction partners. The authors concluded that yeast gene pleiotropy is more often attributable to the same molecular function involved in multiple biological processes (type II pleiotropy) than to multiple molecular functions (type I pleiotropy).

6. CAUSAL RELATIONSHIPS AMONG MULTIPLE EFFECTS OF A MUTATION

As mentioned, pleiotropy can be classified based on whether a single mutation separately affects multiple phenotypic traits (horizontal pleiotropy) or a single mutation starts off a cascade of events that sequentially influence multiple traits (vertical pleiotropy). This classification requires delineating the relationships among the phenotypic effects of a mutation on multiple traits. For example, if a mutation increases the chance of hypertension and reduces life span, it is possible that the reduction in the mutant's life span results entirely, partly, or not at all from the mutational impact on the probability of hypertension. If we no longer find a difference in life span between

wild-type and mutant individuals after controlling for hypertension (i.e., among individuals with similar hypertension status), we can conclude that the mutational effect on life span is entirely due to its effect on hypertension (i.e., vertical pleiotropy). Alternatively, if the life span difference between wild-type and mutant individuals remains unchanged after controlling for hypertension, we can conclude that the mutational effect on life span is not due to its effect on hypertension (i.e., horizontal pleiotropy). It is also possible that the life span difference between wild-type and mutant individuals is reduced (but not to zero) after controlling for hypertension, suggesting that the pleiotropy has both horizontal and vertical components.

Note that distinguishing between horizontal and vertical pleiotropy requires finding the relationships among the mutational effects on multiple traits, not the relationships among these traits per se, as analyzed recently (Geiler-Samerotte et al. 2020). In the context of the above example, the relationship between hypertension and life span is not the same as the relationship between the effects of a mutation on hypertension and life span, which can be different for different mutations. While it remains generally difficult to distinguish between horizontal and vertical pleiotropy, progress is expected given the recent advancement in causal inference by statistical genetics (Pingault et al. 2018, Verbanck et al. 2018).

7. EVOLUTIONARY CONSEQUENCES OF PLEIOTROPY

7.1. Maintenance of Seemingly Deleterious Traits

Mutations causing Huntington's disease, a neurodegenerative disorder in which symptoms typically manifest after the reproductive age, also increase fecundity (Carter & Nguyen 2011). As mentioned, homozygous mutations causing cystic fibrosis also cause male infertility, but heterozygotes show higher fecundity than the wild type (Carter & Nguyen 2011). Such conflicting effects on different traits weaken the negative selection against disease mutations, cause the maintenance of these mutations in the population, and increase the disease incidence. It has been suggested that mutations causing Huntington's disease, cystic fibrosis, sickle-cell anemia, glucose-6-phosphate dehydrogenase deficiency, cancer, and many other diseases are kept in the population because of their benefits to other aspects of life such as development, fecundity, and host defense (Carter & Nguyen 2011). Note that a disease mutation could also hitchhike on linked beneficial mutations; that is, the above phenomenon extends to statistical pleiotropy. For example, mutations associated with myopia were found to be selected for in the current British population due to (presumably statistical) pleiotropic effects on reproduction (Long & Zhang 2021).

Ageing refers to a gradual deterioration of bodily functions that manifests as an increase in the death rate with age after sexual maturity. Ageing is widespread among multicellular organisms and demands an explanation, because natural selection should favor mutations conferring an extended reproductive life span (Reichard 2017). The antagonistic pleiotropy hypothesis posits that mutations contributing to ageing could be positively selected because of their pleiotropic beneficial effects early in life, such as promoting development or reproduction (Williams 1957). Indeed, a recent analysis of human genotype–phenotype data revealed a negative genetic correlation between reproduction and life span (i.e., the same or linked genetic variants have opposite effects on reproduction and life span), supporting the antagonistic pleiotropy hypothesis of ageing (Long & Zhang 2023).

7.2. Cost of Complexity in Adaptation

Orr found that the rate of adaptation of a population (U), measured by its fitness increase per generation, decreases precipitously with mutational pleiotropy (n) (Orr 2000). Because Orr assumed

universal pleiotropy under Fisher's geometric model, n is the total number of traits of the organism, which can be regarded as a measure of organismal complexity. Hence, Orr concluded that the rate of adaptation decreases with organismal complexity, a result dubbed the cost of complexity.

However, Orr made several simplifying assumptions in his formulation. First, as mentioned, empirical data suggest that a mutation typically affects only a small proportion of traits (**Figure 1**). Nevertheless, if we assume that the proportion of traits affected by a mutation is comparable between species of different levels of complexity, Orr's formula still predicts a cost of complexity, albeit a lessened one.

Second, the scaling relationship between the total size (T) of all phenotypic effects of a mutation and n can be written as $T = an^b$, where a and b are two parameters. Orr assumed that T is independent of n (i.e., $b = 0$), while some authors believe that the per trait effect size is independent of n (i.e., $b = 0.5$) (Waxman & Peck 1998). The rate at which U declines with n is much lower under $b = 0.5$ than under $b = 0$ (Wang et al. 2010). In their QTL mapping of skeletal traits of mice, however, Wagner et al. (2008) observed a greater per trait effect size with larger n (i.e., $b > 0.5$). A similar trend was evident from human GWA mapping results (Chesmore et al. 2018). This pattern was also observed for yeast morphological traits, with b estimated at 0.601 ± 0.011 (95% confidence interval) (Wang et al. 2010). Similarly, $b = 0.724 \pm 0.0035$ for a set of eco-phenotypes of *Arabidopsis thaliana* (Frachon et al. 2017). When $b > 0.5$, U no longer monotonically declines with n but is an inverted U-shaped function of n, with U reaching its maximum at an intermediate n (Wang et al. 2010). Thus, with an empirically estimated b value, complexity is much less costly than Orr's original finding and may even promote adaptation. Let n_{optimal} be the level of pleiotropy corresponding to the highest U. It is intriguing that n_{optimal} reaches the highest value when b is in a narrow range from 0.56 to 0.79, which happens to encompass the empirically observed b values (Wang et al. 2010, Frachon et al. 2017). In the *Arabidopsis* study, Frachon et al. further observed the strongest signal of positive selection on genetic variants with intermediate levels of pleiotropy, consistent with the theoretical prediction that an intermediate n yields the highest U.

7.3. Gene Pleiotropy and Evolutionary Rate

It has been hypothesized that the functional constraint of a gene rises with its level of pleiotropy (Hodgkin 1998). Indeed, compared with yeast genes with relatively low morphological or environmental pleiotropy, those with relatively high pleiotropy cause greater fitness reductions when deleted (Cooper et al. 2007) and are more likely to have detectable fly or nematode homologs (He & Zhang 2006). Furthermore, the nonsynonymous nucleotide substitution rate (d_N) of a gene decreases with gene pleiotropy (He & Zhang 2006), even after controlling for the most important d_N determinant—gene expression level (Zhang & Yang 2015).

However, when gene pleiotropy is measured by the number of protein–protein interaction (PPI) partners (N_{PPI}) that the gene has, the result is mixed. A negative correlation was initially reported between the evolutionary rate of a protein and its N_{PPI} (Fraser et al. 2002), but the correlation was later shown to be caused at least in part by a confounder—gene expression level (Jordan et al. 2003). The controversy was subsequently resolved by the discovery of two types of proteins: The first type uses the same set of residues for interacting with different partners such that the increase in N_{PPI} does not alter the fraction of functionally constrained residues, while the second type uses different residues for interacting with different partners such that the increase in N_{PPI} raises the fraction of functionally constrained residues. Consequently, pleiotropy measured by N_{PPI} constrains sequence evolution for the second but not the first type of proteins (Kim et al. 2006).

7.4. Pleiotropy and Mechanisms of Phenotypic Evolution

Based on a summary of case studies and some theoretical reasoning, Carroll proposed different genetic mechanisms for the evolution of morphological and physiological traits (Carroll 2005). Specifically, he posited that morphological evolution more often proceeds by *cis*-regulatory changes than protein functional changes, while the opposite may be true for physiological evolution. The central consideration in his hypothesis is pleiotropy. A protein functional change affects all tissues where the protein is expressed, so it could be too pleiotropic to tolerate in morphological evolution. By contrast, a regulatory change may affect only one tissue, so it could be more tolerable. While the above makes sense, Carroll did not explain why physiological evolution would be different. Regardless, Liao et al. (2010) compared mouse genes that, when knocked out, affect only morphological traits (dubbed morphogenes) and those that affect only physiological traits (dubbed physiogenes). Interestingly, they found many differences between the two groups of genes. For example, morphogenes are enriched with transcriptional regulators, while physiogenes are enriched with channels, transporters, receptors, and enzymes. Compared with physiogenes, morphogenes are significantly more pleiotropic, less tissue specific in expression, and evolve more slowly in their protein sequences but faster in tissue-expression profiles. These results support Carroll's thesis and suggest that physiological evolution can probably tolerate protein functional changes because physiogenes tend to be tissue specific in their expressions.

A change in the expression of a gene can be caused by a *cis*-effect mutation, which is on the same DNA molecule as the gene (e.g., in the promoter or enhancers of the gene), or a *trans*-effect mutation, which can be anywhere in the genome because it acts through diffusible molecules such as transcription factors. It has repeatedly been shown that the expression divergence between orthologous genes is more often attributable to *cis*-effect mutations than *trans*-effect mutations and that this trend becomes more and more prominent when the divergence between orthologs gets larger (Hill et al. 2021). These patterns are explained by the fact that *cis*-effect mutations are less pleiotropic (i.e., affect fewer downstream genes) and therefore less deleterious than *trans*-effect mutations (Vande Zande et al. 2022).

7.5. Antagonistic Pleiotropy and Evolution in Changing Environments

The environment of virtually every natural population changes over time. For a neutral mutation destined for fixation, the time from its initial appearance to its fixation takes on average $4N_e$ generations for diploids. Even for beneficial mutations destined for fixation, the expected time to fixation is $2 \ln (2N_e - 1)/s$ (Otto & Whitlock 2013), which equals ~2,000 generations under $N_e = 10^4$ and ~3,400 generations under $N_e = 10^7$ when $s = 0.01$. Because it is highly likely that the environment varies during the above time, it is important to consider the possibility of environmental pleiotropy of mutations when discussing their behaviors in a population. Of particular interest is antagonistic environmental pleiotropy, under which a mutation would be selected for under some environments but selected against under other environments. Because beneficial mutations are far less common than deleterious mutations, it is likely that any beneficial mutation observed in an environment is deleterious in most other environments. Consequently, these mutations are rarely fixed, although they contribute to the adaptation of the population to the environment in which they are beneficial. Adaptive evolution would then be underestimated by the rate of nonsynonymous substitution relative to that of synonymous substitution (d_N/d_S).

To verify the above prediction, Chen & Zhang (2020) performed yeast experimental evolution in a changing environment that rotated among five media, with a duration of 224 generations per medium, totaling 1,120 generations. The five media were chosen based on the fact that the growth rates of a set of strains tend to be negatively correlated across the five media. For comparison,

they performed experimental evolution in each of the five media separately for 1,120 generations. Because the selection intensity imposed by a medium diminishes as the population adapts to it, the average selection intensity in the constant environments over 1,120 generations should be lower than that in the changing environment where a new selection was imposed every 224 generations. Yet, by comparing the genome sequences of the progenitor and evolved strains, the authors found d_N/d_S in the changing environment to be significantly lower than the average d_N/d_S in the five constant environments. Examining the dynamics of mutations in the changing environment, the authors observed the expected patterns of antagonistic pleiotropy—allele frequencies rose quickly in one medium but dropped precipitously in the next medium.

Given that genome-wide d_N/d_S typically exceeds 1 in microbial experimental evolution performed in constant environments (Tenaillon et al. 2012, Lang et al. 2013, Kryazhimskiy et al. 2014, Tenaillon et al. 2016) and that the environment varies more frequently in nature than in these constant-environment experimental evolution scenarios, we predict that positive selection is frequent in nature. Hence, it is plausible that the observation of $d_N/d_S \ll 1$ between (even very closely related) strains sampled from natural environments (Rocha et al. 2006, Wolf et al. 2009, Maclean et al. 2017) does not reflect a lack of positive selection in nature but rather is due to antagonistic pleiotropy that conceals signals of positive selection (Chen & Zhang 2020).

7.6. Evolution of Pleiotropy

Several mechanisms are known to resolve adaptation-impeding pleiotropy. First, if the expression of a gene is beneficial in some environments or tissues but detrimental in other environments or tissues, gene regulation that promotes gene expression in the former but suppresses gene expression in the latter would be selectively favored. Such regulation reduces gene pleiotropy, because the gene no longer has an effect in the latter environments or tissues. In a yeast study, Qian et al. (2012) reported that deleting the *PRD1*7 gene from a lab strain is deleterious in ETH medium but beneficial in YPG medium, indicating that *PRD1*7 in the lab strain exhibits environmental antagonistic pleiotropy. PDR17 is a phosphatidylinositol transfer protein participating in phospholipid synthesis and transport and is involved in resistance to multiple drugs. If such pleiotropy is (at least partially) evolutionarily resolved in natural strains that experience environments similar to ETH or YPG, the expression of *PRD1*7 is expected to be lower in YPG than in ETH in these natural strains. This was indeed the case. Furthermore, *PRD1*7 expression in YPG and the ratio of *PRD1*7 expression in YPG to that in ETH are both lower in a strain adapted to YPG than in a strain adapted to ETH. Over 80% of genes tested showed signs of evolutionary resolution of antagonistic pleiotropy by gene regulation (Qian et al. 2012).

Second, Qian et al. (2012) discovered that pleiotropy can also be resolved by protein subcellular relocalization. Specifically, MIG1 is a transcription factor functioning exclusively inside the nucleus in glucose repression. Its functional allele was found to be fitter than the null allele in YPD medium, but the opposite is true in OAK medium. In the strain adapted to an environment mimicked by OAK medium, MIG1 is localized to the nucleus under YPD. However, under OAK, where MIG1 would be deleterious, MIG1 is localized to the cytoplasm and hence imposes no harm.

Third, a multi-functional protein may not be able to optimize all of its functions due to tradeoffs, and gene duplication followed by functional specialization is a well-recognized mechanism to resolve such adaptive conflicts (Hughes 1994). For example, mammalian pancreatic ribonuclease (RNASE1) is an enzyme that typically digests both single-stranded RNA (ssRNA) and double-stranded RNA (dsRNA); while the former activity is primarily used for food digestion, the latter is probably involved in host defense against RNA viruses. *RNASE1* was duplicated in an ancestor

of the leaf-eating monkey *Pygathrix nemaeus*; upon duplication, one daughter gene has retained its activity for degrading dsRNA while the other daughter gene (*RNASE1B*) has evolved an elevated activity for digesting ssRNA in the acidic small intestine of leaf monkeys but has almost completely lost its activity toward dsRNA (Zhang et al. 2002). There have been nine amino acid substitutions in the mature peptide of RNASE1B since the gene duplication, and each of them reduced its activity toward dsRNA (Zhang et al. 2002). Therefore, without gene duplication and the relaxation of the functional constraint from the activity toward dsRNA, none of the nine substitutions, some of which are necessary for the improved activity toward ssRNA (Zhang 2006), would have been allowed in RNASE1B (Zhang et al. 2002). In another example, Näsvall et al. (2012) showed in an experimental evolution setting that a gene in *Salmonella enterica* with low levels of two distinct activities was duplicated and each ancestral activity increased in one of the daughter genes in subsequent evolution. The authors found that, although it was not impossible to evolve a single generalist gene with both activities increased, the high rate of gene duplication made it more likely to evolve specialist genes in their study.

Fourth, in principle, mechanisms that generate multiple distinct products from one gene could also help resolve adaptation-impeding pleiotropy. For instance, two functions of a gene may require slightly different protein sequences, which may be accomplished by RNA editing, alternative splicing, alternative translational initiation, and so on (Zhang & Xu 2022).

Because the optimal phenotype can differ between males and females, a mutation may exhibit antagonistic pleiotropy between the two sexes. Evidence for such pleiotropy is abundant (Bonduriansky & Chenoweth 2009). For example, male fitness and female fitness tend to show negative correlations across different laboratory-constructed genotypes of *Drosophila melanogaster* (Innocenti & Morrow 2010). Several mechanisms have been found to alleviate or resolve such sexual antagonism. First, a gene that is beneficial to one sex but deleterious to the other can be differentially expressed in the two sexes (Ellegren & Parsch 2007, Mank 2009, VanKuren & Long 2018). Second, genomic imprinting, where only one parental allele is expressed in the offspring, is believed to reflect a compromise in the conflict of interest between the two parents (Moore & Haig 1991, Wilkins & Haig 2003). For example, for genes that promote embryonic growth, such as the insulin-like growth factor 2 gene in mice, the paternal copy tends to be expressed, while the maternal copy is silenced. Third, sex-specific dominance reversal—the allele beneficial to males is dominant in males but recessive in females and vice versa—can alleviate sexual antagonism (Grieshop & Arnqvist 2018). Fourth, relocating genes beneficial to males but deleterious to females to the (male-specific) Y chromosome should alleviate sexual antagonism. It is, however, debated whether the X chromosome enriches sexually antagonistic genes when compared with autosomes (Rice 1984, Mank 2009, Ruzicka & Connallon 2020, Ruzicka & Connallon 2022).

It is important to distinguish mutations that alter gene pleiotropy from those that alter the pleiotropy of future mutations. For example, a mutation that suppresses *PRD1*7 expression in YPG medium reduces the pleiotropy of *PRD1*7 and is advantageous in YPG. There are also mutations that can be called pleiotropy modifiers, which modify the pleiotropy of future mutations (Pavlicev et al. 2008). Natural selection on pleiotropy modifiers is a second-order selection, so it is expected to be very weak because the modifiers do not alter the fitness of the individuals carrying the modifiers but that of the offspring of these individuals.

8. OUTSTANDING QUESTIONS

First, as discussed, reliably quantifying pleiotropy is difficult, both conceptually and operationally. Consequently, it is debated whether the empirically measured pleiotropy reflects the truth or is an artifact of limited phenotyping sensitivity (Wagner & Zhang 2011, Hill & Zhang 2012, Wagner

& Zhang 2012, Paaby & Rockman 2013a, Paaby & Rockman 2013b, Zhang & Wagner 2013). Theoretical work is needed to determine whether pleiotropy can be reliably measured without substantially increasing phenotyping sensitivity and the number of traits examined. For example, can the SD of the distribution of the effect sizes of a mutation on a moderate number of traits (Wang et al. 2010) serve as a reliable measure of pleiotropy?

Second, as mentioned, adaptation-impeding pleiotropy may be resolved by many mechanisms such as gene duplication and gene regulation. Furthermore, at least in theory, pleiotropy modifiers that minimize the negative impact of the pleiotropy of future mutations are selectively favored. Thus, does pleiotropy constrain evolution only temporarily? Under what conditions is pleiotropy unresolvable, and to what extent does pleiotropy permanently constrain evolution? Theory shows that, when the scaling exponent $b > 0.5$, the adaptation rate U is maximized at an intermediate degree of pleiotropy. However, the pleiotropy that yields the highest U depends on several parameters, so it is unclear whether the observed pleiotropy is larger or smaller than the pleiotropy that yields the highest U. Answering this question will help determine whether there is a cost of complexity and on what type of organisms the cost is imposed.

Third, laboratory microbial evolution in a changing environment showed that antagonistic environmental pleiotropy of mutations causes underestimation of positive selection and conceals adaptive signals. How widespread is this phenomenon in nature? Answering this question will be important for estimating the relative prevalence of neutral and adaptive evolution.

Fourth, how do we reliably separate horizontal from vertical pleiotropy? If a mutation affects trait B because of its effect on trait A (vertical pleiotropy), one could minimize the mutational effect on B by prohibiting the change of A (by any means influencing A). This would not work if the mutational pleiotropy were horizontal. Hence, distinguishing vertical from horizontal pleiotropy can be important in medicine and other applications.

In summary, in less than two decades, pleiotropy research has evolved from a largely theoretical realm with little systematic, empirical data to a rapidly expanding domain with large-scale data, multiple experimental tools and model systems, and ample opportunities for empirical tests of relevant hypotheses. With these developments and the fundamental roles of pleiotropy in so many biological phenomena, pleiotropy research is poised to bring many new insights in the future.

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