

# The pleiotropic structure of the genotype–phenotype map: the evolvability of complex organisms

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**Abstract** | It was first noticed 100 years ago that mutations tend to affect more than one phenotypic characteristic, a phenomenon that was called ‘pleiotropy’. Because pleiotropy was found so frequently, the notion arose that pleiotropy is ‘universal’. However, quantitative estimates of pleiotropy have not been available until recently. These estimates show that pleiotropy is highly restricted and are more in line with the notion of variational modularity than with universal pleiotropy. This finding has major implications for the evolvability of complex organisms and the mapping of disease-causing mutations.

The multifaceted goals of genetics can be summarized as describing, understanding and utilizing the relationship between genotypes and phenotypes, or the genotype–phenotype map (GPM). There are arguably millions of traits in a complex organism such as the human, but the number of genes in the human genome is only slightly above 20,000. Inevitably, there are at least some genes that affect multiple traits. This phenomenon of one gene (or one mutation) affecting multiple traits is referred to as pleiotropy, a concept that is exactly 100 years old<sup>1</sup>. The other side of the coin is the phenomenon that one trait is controlled by multiple genes, which may be epistatic to one another<sup>2–4</sup>; these traits are known as multifactorial, or polygenic, traits. In this Review, we focus on the pleiotropic structure of the GPM, because the multifactorial structure of the GPM, in the context of human common disease, has been the subject of a number of recent reviews<sup>5–9</sup>.

Pleiotropy is a common phenomenon. For example, mutations in the human homeobox gene *ARX* cause phenotypes as diverse as ambiguous genitalia and lissencephaly (in which whole or parts of the surface of the brain appear smooth). Mutations in human *AMT*, a phosphatidylinositol-3 kinase gene, cause the symptoms of cerebellar ataxia, telangiectases (visibly dilated blood vessels on the skin or mucosal surface), immune defects and a predisposition to malignancy. Although some definitions of pleiotropy require that the affected traits are seemingly unrelated, we feel that this criterion is too subjective to be useful, and so here we adopt a more liberal definition, unless otherwise noted.

Pleiotropy is a central feature in genetics and has broad implications for evolution<sup>10–14</sup>, development<sup>15</sup>, canalization<sup>16</sup>, ageing<sup>17</sup> and disease<sup>18,19</sup>. Pleiotropy causes compromises among adaptations of different traits, because a mutation that is advantageous to one trait may be harmful to another trait. The quantitative modelling of this idea led to the so-called ‘cost of complexity’ hypothesis (BOX 1), which posits that complex organisms are inherently less evolvable or adaptable to changing environments than are simple organisms, because their mutations are more pleiotropic<sup>10–11</sup>. It is also believed that the compromises among adaptations of different traits underlie many fundamental principles and phenomena in biology, including senescence<sup>17</sup>, speciation<sup>20</sup> and cooperation<sup>21</sup>. In relation to senescence, for example, it was proposed that mutant genes that are beneficial to development and reproduction are deleterious after reproductive age, which may explain why all species have a limited lifespan<sup>17</sup>.

Despite the importance of pleiotropy, few empirical data were available on this phenomenon until a few years ago, when large data sets from several model organisms were generated by functional genomics and QTL mapping. The arrival of the data not only led to revelations of general patterns of pleiotropy, but also stimulated assessments of mathematical models of pleiotropy and their evolutionary predictions. Many findings are in sharp contrast to what has been presumed true and taught for 80 years. For instance, the model of universal pleiotropy — that every gene affects every trait — is not empirically supported.

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### Box 1 | Fisher's geometric model and the 'cost of complexity'

Concerns about how random mutations and natural selection can lead to complex organisms and adaptations are as old as evolutionary theory<sup>61</sup>. The most influential attempt to give this topic a rigorous basis is Fisher's geometric model (FGM)<sup>10</sup>, which is the framework of most research on the genetic and evolutionary consequences of pleiotropy.

Fisher used a metaphor. Consider a microscope with many knobs to adjust the lenses so that one obtains a sharp image. Now ask yourself what is the chance of obtaining an optimally functioning microscope by randomly turning the knobs on the microscope. The chances are not bad as long as the number of knobs is small, say one or two, but intuition suggests that the chances will decrease dramatically if the number of adjustable parameters (knobs) is larger than two or three. To back up this intuition, Fisher introduced a geometric metaphor, which eventually became known as the FGM.

In his model, Fisher argues that the functioning of the microscope is analogous to the fitness of an organism. The performance of the microscope depends on the state of various tunable knobs, corresponding to distances and orientations of various lenses, whereas the fitness of an organism depends on the state of various phenotypic characters such as body size, beak length and beak depth. The increase in the fitness of an organism by random mutations is then analogous to the attempt to improve the performance of a microscope through randomly changing the positions of the knobs on the microscope.

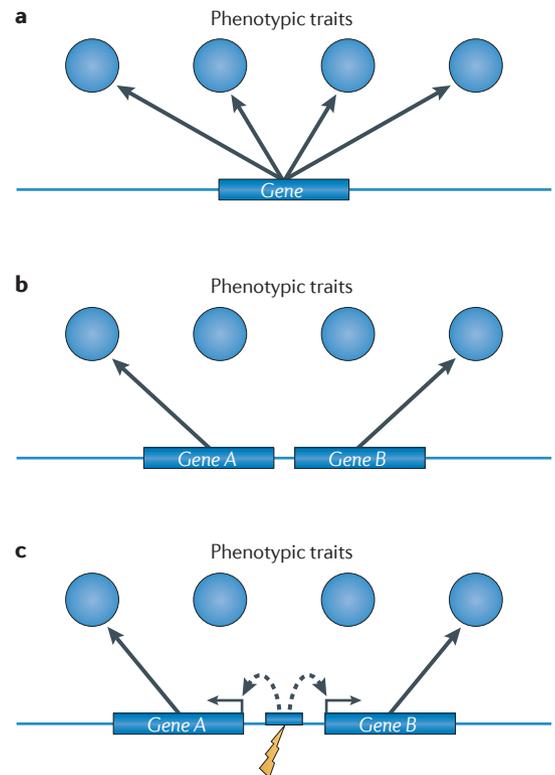
The analogy between the microscope and an evolving organism can be formalized by representing the phenotype of an organism as a point in a high-dimensional space, where the dimensions of that space correspond to the traits of the organism. The more independent dimensions of variation the phenotype has, the more difficult is improvement resulting from random changes. The reason is that, if there are many different ways to change a phenotype, it becomes very unlikely that a random mutation affects the right combination of traits in the right way to improve fitness. Fisher noted that, the smaller the effect, the higher the chance that the mutation is beneficial. At one extreme, mutations with infinitesimally small effect have a 50% chance of improving fitness. This argument led to the widely held position that evolution proceeds by small mutations.

Furthermore, Orr discovered that both the fixation probability of a beneficial mutation and the fitness gain that is conferred by the fixation of the beneficial mutation decrease with organismal complexity<sup>11</sup>. Thus, the predicted rate of adaptation decreases quickly with the rise in organismal complexity, a theoretical finding known as the 'cost of complexity'.

In this article, we review the recent literature on the measurement of pleiotropy, and describe the extent and patterns of pleiotropy. We discuss what is known about its molecular basis, how our current findings relate to the mathematical models of pleiotropy, and the implications of studies of pleiotropy for evolvability and adaptation. We argue that most genes in a genome are not highly pleiotropic, that the gene–trait relationship is strongly modular, and that pleiotropy does not hinder the evolution of complex organisms.

#### Measuring pleiotropy

The standard definition of pleiotropy is as intuitive as it is ambiguous. It simply states that a mutation is pleiotropic if it affects more than one trait or character (FIG. 1a). The degree of pleiotropy, then, is just the number of traits that are affected by a mutation, and all that seems to be required is to count those affected traits. This is a simple and intuitive definition but, for technical and conceptual reasons, it is difficult to put into scientific practice. Pleiotropy inherits all the ambiguities that exist in making both genetic and phenotypic concepts precise and operational. On the genetic side of



**Figure 1 | Possible causes of co-inheritance of phenotypic traits.** **a** | Pleiotropy. Variation at a genetic locus affects more than one trait. **b** | Two closely linked genes, each affecting one character, can lead to co-inheritance of phenotypic differences as a result of linkage disequilibrium. **c** | So-called 'artefactual' pleiotropy describes cases in which a mutation (represented by the lightning bolt) that affects a shared *cis*-regulatory element also affects the traits that are controlled by the two genes.

the genotype–phenotype relationship, the problems are largely technical. For instance, how does one distinguish between a pleiotropic mutation and two closely linked mutations (FIG. 1b)? The problems can also be conceptual. For instance, a mutation in a shared *cis*-regulatory element can affect the expression of two neighbouring genes, each affecting a separate trait (FIG. 1c). Is this pleiotropy real, or artefactual? If pleiotropy is a property of a mutation, then this second scenario would be considered to be a case of pleiotropy; if pleiotropy is a property of genes, then it would be classified as an artefact<sup>22</sup>.

On the phenotypic side, the problems are more conceptual. For instance, what exactly is a phenotypic character? Are different measures taken from the skull of a mouse really different characters, or just different attributes of the same character? Shall we measure pleiotropy directly by counting effects, or indirectly by measuring the consequences of pleiotropy on other observable variables using mathematical models? In the following two sections we discuss problems that affect both the genotypic and phenotypic characterization of pleiotropy, and we point to possible routes to resolve them.

Box 2 | Indirect methods to estimate pleiotropy

Poon and Otto<sup>51</sup> and Orr<sup>11</sup> were the first to propose using the results from Fisher's geometric model (FGM) (BOX 1) to indirectly measure the complexity or degree of pleiotropy of mutations in real organisms. For instance, Poon and Otto showed that the genetic load of a population ( $L$ ), under the FGM, should depend only on the effective population size ( $N_e$ ) and the number of dimensions (or characters, denoted  $n$ ), assuming universal pleiotropy:

$$L = \frac{n}{n + 2N_e} \quad (1)$$

Hence, measuring how the genetic load increases with decreasing effective population size allows one to estimate the effective dimensionality of the phenotype, or, more likely, the average pleiotropy of mutations. Using similar results, Tenaillon and colleagues<sup>62</sup> estimated the dimensionality of the vesicular stomatitis virus (VSV) and the bacteriophage  $\Phi$  X174. They found the effective number of dimensions to be 45 for  $\Phi$  X174 and ten for VSV. This is consistent with the ordinal difference of genome complexity, as  $\Phi$  X174 has 11 genes and VSV has five.

Another approach to estimating effective phenotypic complexity is to utilize predictions of the FGM about the distribution of fitness effects<sup>53</sup>. This method was applied to data from a wide range of organisms (from viruses to plants). However, the estimates of the effective number of characters from this method are low. For instance, they estimate an  $n$  for VSV of about 1. The reason for the discrepancy between this estimate and that obtained by Tenaillon and colleagues<sup>62</sup> is not clear. Also, the highest estimate from fitness distributions for *Caenorhabditis elegans* ( $n = 2.58$ ) seems low for a metazoan organism, if taken as an estimate of phenotypic complexity. However, if these numbers are interpreted as average pleiotropy rather than phenotypic complexity, then they do not seem to be out of the ordinary in light of the results of direct pleiotropy estimates. It could be that the methods used by Poon and Otto and by Tenaillon and colleagues are actually measuring phenotypic complexity, whereas those that use fitness effect distribution measure average pleiotropy.

Finally, Su and colleagues<sup>63</sup> used the FGM prediction of the variance in amino acid substitution rates to analyse protein evolution data from mammals<sup>64,65</sup>. These estimates of pleiotropy have a modal value of six, which is similar to the estimates from more direct methods of measuring pleiotropy.

**The genetics of pleiotropy**

Operationally, pleiotropy is detected by the co-segregation of some phenotypic differences. If two or more traits are affected, their co-segregation can be due to linkage between two loci, each of which has a mutation that affects one or a subset of the traits. There are a number of methods that test for pleiotropy in QTL data. One of the more frequently used techniques is that of Knott and Haley<sup>23</sup>. The bias that is introduced in estimating the degree of pleiotropy by failing to detect closely linked genes is an upward bias, meaning that in QTL data we will tend to overestimate pleiotropy.

**Gene knockout and gene knockdown genotypes.** An alternative and increasingly popular method of measuring pleiotropy is to use knockout genotypes in a homogeneous background<sup>24</sup>. This method avoids the problem of closely linked genes, but it assesses only mutations that lead to the complete loss of gene activity. As pointed out by David Stern<sup>25</sup>, one has to distinguish between the pleiotropy of the gene, that is, the totality of functions a gene performs, and the pleiotropy of mutations, which tends to affect only a subset of the functions of a gene. Mutational pleiotropy is arguably more relevant to evolutionary change than is total gene pleiotropy, as evolution proceeds mostly by replacing one allele by another

rather than by removing or creating an entire gene. Mutational pleiotropy tends to be smaller than gene pleiotropy (for examples, see REF. 25). Nevertheless, one can argue that the pleiotropy of knockout genotypes gives an upper limit of mutational pleiotropy, as it is plausible that a complete loss of gene activity has more widespread effects than do mutations that affect only some aspects of gene activity. The above reasoning applies mainly to knockout genotypes that are not lethal. If the knockout is lethal at a certain stage, any functions that the gene may have in later stages will remain undetected.

In recent years, functional gene knockdown experiments have also been used to estimate gene pleiotropy. The idea is that a functional knockdown will mimic the effect of a genetic knockout, at least in the stage of the life cycle in which the knockdown is carried out. Again, these estimates have to be taken as an upper limit of the pleiotropy of allele substitutions, with the additional caveat that the knockdown phenotypes are usually limited to a particular time in development. For instance, the knockdown strategy has been used to assess pleiotropy of genes that function in early nematode embryogenesis<sup>26</sup>.

**Interpreting measures of pleiotropy.** Another methodological consideration is that pleiotropy can be detected only for characters that are in fact recorded or measured in any given study. Hence, a statement such as "this gene has  $N$  pleiotropic effects" is meaningless, because  $N$  depends on the number and kind of phenotypic traits that have been recorded in a study. There are of course many and deep problems with defining the units of phenotypic variations<sup>27</sup>, as briefly discussed in the next section. Here, we only want to point out that pleiotropy is better expressed in terms of the percentage of the phenotypic characters that have been studied. This approach also makes estimates of the degree of pleiotropy more comparable between studies; however, it is not yet formally established that this is the best way to scale observed pleiotropy with the size of the study (that is, the number of characters scored). Of course, choosing an unbiased sample of traits to score is important for an accurate measure of pleiotropy.

**Detection limits.** Finally, there is the vexing problem of detection limits, which makes estimates of pleiotropy difficult to compare from study to study. This problem is particularly acute when the phenotypic traits are quantitative. Of course, the size of an effect that can be detected in any particular study depends on the experimental design (chiefly, its power) and the variance of the characters studied. Hence, it is perfectly rational, but not very useful, to say that we cannot know the pleiotropy of any mutation because there can always be more effects than a particular study can detect.

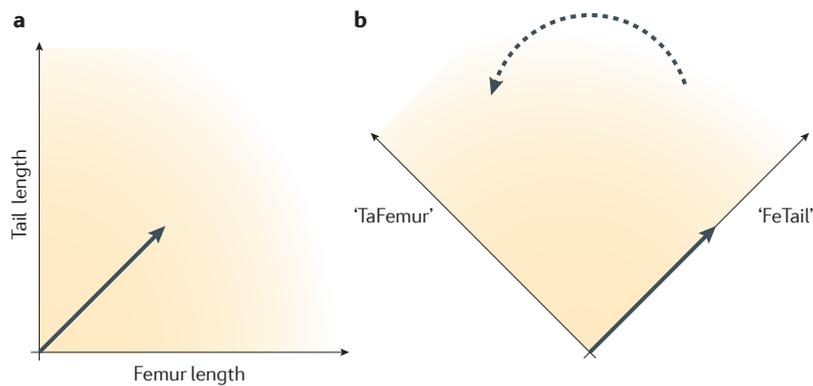
Although the above argument is correct, it is also true that, for each experimental design, the detection limit can be assessed; thus, we can be reasonably sure that any phenotypic effects we miss will be substantially smaller than the detected ones. Recent estimates of pleiotropy usually find a significant degree of pleiotropy for  $\leq 10\%$  of characters scored. Let us assume that

**Genetic load**

The difference between the mean fitness of the population and the fitness of the fittest genotype in the population. The more deleterious the mutations in a population, the lower the mean fitness and the higher the genetic load.

**Effective population size**

( $N_e$ ). A measure of the strength of random genetic drift in a population. The lower the effective population size, the stronger the genetic drift.  $N_e$  is influenced by the census population size, the breeding system, the fitness differences among individuals, the sex ratio and other factors.



**Figure 2 | The degree of pleiotropy depends on the definition of phenotypic traits.** **a** | A mutation affecting femur and tail length can be considered as having two pleiotropic effects. **b** | However, if we choose another coordinate system that is mathematically equivalent to that in part **a**, the ‘number of pleiotropic effects’ can be one. As shown in the diagram, this can occur if we choose a coordinate that coincides with the direction of the effect in phenotype space. For instance, if we calculate a trait called ‘FeTail’, which is the sum of femur length and tail length, and ‘TaFemur’, which depends on the negative femur length and the tail length, we obtain a rotated coordinate system that represents the same space as the original one. To make the idea of pleiotropic effects non-ambiguous, the traits or characters need to reflect biologically meaningful partitions of the phenotype, meaning that the distinction between traits needs to be biologically non-arbitrary.

we have a study in which 30 characters are examined, and we find significant effects for three of them, with a power of 80%; then, the likelihood that we miss 27 effects of the same or larger size by chance is less than  $10^{-19}$ . In cases in which we detect few effects and the number of characters studied is high, and in which the power of the test is reasonable, it is unlikely that the true number of above-threshold effects is much higher. Of course, this limitation introduces a downward bias in estimates of pleiotropy, but this bias tends to be small in well-designed studies.

**Revising the experimental design.** The above discussion shows that counting statistically significant effects is a pragmatically reasonable thing to do, as long as we have no better method. However, it also shows that our current approach is not entirely satisfying. For example, counting significant effects leads to results that are conditional on the experimental design and the arbitrary choice of the critical type I error rate, suggesting that it might be more meaningful to abandon measuring pleiotropy by the number of significantly affected traits and to conceptualize pleiotropy as a statement about the distribution of effect sizes across characters. Then, pleiotropy can be measured by using the effects on all traits examined, rather than just those that are significant at an arbitrary level of type I error. For instance, in the hypothetical example above, instead of counting significant effects, one may want to estimate the shape of the distribution of effect sizes of a mutation on all scored traits. This distribution was recently reported to be approximately normal, with more pleiotropic mutations having greater standard deviations<sup>28</sup>. Thus, the standard deviation of the effect size distribution

may be a natural measure of the level of pleiotropy. It would be important to develop such a measure and an appropriate sampling theory.

### The phenetics of pleiotropy

Measuring pleiotropy leads to many conceptual and methodological problems. Below, we describe the direct methods that are available to estimate pleiotropy. Indirect methods have also been developed; these methods are based on Fisher’s geometric model (BOX 1) and are briefly discussed in BOX 2.

**How do you define a phenotype?** Measuring pleiotropy raises difficult questions about what we mean by the statement that “a mutation affects more than one trait or character”. Operationally, any effect on two or more distinguishable traits can count as pleiotropy. However, this definition becomes problematic if we attempt to measure pleiotropy. To measure an attribute requires that we eliminate ambiguity about its meaning by removing arbitrary factors that influence the numerical assignment<sup>29</sup>. To illustrate the point, let us assume that we find a QTL in the mouse genome with significant effects on tail length and femur length out of 20 quantitative measurements taken from the post-cranial skeleton. This can be seen as evidence that this QTL has at least two pleiotropic effects (FIG. 2a). However, a mathematician would probably point out that this estimate of pleiotropy is arbitrary, because we can define a new coordinate system, in which one dimension is defined as ‘FeTail’ = femur length + tail length. A rotation of the coordinate axis leads to an equally valid representation of the phenotype space (FIG. 2b). The pleiotropy of our QTL can take the value of 2, or 1, or even 20, simply by changing the coordinate system.

The question raised by our mathematician friend cannot be answered by mathematics alone. At its core, the question is whether there are natural units of which the phenotype is composed<sup>27</sup>. Specifically, the question is whether, in fact, the femur and the tail are more natural units of the phenotype than is the artificial character FeTail. Intuitively, at least to biologists, it seems that femur and tail are more natural characters, that ‘carve nature at its joints’, than FeTail (FIG. 2). But is there a principled way to defend this position? And can this way of identifying natural characters, if it ever becomes available, help us to correctly count gene effects?

**Accounting for correlations among traits.** Another, related, problem is whether the effects of a gene on correlated traits can be counted as independent contributions to the degree of pleiotropy. For instance, are the depth and the width of a bird beak really two different characters? If they always change together, then perhaps the beak depth and width are two different measures of the same thing, and any mutation that affects both really has only one effect. Again, the question boils down to the problem of identifying the basic building blocks of the phenotype, if they exist. Ignoring trait correlations leads to a systematic upward bias in estimates of pleiotropy, but it also creates uncertainty as to what is the

'effective number of characters scored'. In other words, the denominator for calculating the pleiotropy percentage is uncertain.

The problem of correlated traits can, to some degree, be addressed by calculating an 'effective' number of traits, which takes the strength of the correlations among the traits into account (for example, see REF. 30). A way to measure the overall integration of the traits, on the basis of their correlations, is to consider the eigenvalue distribution of the correlation matrix, which gives one overall measure of how strongly the traits are correlated<sup>31</sup>. The more different the eigenvalues, the more integrated the traits.

An empirical approach to estimating the 'true' number of characters could be based on an analysis of mutational effects themselves. One could count two traits as (quasi-) independent if there is a mutation that dissociates the two traits, meaning that there has to be a mutation that affects one trait but not the other. This idea was first used by Antónia Monteiro and collaborators in an attempt to determine the degree of modularity among the different eye spots on the wing of the butterfly *Bicyclus anynana* using X-ray-induced mutations<sup>32</sup>. We are not aware of a study of pleiotropy that uses this correction for trait independence, but it seems to be a reasonably operational way to ensure that the traits counted are in fact quasi-independent. Of course, this method is limited by the array of mutations used to assess trait independence; thus, the number of quasi-independent characters tends to be underestimated, which therefore also leads to an underestimation of the degree of pleiotropy.

Most of the conceptual and methodological problems associated with measuring pleiotropy that are described above are seemingly solvable, even though too little effort has been expended in addressing them.

### The extent of pleiotropy

**QTL studies of morphological traits.** The extent of pleiotropy has been a subject of long-standing contention. Whereas some geneticists consider pleiotropy to be rare, others believe that every gene affects every trait<sup>10</sup>. Empirical data on pleiotropy had been scarce until recently. In 2008, a study was published in which the QTLs underlying a set of traits that represented all major subsystems of the bony skeleton were mapped in inbred mice that were selected for increased or reduced body size<sup>30</sup>. A total of 102 QTLs for 70 traits were identified. Despite the finding that the most pleiotropic QTL affects 30 traits, the median degree of pleiotropy is only six traits, or 8.6% of the traits examined. The frequency distribution of the degree of pleiotropy is L-shaped, indicating that most QTLs affect only a few traits, whereas a small number of QTLs affect many traits (FIG. 3Aa). Because the phenotypic variations in these mice resulted from strong artificial selection, one wonders whether the result applies to natural variations. Albert and colleagues mapped QTLs of 54 position traits describing body shapes of two threespine stickleback species: an ancestral Pacific marine form and a derived species that inhabits a geologically young lake<sup>33</sup>. Their finding is similar to that of the mouse study: the

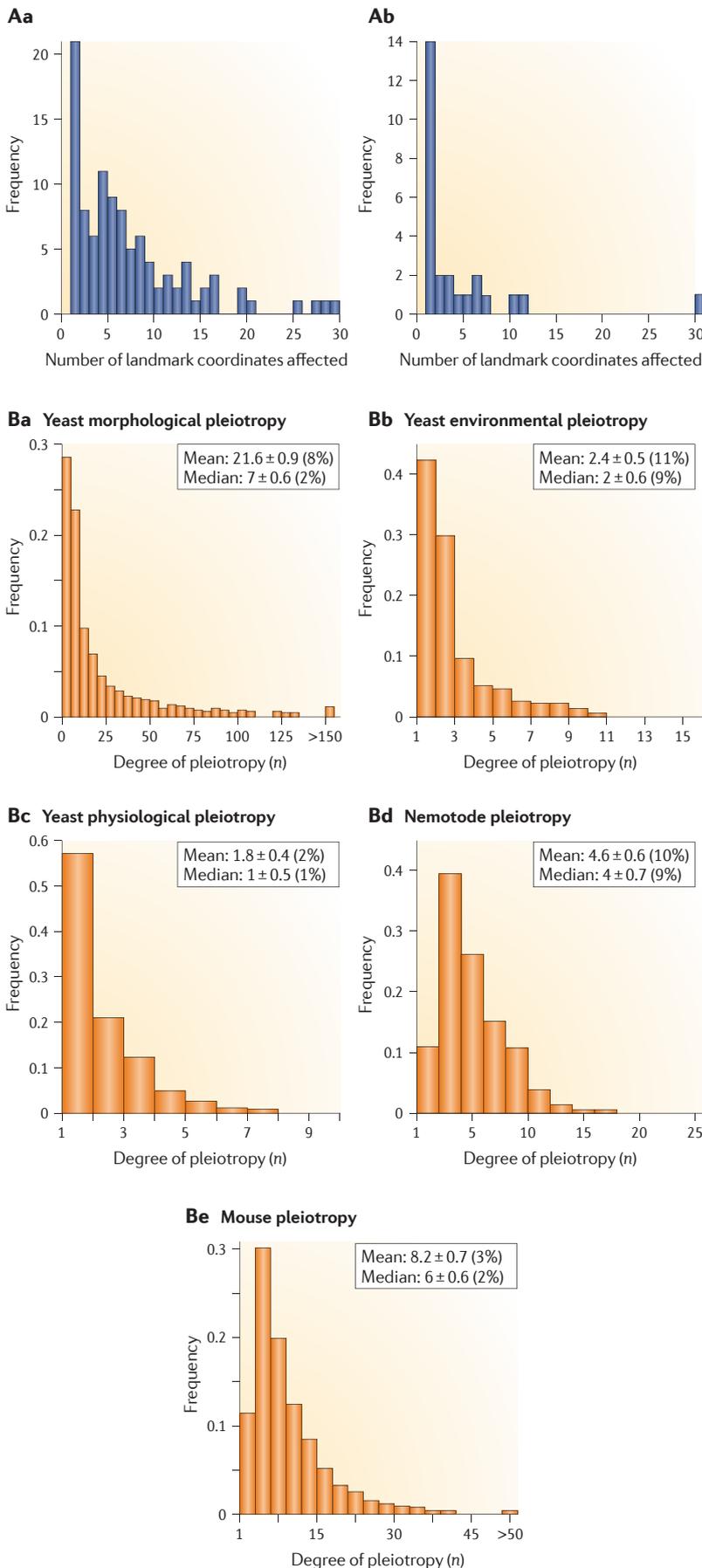
Figure 3 | **Distribution of the degree of pleiotropy.** In all cases the pleiotropy distribution is largely L-shaped, with a low median degree of pleiotropy. **A** | Distribution from QTL mapping. **Aa** | Pleiotropy of QTLs affecting skeletal traits in a mouse intercross experiment<sup>30</sup>. The median degree of pleiotropy is six out of 70 skeletal traits (8.5%). **Ab** | Pleiotropy of QTLs affecting body shape and skeletal traits in stickleback<sup>33</sup>. The median pleiotropy per QTL is only one trait out of 54 traits scored (1.9%). **B** | Distribution from gene knockout and knockdown studies. The mean and median degrees of pleiotropy and their standard deviations are shown in the inset boxes. The fraction of traits affected is given in parentheses. **Ba** | Pleiotropy of genes affecting yeast morphological traits<sup>28</sup>. A total of 2,449 genes and 253 traits are considered, after the removal of genes that do not affect any trait and traits that are not affected by any gene. **Bb** | Pleiotropy of genes affecting yeast growth in different environments<sup>28</sup>. A total of 774 genes and 22 traits are considered. **Bc** | Pleiotropy of genes affecting yeast physiological traits<sup>28</sup>. A total of 1,256 genes and 120 traits are considered. **Bd** | Pleiotropy of genes affecting nematode embryonic developmental traits<sup>28</sup>. A total of 661 genes and 44 traits are considered. **Be** | Pleiotropy of genes affecting mouse morphological and physiological traits<sup>28</sup>. A total of 4,915 genes and 308 traits are considered. Part **Aa** is modified, with permission, from REF. 30 © (2008) Macmillan Publishers Ltd. All rights reserved. Panel **Ab** is modified, with permission, from REF. 33 © (2008) John Wiley and Sons. Panels **Ba–Be** are modified, with permission, from REF. 28 © (2010) US National Academy of Sciences.

median degree of pleiotropy is only one trait, or 1.9% of the traits examined, although the most pleiotropic QTL underlies 30 traits (FIG. 3Ab).

**QTL studies of expression traits.** In addition to morphological traits, QTLs of many gene expression traits have been mapped in several model organisms. Gene expression is under the control of both *cis*-elements and *trans*-factors. A *cis*-element tends to have low pleiotropy, unless it regulates the expression of a *trans*-factor, which tends to affect the expression of many genes. Brem and colleagues identified QTLs underlying expression differences of 570 genes between a common laboratory strain and a vineyard-isolated strain of the budding yeast *Saccharomyces cerevisiae*<sup>34</sup>. They found that 32% of the expression traits showed 'self-linkage', meaning that the QTL of the expression difference of a gene is located within 10 kb of the gene. Such expression variation is likely to be caused by *cis*-element changes. After dividing the yeast genome into 661 segments of 20 kb, they found eight segments that affect 7–94 expression traits *in trans*, whereas the rest of the segments each affect zero to a few expression traits. Even when the segments that affect no trait are excluded, the median number of expression traits that is affected by a segment is only one (or 0.18% of the traits examined). In another study, the QTLs underlying the expression variations of 984 human genes in immobilized B cells were identified<sup>35</sup>. After dividing the genome into 491 segments, each with 5 Mb of DNA, the authors found

#### Eigenvalue

A characteristic parameter of a matrix. In the case of co-variance matrices, the eigenvalues are equal to the amount of variance that is associated with the principal components of the co-variance matrix.



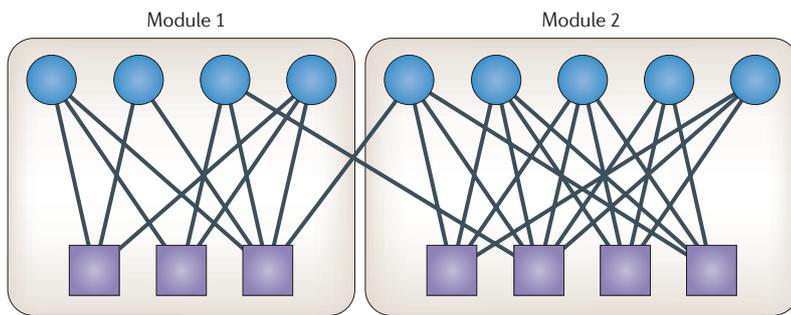
that the most pleiotropic segment affects 31 of the 984 expression traits and that the second-most pleiotropic segment affects 25 traits. However, the vast majority of the 491 segments affect no more than two traits, or 0.2% of all examined traits.

**Knockout and knockdown studies.** One drawback of QTL studies is that the mapping resolution may be so low that one QTL may include multiple genes. Consequently, the degree of pleiotropy can be overestimated.

In recent years, many genes have been either knocked out or knocked down in several model organisms. Phenotypes of these mutants provide genome-scale information about gene pleiotropy without the confounding effects of linked mutations. Wang and colleagues reported recently that the median degree of gene pleiotropy is only a few per cent of the total number of traits examined in yeast, nematode worms and mice<sup>28</sup> (FIGS 3Ba–Be). For example, 2,449 genes were found to affect at least one of the 253 morphological traits of yeast that were examined systematically. Despite the fact that the most pleiotropic gene affects more than 150 traits, the median degree of pleiotropy is only seven (or 2% of traits). One potential caveat of such a meta-analysis is that sometimes not all mutants of a species are phenotyped for the same set of traits (although this is not a problem in the yeast study above). For example, 4,915 mouse genes are known to affect one or more of 308 traits, but most mouse mutants have been phenotyped for only a small subset of these traits. Because scientists tend to phenotype traits that are suspected to be affected in a mutant, incomplete phenotyping tends to overestimate gene pleiotropy that is measured by the percentage of affected traits. Furthermore, on average, natural mutations are much milder than are knockout or knockdown effects. Thus, it is likely that the detectable number of affected traits is lower for natural mutations than for knockout or knockdown effects.

In summary, overwhelming empirical data, from unicellular eukaryotes such as yeast to complex vertebrates such as humans and mice, show that pleiotropy is generally low. Given the potential upward biases of current methods in estimating pleiotropy, the actual level of pleiotropy may be even lower. Nonetheless, all empirical measures of pleiotropy are limited by the power of the measurement, as mentioned in the previous section. Admittedly, some of the studies have limited power. For instance, we estimate that, in the yeast morphological pleiotropy data (FIG. 3Ba), a mutational effect with an expected size of one standard deviation in the wild-type distribution is detected in only 17% of cases. This makes it also possible that the actual degree of pleiotropy is greater than what has been revealed. But the unrevealed degree of pleiotropy must have much lower functional and evolutionary significance, owing to a much smaller effect size. In addition to the generally low pleiotropy, the GPM is highly modular (BOX 3) and the per-trait phenotypic effect of mutations increases with the number of pleiotropic effects (BOX 4). These findings have significant implications for our understanding of disease, development and evolution.

## Box 3 | Evidence for modular pleiotropy



It is often observed that certain groups of traits tend to co-vary among individuals of the same species or across different species, forming variational modules<sup>66</sup>. Variational modularity occurs when a set of traits are co-determined by a set of genes, a phenomenon known as modular pleiotropy<sup>48</sup>. The genotype–phenotype map (GPM) can be viewed as a bipartite network that is composed of two types of nodes: gene nodes and trait nodes. A link between a gene node and a trait node indicates that the gene affects the trait. It was recently shown in yeast, nematode worms and mice that the bipartite network of the GPM is highly modular, forming groups of traits that are co-affected by groups of genes<sup>28</sup> (see the figure; circles represent genes and squares represent traits). For instance, the modularity in the mouse gene–trait bipartite network is 238 standard deviations greater than that expected from the same network in which the gene–trait links are randomly rewired. The evolutionary origin of the modularity in the GPM is unclear, and several models, either with or without natural selection for modularity, have been proposed<sup>66</sup>. Regardless of its origin, modular pleiotropy further constrains the pleiotropic effects of mutations, because a mutation with modular pleiotropy is more likely to have smaller effects on unrelated traits than on related traits.

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### The molecular basis of pleiotropy

**Type I and type II pleiotropy.** Despite the importance of pleiotropy and its long history of study in genetics, little is known about its molecular basis. A central question in this regard is whether pleiotropy is conferred by multiple molecular functions of a gene product, which is referred to as type I pleiotropy, or by multiple morphological and physiological consequences of a single molecular function, which is referred to as type II pleiotropy. This distinction goes back to 1938, to Grüneberg, who called them ‘genuine’ and ‘spurious’ pleiotropy, respectively<sup>36</sup> — terms that, in retrospect, seem unfortunate. In the wake of the acceptance of the ‘one gene, one enzyme’ hypothesis, it was assumed that type II pleiotropy would be the most frequent, if not the only, form of pleiotropy<sup>1</sup>. However, the discovery of multiple functional domains in the same protein and the discovery of alternative splicing, which produces different molecular species from the same gene locus, reopened the issue.

An example of type I pleiotropy comes from human serum albumin, which has a crucial role in maintaining the osmotic pressure that is needed for proper distribution of body fluids between intravascular compartments and body tissues. Serum albumin also acts as a plasma carrier by nonspecifically binding several hydrophobic steroid hormones, and as a transport protein for haemin and fatty acids. Furthermore, it is involved in the oxidation of nitric oxide by binding this molecule

in its hydrophobic core<sup>37</sup>. Structural analysis revealed two distinct binding sites in serum albumin that allow its interaction with different ligands<sup>38</sup>. The yeast *HIS7* gene represents a case of type II pleiotropy. *HIS7* encodes glutamine amidotransferase, which is used in both histidine biosynthesis and purine nucleotide monophosphate biosynthesis. Thus, loss of *HIS7* leads to the shortage of multiple necessary metabolites (see the *Saccharomyces Genome Database*).

Is pleiotropy mostly of type I or type II? This question was addressed by analysing yeast gene pleiotropy<sup>39</sup>. Interestingly, no significant correlation was found between the degree of pleiotropy of a gene and the number of molecular functions of that gene. There is also no correlation between gene pleiotropy and the number of domains in a protein. Among enzyme genes, there is no correlation between pleiotropy and the number of catalytic activities of the enzyme. By contrast, there is a positive correlation between pleiotropy and the number of cellular components to which the gene product is localized. There is also a positive correlation between pleiotropy and the number of biological processes in which a gene engages. These findings indicate that, at least in yeast, pleiotropy is mostly of type II<sup>39</sup>.

### Evolutionary and disease implications of type II pleiotropy.

If we measure organismal complexity by the number of recognizably different types of cells in an organism, then this property can be said to have increased markedly from prokaryotes to advanced multicellular eukaryotes such as vertebrates and flowering plants<sup>40</sup>. However, the number of genes has increased only about fourfold<sup>41</sup>. This disparity has necessarily resulted in an increase in average gene pleiotropy during the evolution of complex organisms. The finding that pleiotropy is primarily of type II suggests that the increase in average gene pleiotropy during evolution is probably realized by the recruitment of existing genes into new biological processes (rather than the acquisition of new molecular functions), which presumably can occur, for example, by changes in tissue expression, subcellular localization and interaction partners, as well as by context-sensitive transcription.

As mentioned, pleiotropic effects of mutations underlie some human diseases. In principle, the GPM constructed from model organisms such as the laboratory mouse can guide the search for genes that, when mutated, cause human diseases. Such an approach can also aid the construction of the gene–disease map, which is also known as the diseaseome<sup>42</sup>. Conversely, the diseaseome informs us about the relationship between genes and phenotypic defects, and thus can be used for inferring the prevalence and role of pleiotropy in human disease. It is commonly believed that different alleles of a gene will exhibit pleiotropic effects on different subsets of traits<sup>43</sup>. Although this situation is possible<sup>44</sup>, if the pleiotropic effects of a gene are usually conferred by the same molecular function<sup>39</sup>, it would be difficult to isolate alleles of pleiotropic genes that affect only one trait. This consideration is relevant to human genetics, in which isolation of symptom-specific alleles is thought to be important

Box 4 | **Pleiotropic scaling of gene effects**

What is the total effect size of a mutation on all traits that are affected by the mutation? Intuitively, one may think that the total effect size of a mutation is a constant, such that mutations that affect more traits have a smaller average effect per trait. This is known as the invariant total effect model<sup>11</sup>. Alternatively, one may think that the effect size of a mutation is constant per trait, such that the total effect size rises with the number of traits that are affected by the mutation. This is known as the Euclidean superposition model<sup>12,58,67</sup>. The total effect size of a mutation can be calculated by the Euclidean distance:

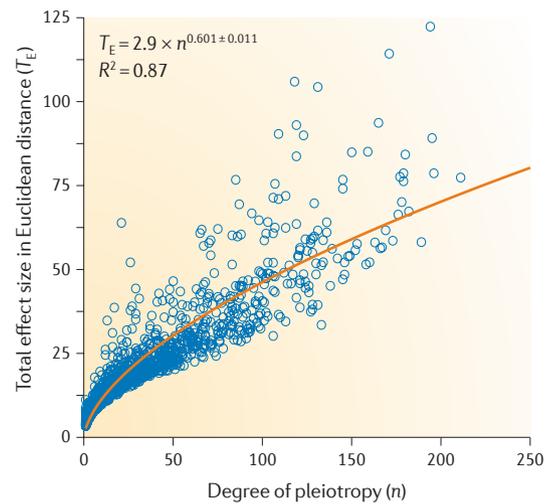
$$T_E = \sqrt{\sum_{i=1}^n Z_i^2} \quad (2)$$

where  $n$  is the mutation's degree of pleiotropy and  $Z_i$  is the mutation's effect on trait  $i$  measured by the  $Z$ -score<sup>30</sup>. If we express the scaling relationship between  $T_E$  and  $n$  by  $T_E = an^b$ , the invariant total effect model predicts  $b = 0$ , whereas the Euclidean superposition model predicts  $b = 0.5$ . Unexpectedly, it was found that  $b$  is significantly greater than 0.5 in the mouse skeletal QTL data<sup>30</sup>, suggesting that the per-trait effect size of a QTL increases with the number of traits affected by the QTL.

One important caveat, however, is that each QTL in the mouse study may include multiple genes, which could lead to an overestimation of  $b$ <sup>68</sup>. To rectify this problem, Wang and colleagues estimated  $b$  using the phenotypic data of yeast gene knockout strains<sup>28</sup>. They again observed  $b$  to be greater than 0.5, with its 95% confidence interval between 0.590 and 0.612 (see the figure;  $R$  is the correlation coefficient). Interestingly, they found that the conditions that are sufficient for  $b$  to exceed 0.5 are that the effect-size distribution of each gene is normal and that the standard deviation of the normal distribution varies among genes. They also excluded the possibility that the observation is simply an artefact of limited accuracy in measuring effect size. Thus, the counterintuitive phenomenon of  $b > 0.5$  may have a simple cause. Because the conditions for  $b > 0.5$  are easy to satisfy,  $b > 0.5$  is probably a widespread phenomenon across the tree of life.

The observation of  $b > 0.5$  means that the per-trait effect size of a mutation increases with the number of traits that are affected by the mutation. When this scaling property is considered, the cost of complexity or cost of pleiotropy is substantially alleviated, such that the adaptation rate is no longer highest for the least pleiotropic genotype–phenotype map<sup>28</sup>. The reason is that this scaling property causes a greater probability of fixation and a larger amount of fitness gain when a beneficial mutation occurs in a more pleiotropic gene than in a less pleiotropic gene. This counteracts the lower frequency of beneficial mutations that is seen in more pleiotropic genes, resulting in an interesting situation in which organisms with intermediate levels of pleiotropy are predicted to adapt most rapidly<sup>28</sup>. The pleiotropy level associated with the highest adaptation rate is maximized when  $b$  falls in a narrow range between 0.56 and 0.79, which happens to include the observed value of 0.60 from yeast<sup>28</sup>.

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for developing effective treatments<sup>24</sup>. Our current understanding of the molecular basis of pleiotropy suggests that developing drugs that target only one particular phenotypic effect of a pleiotropic gene could be futile.

Another question raised about the mechanistic nature of pleiotropy is the degree to which pleiotropy is itself genetically variable. Cheverud and collaborators have addressed this issue by developing a new QTL mapping method. This method identifies genetic factors that change the statistical relationship between two or more characters<sup>45,46</sup>. These factors have been called relationship QTLs (rQTLs). A number of studies<sup>45,46</sup> have shown that a considerable number of rQTLs can be identified in interbreeding populations of mice, suggesting that there is ample opportunity for variation in pleiotropic effects.

**The ‘cost of pleiotropy’**

The analysis of Fisher’s geometric model (FGM) of adaptation (BOX 1) predicts that the rate of adaptation should

greatly decrease with the complexity of the organism. The concept of the ‘cost of complexity’, however, should be more properly called the ‘cost of pleiotropy’, as the classical versions of the FGM assume that every mutation potentially affects all traits of an organism. This position has been called ‘universal pleiotropy’ and has been the reigning paradigm in population genetics theory<sup>43</sup>. With universal pleiotropy, the complexity of an organism, measured by the total number of quasi-independent traits, equals that of pleiotropy. However, there is an alternative model that assumes that mutations are more structured and tend to affect only subsets of characters. This model is variously known as ‘variational modularity’ or ‘restricted pleiotropy’ (REFS 47,48). Empirical evidence against universal and in favour of restricted pleiotropy, reviewed above and in BOX 3, shows that restricted pleiotropy is the predominant pattern. With restricted pleiotropy, the rate of evolution depends on the degree of pleiotropy of individual mutations rather than on the complexity of the organism<sup>49</sup>.

Of course, the FGM is in many ways a simplification. A lot of recent work has generalized the model by relaxing some or all of these assumptions<sup>50–57</sup>. The main result of these studies is that in a more general model the number of traits has a smaller effect on the rate of evolution than in the original FGM. For instance, allowing for heterogeneity in mutation effects and in selection intensities decreases the impact of the total number of traits on evolvability. This result is consistent with the perspective expressed above about the cost of pleiotropy, which states that it is the amount of pleiotropy per mutation that is important, rather than the total number of characters affected. Differences in mutational effects, statistically, lead to restricted pleiotropy, in which only a small set of characters is greatly affected by the mutation, and others much less so.

Another simplification of the FGM is that the rate of evolution is limited by the arrival of new mutations, rather than by the amount of genetic variation that segregates in a population. Models that estimate the evolvability of genetically variable populations, using quantitative genetic models, come to qualitatively different results than do those that follow the FGM mutation

accumulation approach. For instance, in quantitative genetic models, the dimensionality of the phenotype does not affect the rate of evolution<sup>58</sup>; higher levels of pleiotropy are predicted to increase the rate of evolution<sup>59</sup>, and evolvability depends only on genetic co-variance rather than pleiotropy *per se*<sup>60</sup>. It will be important to reconcile the results from these different modelling approaches.

**Conclusion: whence evolvability?**

The problem of explaining the evolvability of complex organisms has fundamentally changed because of recent advances in data acquisition and theoretical modelling. The new data show that previous estimates of the cost of complexity are fundamentally flawed, because their basic assumptions are not empirically supported. The most important differences from the traditional picture are that it is now recognized that: pleiotropy is highly restricted; the total effect size scales more than additively with the number of pleiotropic effects (BOX 4); and the rate of evolution that is predicted from the FGM is a function of pleiotropy rather than of organismal complexity (BOX 1). Now the problem is to explain how the GPM has acquired this structure.

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**Competing interests statement**

The authors declare no competing financial interests.

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