Selection and Complex Multigene Traits

Advanced article

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Phenotypic characters that display continuous variation are usually called 'quantitative traits' or 'complex traits'. Alternatively, geneticists refer to them as 'multigene traits', because the underlying genetic architecture is assumed to be polygenic. Analyses of the genetic architecture of diverse quantitative traits suggest that the number of loci (quantitative trait loci, QTLs) affecting trait variation can be very different. Moreover, experimental studies report contrasting genetic architectures, where either large-effect QTLs or small-effect QTLs explain most of the phenotypic variation. In addition, recent reports highlight the pervasiveness of epistasis. Considerable evidence, obtained with the QST–FST methodology, supports the idea that natural selection plays a key role in the evolution of complex traits. Nevertheless, the identification of a representative number of genes underlying QTLs is necessary to determine the contribution of selection, drift and gene flow for the evolution of complex traits.

Introduction

For centuries scientists have examined variation in morphology, behaviour and physiology without knowing the underlying genetic causes of this variation. In the nineteenth century, Darwin proposed that variation within populations was the raw material upon which natural selection could act. Advantageous traits would be selected by nature in the course of evolution, as breeders artificially select for beneficial characteristics in animals and plants. Despite his revolutionary conclusions, Darwin himself was completely ignorant of the bases of inheritance and variation. The advent of genetics, in the beginning of the twentieth century, brought about the opportunity to explore the relationship between phenotypic variation and genetic variability. Nowadays, we know that variation in most characters has a strong genetic component. However, we should never forget that the environment and stochastic factors also play a role in shaping phenotypic variation.

Many organismal characters exhibit continuous variation when measured in a group of individuals, as opposed to characters that can be grouped into discrete categories (**Figure 1a**). Characters that exhibit continuous variation display a wide range of phenotypes and, often, the distribution of phenotypes can be adjusted to a normal curve (**Figure 1b**). Characters that display continuous variation are sometimes called 'quantitative traits' or 'complex traits'. Alternatively, geneticists refer to them as 'multigene traits', because the underlying genetic architecture is assumed to be polygenic (i.e. determined by multiple loci). In effect, if the phenotype under scrutiny has a strong genetic basis, continuous variation can only be explained by the segregation of alleles at multiple loci. The multigenic nature of quantitative traits complicates their genetic dissection and, furthermore, imposes additional limitations for understanding the evolution of these characters. **See also**: Quantitative Genetics

Being aware of the genetic complexity of continuous characters, geneticists have tried to focus on different aspects of the problem:

1. How many loci compose the genetic architecture of a quantitative trait? In theory, character traits could be influenced by a large number of loci, as the nuclear genomes have hundreds or thousands of genes. However, genetic variation in less than 10 genes could account for the occurrence of continuous phenotypic variation. Considering the above possibilities, a new

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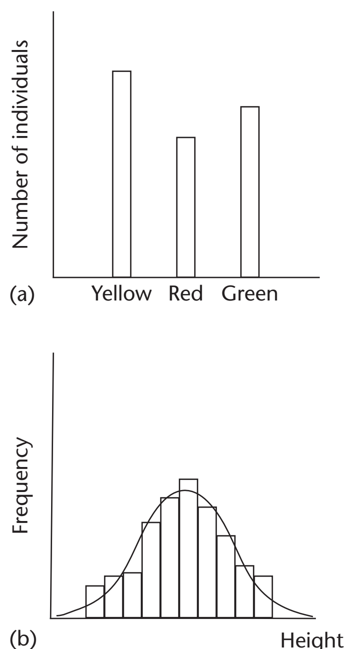

Figure 1 Types of phenotypic characters. Some phenotypic characters can be grouped into discrete categories. For example, plumage colour in a population of birds. (a) The size of the bar represents the number of individuals that have that character state in the population. In contrast, other characters (such as height) exhibit continuous variation, and individual values have to be assigned to arbitrary bins in a histogram. (b) These characters usually follow a normal distribution.

question arises: are most complex characters affected by a similar number of loci? Or, depending on the character type, could the number of loci be very different? At this point, it is important to clarify that the number of genes contributing to variation in a particular phenotype might be different from the number of genes that are involved in the production of that phenotype. This could happen if the genes needed to produce a phenotype either display variation that does not affect the phenotype or do not display genetic variation that influences the phenotype. Thus, when we search for genes that underlie the evolution of complex traits we are only looking at the loci displaying genetic variation that is expressed in the phenotype.

2. What is the magnitude and distribution of allelic effects of the loci underlying a quantitative trait? What are the genetic steps during quantitative trait evolution? Imagine an ancestral species 'A' that has a phenotypic value of 100 (measured in arbitrary units) for a hypothetical trait 'X' and a descendant species 'B' that evolved by natural selection a phenotypic value of 200 in trait 'X'. How did the increment of 100 units happen? Was it due to a 100 adaptive bouts of 1 unit or through four big leaps of 25 units? The first option fits the ideas of Fisher's infinitesimal model (Fisher, 1930) and Darwin's gradualism. Evolutionary change occurs through small changes in the phenotype. In this scenario, a large number of variable loci with small effect on the phenotype would have to exist or arise in a population. Alternatively, evolution may take larger steps from 'A' to 'B', following a 'macromutational' path (Goldschmidt, 1940). Inspired by these classical views, the issue of the number and size effects of loci involved in adaptation is still debated in the field of Evolutionary Biology (Orr, 2005). In this context, it is common to talk about 'major' and 'minor' genes, referring to those loci that have a big or small effect on the phenotype, respectively.
3. Are the effects of multiple loci strictly additive or is the phenotype the result of complex genetic interactions between loci (epistasis)? The discussion about the relevance of epistasis constitutes a long-standing controversy among geneticists (Fisher, 1930; Wright, 1931; Hill *et al*., 2008; Huang *et al*., 2012). To what extent is the effect of a particular allele dependent upon the genetic background? The latter is not a trivial question if we are to understand the evolution of phenotypes with complex genetic architectures. Consider an evolutionary transition from phenotype 'A' to phenotype 'B' that involves three novel mutations at three different loci. If mutations had completely additive effects, the order of fixation of mutations would not be important. However, if the phenotypic effect of a mutation is completely dependent on the presence of a previous one, then, the possible evolutionary paths become restricted.
4. What are the evolutionary forces acting on complex traits? It is a common assumption that strong selection shapes the evolution of complex phenotypes. But is this a fair assumption? Genetic drift and demographic events should always be considered as the possible players. Necessarily, claims about selection in natural populations should be backed up with convincing evidence.

In the following sections we describe the common techniques for genetic dissection of complex traits and discuss some experimental examples that have shed light on the architecture and evolution of complex traits. In addition, we discuss ways to uncover the evolutionary forces acting on complex traits without knowing the genetic determinants of the traits themselves.

Genetic Dissection of Complex Traits

Throughout most of the field's history, the genetic factors responsible for naturally occurring continuously varying traits were unknown. The genetic analysis consisted mainly of general statistical inferences based on phenotypic comparisons (e.g. rough estimates of the number of contributing loci and of the independence (additivity) or nonindependence (epistasis) of their interactions). Until recently, our knowledge of the genetic architecture of quantitative traits was limited to estimates of heritability or

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dominance effects and inferences of pleiotropic effects stemming from the genetic correlations between traits (Falconer and Mackay, 1996). Nowadays, technical advances allow us to explore in depth the genetic underpinnings of phenotypic variation. Geneticists try to hunt for the genes and polymorphisms that cause functional differences in complex traits within populations or between species. In this vein, a central aim of evolutionary biology is to understand how selection and drift act on these genes during evolutionary change. Therefore, it is important to comprehend how genotypic variation translates into phenotypic variation, and, also, how complex traits are shaped through interactions between genotypes and the environment. One way to study the relationship between genotype and phenotype, in terms of its components and mechanisms of change, is through the dissection of the genetic architecture of complex traits. Complex traits are affected by multiple loci whose effects are usually environmentally sensitive (Falconer and Mackay, 1996; Lynch and Walsh, 1998).

An understanding of the genetic architecture of complex traits begins with the identification and characterisation of quantitative trait loci (QTLs) (**Figure 2**). QTLs are regions of the genome that contain the gene (or genes) affecting the variation of a quantitative phenotype. Disentangling multigene characters typically involves: (1) mapping genetically varying QTLs in genomes of divergent populations or species, (2) determining if there are genetic interactions between the multiple QTLs, (3) finding candidate genes (within QTL regions) that may explain phenotypic differences and (4) pinpointing candidate molecular differences defining gene variants. Having identified genomic regions responsible for phenotypic differences, it is possible to detect the footprint of natural selection in these regions with custom-made genetic tests (Anisimova and Liberles, 2007; Zhen and Andolfatto, 2012).

Substantial progress in identifying genes involved in the formation of complex characters has been achieved through the analysis of gene function in model organisms such as *Drosophila melanogaster*, *Mus musculus*, *Arabidopsis thaliana* and *Caenorhabditis elegans* (Davis, 2004). In fact, different approaches have allowed the association of individual genes with complex phenotypes. However, as mentioned above, the functional connection between a gene and a complex phenotype does not imply that the gene in question contributes to variation or evolution of that particular phenotype. Thus, if we want to find the genes that generate phenotypic variation and contribute to evolutionary change, it is advisable to take a forward-genetics approach (i.e. crossing phenotypically different individuals of the same or different species in order to find the genetic variants that underlie the phenotypic differences).

The search for the genes that harbour naturally segregating variation affecting the quantitative traits is commonly performed through linkage QTL mapping (Lynch and Walsh, 1998). The theory of linkage QTL mapping was originally developed in model organisms (Mackay, 2001) and, motivated by direct applications, it was later extended to crops and livestock. It is also used in medicine to map disease genes. As the phenotypically divergent populations of both model organisms and crops can be inbred and crossed, simple experimental designs are used for mapping QTLs (**Figure 2**). By crossing two parental lines with divergent phenotypes, we obtain hybrid individuals (F1 generation; **Figure 2**). Later, fertile individuals of the F1 are backcrossed to one or both parents to obtain a backcross (BC1) generation (**Figure 2**). Alternatively, if both sexes of the F1 are fertile, F1 males and F1 females can be crossed to obtain an F2. More advanced segregating generations can be obtained with further crosses. For example, recombinant inbred lines can be obtained by crossing F2 individuals and successive generations (see Crow, (2007) for experimental details). Next, the phenotypes of the individuals of the mapping population (F2, BC1 or a more advanced cross) are scored. Concomitantly, the phenotyped individuals are genotyped (usually using molecular markers scattered throughout the genome). Finally, different statistical methods are used to determine if a genomic region flanked by two markers (e.g. QTL) is associated with our phenotype of interest (**Figure 2**; Falconer and Mackay, 1996; Lynch and Walsh, 1998). **See also**: Quantitative Trait Loci (QTL) Mapping; Quantitative Trait Loci (QTL) Mapping Methods

A limitation of QTL mapping is that it requires large sample sizes and high marker densities to provide a high resolution map. Usually, average genome scans detect large QTLs (typically 5–20 cM) that encompass a large number of genes (in the order of hundreds). Two general strategies are commonly adopted to identify the causal gene(s) underlying the QTL effect. The first is the candidate gene approach, inferring which genes within the QTL interval are likely to affect the trait based on previous functional knowledge. The second is positional cloning of the causal gene(s) via dissection of a QTL into smaller regions (Mackay, 2001). Additional recombinant individuals and a high density of molecular markers are required for this laborious effort. Several species-specific genetic tools can be used in combination with QTL mapping to aid in the search for the causal genes. For example, in the fruit fly *D. melanogaster* it is possible to take advantage of deficiency strains (Fanara *et al*., 2002; Cook *et al*., 2012) in order to refine QTLs. In this approach, fly strains that carry genomic deletions within the QTL region are scored for the specific phenotype. Despite the wealth of experimental options, genetic dissection of quantitative traits using QTL mapping in combination with other methodologies remains a challenging task. The power to detect the causal genes (QTGs) and causal nucleotides (QTNs) depends mostly on the rate of recombination in the QTL region, the effect size of the QTL (alleles with larger effects being easier to map), and the absence of strong epistasis between QTLs and/or minimal environmental sensitivity of the QTL. These experimental issues delay the identification of individual genes. In this context, the lack of genetic resolution limits our understanding of the evolutionary forces acting on complex traits. So far, only a few

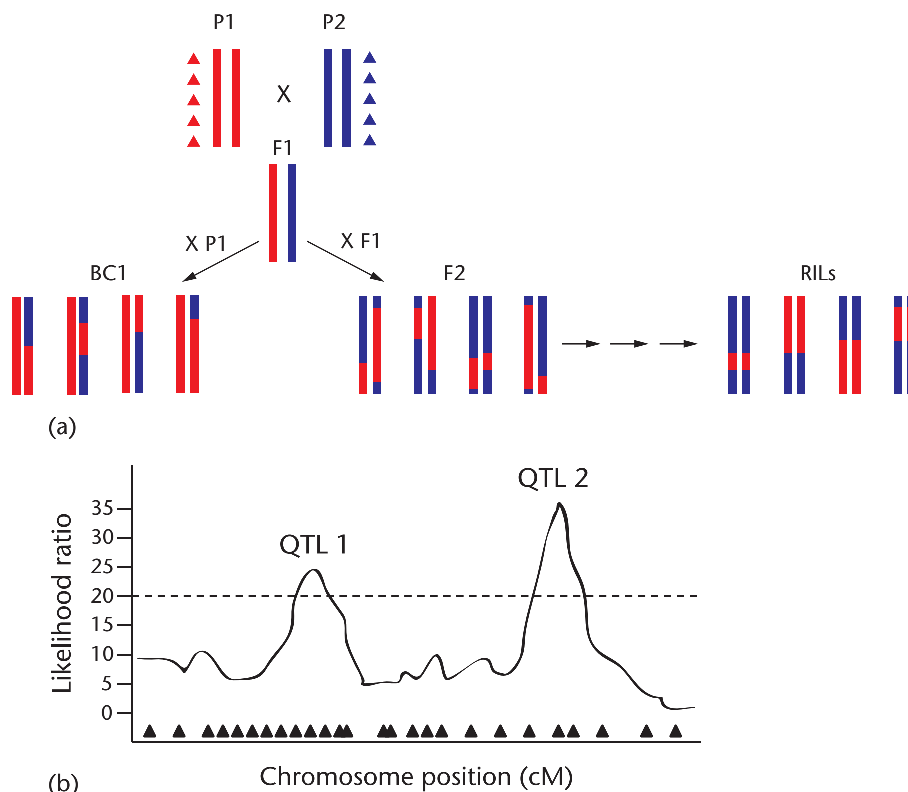

Figure 2 Quantitative trait locus mapping. (a) Two inbred parental lines (P1 and P2) are crossed to produce the F1 generation. Blue/red bars represent a pair of homologous chromosomes. Triangles indicate molecular markers specific for each parental line. F1 individuals can be crossed to P1 and/or P2 to generate a backcross (BC) mapping population or to each other to generate an F2 mapping population. Recombinant inbred lines (RILs) are generated by performing full-sibling matings for many generations. (b) Identification of QTLs by linkage. Triangles on the *x*-axis denote the locations of molecular markers. The likelihood ratio (*y*-axis) is the quotient of the likelihood of two contrasting hypotheses: (H_1) a QTL is linked to a specific marker and (H_0) there is no QTL linked to that specific marker. The horizontal dotted line is the significance threshold for the likelihood ratio based on permutation tests. Genomic regions with markers above this line contain putative QTLs. The most likely location of a QTL is the position on the *x*-axis associated with the highest likelihood value. For a detailed explanation of the likelihood ratio test and permutation tests see Lynch and Walsh (1998).

studies have detected selection acting on QTGs (Carbone *et al.*, 2006; Gerke *et al.*, 2009; Mackay *et al.*, 2012). Despite the difficulties in finding the causal genes, QTL analyses have shed light on the architecture of complex traits. **See also**: Gene Mapping and Positional Cloning

An analysis of the genetic architecture of different characters suggests that the number of QTLs contributing to a trait can be very different. As mentioned above, sample sizes affect the statistical power to detect significant QTLs and, thus, the results of different investigations cannot always be compared. However, studies that have analysed multiple traits in the same pool of segregants (i.e. they have the same statistical power for each trait) uncovered substantial variation in the number of QTLs that confer resistance to toxic chemicals (Ehrenreich *et al.*, 2010, 2012). It has also become clear that tens of loci affect adaptive traits in plants (Buckler *et al.*, 2009; Li *et al.*, 2010; Pelgas *et al.*, 2011). In contrast, the number of loci affecting phenotypic variation in dog breeds seems to be rather small (Boyko *et al.*, 2010).

The relevance of major and minor genes for character evolution constitutes a continuing debate (Hill, 2012). Different experimental studies report contrasting architectures, where major or minor QTLs explain most of the variance. An interesting example is given by a recent paper that analysed the evolution of complex traits in populations of the stickleback fish (*Gasterosteus aculeatus*) (Rogers *et al.*, 2012). All extant populations analysed in this study diverged recently from a common ancestor and adapted to new habitats. This article suggests that the effect size of QTLs fixed during adaptation to new environments is related to the phenotypic distance between the ancestral state and the present population optimum. Extant populations exhibiting phenotypes that are closer to the ancestral phenotype evolved mostly through small-effect QTLs. In contrast, populations whose phenotypes lie further from the ancestral phenotype evolved through both small-effect and large-effect QTLs (Rogers *et al.*, 2012). This experimental study strengthens the idea that, sometimes, big ‘jumps’ might move species away from the optimum phenotype (Fisher, 1930). Nevertheless, there are two problematic issues when discussing the predominance of major versus minor QTLs. First, we may not have the statistical or experimental tools needed to detect all minor

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QTLs. Consequently, there could be a bias for large-effect QTL discovery (Rockman, 2012). Second, the identity of QTGs/QTNs within QTLs is known only in very few cases. Therefore, we are almost completely ignorant about the composition of QTLs. Certainly, a single QTL with large effect may harbour several QTGs/QTNs with small effects (Frankel *et al.*, 2011). To complicate matters, Genome Wide Association Studies (GWAS, see perspectives) have revealed that statistically significant QTGs/QTNs generally explain a small proportion of the variation in complex traits (Gibson, 2010). Different hypothesis have been proposed to explain the cause of this 'missing heritability' (Gibson, 2011). **See also**: Genome-wide Association Studies; Identifying Genes Underlying Human Inherited Disease; Molecular Basis of Complex Traits

The seminal works of Fisher (1930) and Wright (1931) established different views on epistasis. Fisher believed that the genetic architecture of a trait is determined mostly by genes with additive effects, so that the phenotype is the result of the sum of the effects of each individual gene combined with environmental effects. Under this scenario, which considers an infinite population, natural selection is the primary evolutionary force that shapes phenotypic evolution (Fisher, 1930). In contrast, Wright (1931) postulated that both additive effects and epistatic interactions among genes play a fundamental role in evolution. In this model, the population is structured in small units (which occupy local adaptive peaks) with little gene flow. This model posits that evolutionary change is governed by a fine balance between natural selection and genetic drift. Recent experimental studies have highlighted the pervasiveness of epistasis (e.g. Huang *et al.*, 2012; Lorenz and Cohen, 2012), giving some support to Wright's ideas. **See also**: Epistasis; Evolution: Shifting Balance Theory

The Search for the Footprint of Natural Selection in Quantitative Traits

> "…I have encountered with dismay a number of occasions in which natural selection is invoked as a panacea to explain virtually any aspect of evolution and variation. It is easy to invent a selectionist explanation for almost any specific observation; however, proving it is another story. Such facile explanatory excesses can be avoided by being more quantitative…"
>
> (Kimura, 1983).

A major tenet of the evolutionary theory is that the pace of evolution depends on the interplay between natural selection, random genetic drift, mutation and gene flow. However, it is not always easy to unravel the relative importance of these factors for evolutionary change. Whether restriction of gene flow and random genetic drift alone are sufficient to explain phenotypic divergence or whether selection can promote divergence in the face of gene flow are still hotly debated topics.

It is generally accepted that the fate of variation in quantitative behavioural, physiological and morphological traits is governed by natural selection. Although it may be relatively simple to invent a selectionist explanation for any specific observation, it is wise to avoid verbal arguments and search for evidence that validates adaptive hypothesis. In other words, it is critical to test the hypothesis that natural selection is primarily responsible for patterns of phenotypic variation in natural populations. Thus, we would like to distinguish between natural selection and neutral processes such as genetic drift and gene flow. Tests of adaptive variation in quantitative traits should compare experimental observations with predictions of a null hypothesis – the assumption that variation is selectively neutral (Lande, 1992). The Neutral Theory of Molecular Evolution was originally developed to account for patterns of variation at the level of protein and DNA sequences (Kimura, 1983). Despite not being able to explain patterns of polymorphism and the abundance of cases of positive selection, Kimura's theory became an extremely useful null hypothesis in evolutionary genetics. A rejection of this null hypothesis is considered as a proof of natural selection at the molecular level. A similar body of theory has also been developed for quantitative traits (Lande, 1992). **See also**: Molecular Evolution: Neutral Theory; Neutrality and Selection in Molecular Evolution: Statistical Tests

A vast variety of supposedly neutral genetic markers were discovered in the past 30 years. This breakthrough boosted studies of intraspecific variation and offered new tools to evaluate the roles of gene flow and historical demographic events in evolution. These data provide a baseline measure of neutral divergence with which to compare divergence in quantitative traits of interest (Merilä and Crnokrak, 2001; McKay and Latta, 2002; Leinonen *et al.*, 2008; Whitlock, 2008). Thus, genetic divergence among populations measured by means of Wright's FST statistic (or related statistics) provides a standardised measure of the degree of population genetic structure at known single loci (e.g. microsatellites) or anonymous genomic regions (AFLPs, RAPDs, etc.). Specifically, FST and related statistics quantify the proportion of allelic variation between populations relative to total variation (within and between populations). Sewall Wright (1978) showed that the genetic variance of a quantitative polygenic trait can be partitioned into within ($\sigma^2_{g(w)}$) and between ($\sigma^2_{g(b)}$) population variance components. The ratio of between population differentiation to total additive genetic variation ($\sigma^2_{g(b)}/(\sigma^2_{g(b)} + 2\sigma^2_{g(w)})$) is known as QST. QST can be considered as an analogue of FST for quantitative trait variation. The comparison of FST and QST became one of the most popular methods employed to search for the signature of natural selection on quantitative traits. The procedure to compare these two parameters involves capturing individuals from various populations (**Figure 3**). These individuals are genotyped for neutral

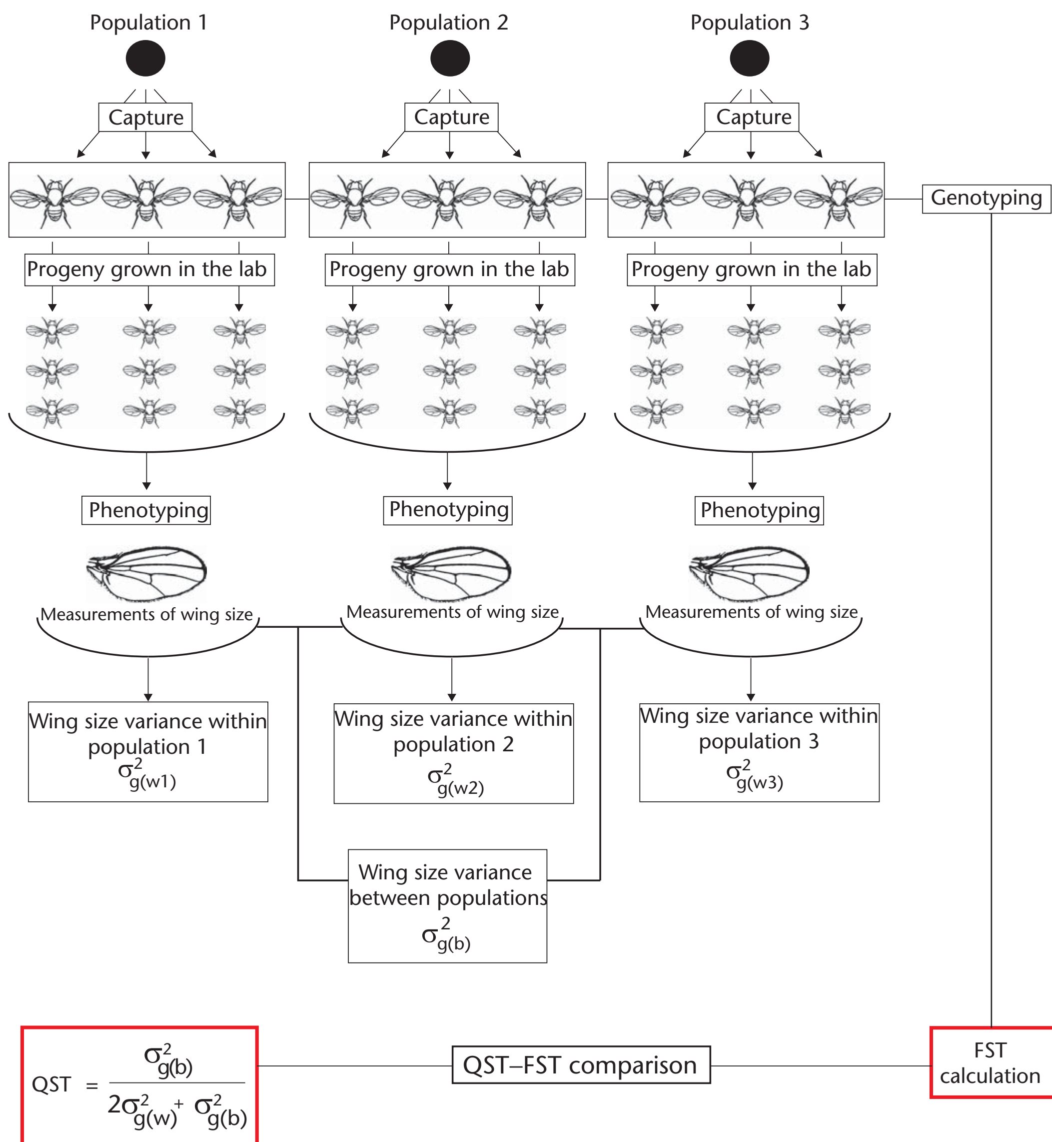

Figure 3 The workflow of the QST–FST method, illustrated with fly populations. The first step involves the capture of wild flies. The progenies of wild inseminated females are bred in controlled laboratory conditions. Wild caught females are genotyped to obtain measures of genetic differentiation within and between populations (FST) and the lab-raised progeny is analysed for a particular trait (or set of traits). In this case, wing size is measured to obtain within and between population variances. These values represent within and between population additive genetic variances. QST is calculated with these values. Finally, the evolutionary forces acting on a trait (or suite of traits) may be inferred on the basis of the results of the comparison between QST and FST.

markers (AFLPs, RAPDs, SNPs and microsatellites) to infer relatedness (FST; see **Figure 3**). The next step involves phenotyping the offspring of the wild-caught individuals in 'common garden breeding experiments' ('the laboratory population') to estimate the genetic component underlying a quantitative trait or suite of traits (QST; see **Figure 3**). As shown by Lande (1992) for the island population model and Whitlock (2008) for any population structure, QST for neutral traits is expected to be equal to FST for neutral markers (**Figure 3**). The method assumes that quantitative trait variation is under the control of genes with additive effects. Departures from the neutral expectation (QST = FST) may be considered as indications of selection on the trait. Thus, a QST < FST is usually construed as evidence of homogeneous selection across populations, or, in other words, a type of natural selection named stabilising selection. In this case, selection favours the same phenotype in all populations. Conversely, a QST > FST indicates that the quantitative variation in the different populations is influenced by heterogeneous selective pressures (i.e. different optima are being favoured in each population; Merilä and Crnokrak, 2001).

Comparative studies of within-species divergence in quantitative traits and neutral genetic markers became increasingly popular in the first decade of this century. Recent analyses of quantitative variation in a wide variety

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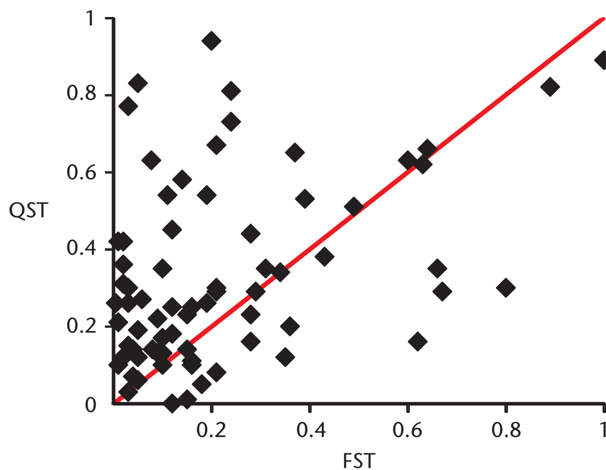

Figure 4 Experimental studies show that QST is higher than FST for most cases. Data points (black diamonds) are QST–FST values obtained for single traits or averaged across several traits. The red line marks the neutral expectation (QST = FST). Empirical data from Rogell *et al.* (2010), Chun *et al.* (2009), Leinonen *et al.* (2008), Richter-Boix *et al.* (2010), Santure *et al.* (2010) and Volis and Zhang (2010) were used to construct the graph.

of traits (morphological, life history and behavioural) and species (plants, invertebrates, vertebrates and fungi) showed that most QST values (calculated for single traits or averaged across several traits) were greater than FST (Rogell *et al.*, 2010; Chun *et al.*, 2009; Leinonen *et al.*, 2008; Richter-Boix *et al.*, 2010; Santure *et al.*, 2010; Volis and Zhang, 2010). These data, depicted in **Figure 4**, show that most of the data points are positioned above the diagonal, which represents the expected pattern under the null hypothesis of equality of QST and FST. This observation suggests not only that selection is the main force shaping variation in quantitative traits but also that differentiation between populations of the same species is shaped by different selective pressures (divergent selection). However, these conclusions have to be taken with caution, due to inherent caveats of the QST/FST method. First, most studies of quantitative trait variation investigate the relative role of drift and selection in phenotypic differentiation and, as a consequence, sampled populations and traits are very likely not randomly chosen. Studies might select populations from contrasting environments and/or traits with high degrees of differentiation (Whitlock, 2008; Leinonen *et al.*, 2008). Second, it is assumed that the traits being studied are determined by genes that interact additively both within (i.e. no dominance) and between (i.e. no epistasis) loci. Dominance and epistasis would be a major obstacle in interpreting low QSTs, since both may produce the false impression of stabilizing selection (QST < FST). Third, direct or indirect selection on presumptive neutral markers may produce extremely heterogeneous estimations of FST among loci, compromising the reliability of QST–FST comparisons. Moreover, FST can also vary across marker loci, as each genomic region has its own genealogical history. Let us imagine two populations with limited gene flow and small population size, where random fluctuations for neutral markers occur. Thus, when we measure FST at multiple loci in a given point in time, FST may be high for some loci and low for others. Such heterogeneity in FST among loci, caused by drift, is the largest source of variance in FST estimates. Finally, there might be methodological problems biasing QST estimation. For many species 'common garden experiments' needed to estimate the additive component of genetic variation are almost impracticable (Leinonen *et al.*, 2008). In these cases, quantitative divergence can be estimated from wild phenotypes, a procedure that may confuse genetic variation with environmental effects. If phenotypic divergence reflects mainly plastic responses to different environments, population divergence can be overestimated or underestimated in cases in which environmental effects reduce phenotypic variation despite high levels of genetic divergence (Leinonen *et al.*, 2008).

Before the proliferation of molecular markers, researchers used alternative methodologies to determine the evolutionary forces acting on complex traits. For example, a multivariate approach was developed based on the premise that selection is unlikely to act independently on single traits (Lande and Arnold, 1983). The multivariate approach proposes a multivariate equivalent of the breeder's equation, predicting the response to selection on multiple traits. Under neutrality, the expectation is to find a proportional relationship between within-population and among-population genetic covariance, as drift is expected to affect all additive variances and covariances by the same factor. Departures from proportionality are considered as the signature of natural selection. However, as noted by Schluter (1996), some natural selection regimes can also produce proportionality between covariances, casting doubts on the sufficiency of this methodology to disentangle the effect of drift and selection. Merilä and Bjorklund (2004) combined the multivariate approach with the QST–FST methodology to produce a multivariate extension of the classic QST–FST comparison. Using this approach, Chapuis *et al.* (2008) reported strong evidence for selection on complex phenotypes in a snail that inhabits a spatially heterogeneous environment.

Perspectives

It could be argued that phenotyping methods have not changed that much in recent years. However, it is unquestionable that technological breakthroughs allow for a better (and easier) quantitation of behaviour, physiology and morphology. However, it is the new genotyping methods that promise to revolutionise research. In fact, advances in DNA-sequencing technologies (the advent of 'Next Generation Sequencing', also known as massive parallel sequencing) and bioinformatics propelled the utilisation of new methods for identifying the genetic variation underlying complex adaptive traits (Stapley *et al.*, 2010). In particular, the power of genome wide association

studies (GWAS) to identify genes linked to complex traits has been demonstrated in flies, humans, maize (Mackay *et al*., 2012; Visscher *et al*., 2012; Tian *et al*., 2011) and other organisms. GWAS allow researchers to link genomic polymorphisms (usually single-nucleotide polymorphisms, better known as SNPs) present in populations with relevant traits. As such, GWAS are an excellent complement to linkage QTL mapping. **See also**: Next Generation Sequencing Technologies and Their Applications

Considerable evidence supports the idea that natural selection is a key player in the evolution of complex traits. Yet, the identification of a representative number of QTGs is necessary to determine the contributions of selection, drift, and gene flow for the evolution of complex traits. In this vein, a better understanding of the evolutionary history of quantitative traits may result from combining experimental methodologies such as QTL mapping, GWAS and the analysis of candidate genes in model organisms.

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