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Review

Detecting Polygenic Evolution: Problems, Pitfalls, and Promises

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Unraveling the genetic basis of organismal form and function remains one of the major goals of evolutionary biology. Theory has long supported a model of polygenic evolution in which quantitative traits are underpinned by many genes of small effect, but empirical methods have lacked the power to detect causative loci when effect sizes are small or moderate. We (i) review traditional approaches used for identifying the molecular basis of phenotypic traits, to highlight the inherent problems and pitfalls that bias them towards the detection of large-effect loci. We then (ii) outline the promises of recent statistical frameworks to detect polygenic signatures of trait evolution, and discuss some of the first studies in evolutionary biology employing these approaches. Lastly, we (iii) outline future directions and point to areas that still need development.

The Search for the Loci that Matter in Evolution

A fundamental goal in evolutionary biology is to identify the genes shaping phenotypes [1]. Achieving this goal has been anything from straightforward, however. Theoreticians have long described phenotypic evolution as a slow process that is driven by weak selection that typically extends long time-periods. The mathematical interpretation of this process is the infinitesimal model, which was introduced in 1918 by Fisher when he demonstrated that the inheritance and evolution of **quantitative traits** (see Glossary) proceeds via selection on an infinite number of unlinked and non-epistatic polygenes of small effect [2,3]. An abundance of theoretical treatments have since emerged corroborating that the majority of quantitative traits are caused by many genes of small and equal effect, suggesting that evolutionary change can be represented as a flux in allele frequency changes of these polygenes (e.g., [4–6]).

While theoretical models overwhelmingly support a model of **polygenic** evolution, the empirical demonstration of polygenes has proven difficult [7]. In the early days, the demonstration of polygenes was hampered by a lack of molecular knowledge and technologies, and it was only after 1980 that it was possible to use polymorphic marker systems [e.g., allozymes, amplified fragment length polymorphisms (AFLPs), microsatellites] to initiate the search for the genes responsible for quantitative phenotypic variation within a formalized framework [8]. Mapped loci via this framework were redubbed **quantitative trait loci** (QTLs), and thereupon became a popular research pursuit. The identification of QTLs can in principle estimate the number of genes responsible for quantitative variation and the size of their effects, but in practice the majority of current approaches carry significant problems. First, the vast majority of study designs are underpowered for detecting polygenes, and thus show an ascertainment bias towards large-effect loci [9–12]. Second, spurious QTLs and skewed effect sizes occur due to non-representative allele frequencies in the mapping population (e.g., few founders), population stratification (e.g., caused by population structure or family structure), or to low environmental

Trends

Understanding the genetic basis of organismal form and function is fundamental in evolutionary biology.

Theoretical work supports models of polygenic evolution, but years of underpowered mapping analyses have biased the literature in favor of large-effect QTLs.

The disconnect between theoretical models and empirical data is troublesome because it distorts our understanding of the molecular targets of selection.

Recent methodological advancements, and improvements in statistics and experimental designs, promise a less-biased empirical evaluation of the causal variants of phenotypic evolution.

Despite these advancements, the application of new methods has been slow, and empirical data powerful enough to genetically dissect polygenic traits are only starting to emerge.

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variance in laboratory experiments (e.g., leading to an overestimation of the genetic components) [13]. Third, many of the commonly employed experimental designs use **candidate gene** approaches that effectively target large-effect variants *a priori* [14].

In the past years, more powerful methods have been developed that can potentially overcome many pitfalls inherent in the traditional approaches. These approaches, coupled with advances in **next-generation sequencing** (NGS), allows the generation of thousands of markers in any organism, in more detail, and at lower cost than ever before [15], and hold immense promise to obtain a less-biased empirical evaluation of the causal molecular variants of phenotypic evolution. Despite this appealing promise, applications of the new polygenic frameworks are still in their infancy in evolutionary biology, but are already being increasingly applied in the fields of human medicine and agriculture [16–18]. We review here the methods available to generate genotype–phenotype maps by (i) briefly outlining the traditional approaches and discussing their underlying problems and bias towards the detection of the types of genes underlying phenotypic evolution. Then, we (ii) turn to the very recent methodological developments and statistical models that now allow a more-powerful dissection of polygenic evolution. Finally, we outline (iii) how these new developments can be applied to detect polygenic evolution in evolutionary biology, and highlight areas where conceptual uncertainties remain that require further development.

Traditional Approaches and their Problems and Pitfalls

In the pre- and early-**genomics** era, the mapping of genes underlying phenotypic traits employed different approaches that can be coarsely classified as either forward genetics ('top-down') or reverse genetics ('bottom-up'). We do not intend to provide a comprehensive review of the statistical frameworks and assumptions of the methods here, which can be found elsewhere [19–23], but instead we aim to briefly discuss their inherent biases and how these may impact on their suitability to detect genomic regions that correspond to phenotypic traits.

Forward-Genetics Approaches

Forward-genetics approaches start with the measurement of a phenotype followed by associating markers and phenotypic variation to detect causative genes or chromosome regions. The two main procedures for phenotype-driven mapping approaches are (i) **QTL mapping** analysis and (ii) **genome-wide association study** (GWAS) [13,24]. These approaches depend on the existence of a positioned genome-wide marker map, but differ in how the association to the phenotype variation is modeled. QTL mapping measures, loosely speaking, the correlation between marker and phenotype variation in experimental crosses or pedigrees with related, but phenotypically variable, individuals [23]. GWAS aims at obtaining statistical genotype–phenotype associations with physically positioned markers in a set of phenotypically variable but typically unrelated individuals (e.g., in humans [25]). Thus, the main distinction between QTL mapping and GWAS is that the former examines genotype–phenotype associations within controlled crosses or wild pedigrees, and therefore exploits recent recombination events, whereas the latter detects such associations in populations with an old history of recombination and thus with low levels of **linkage disequilibrium** (LD). As a consequence, QTL mapping has low precision but requires fewer markers (one marker every ~1–10 cM), whereas GWAS has higher precision but requires much denser marker maps.

In addition to this main distinction, the two approaches also differ in their power to detect QTLs and in their experimental design flexibility. For example, the power of QTL mapping studies ultimately relies on large families, and these can be difficult to obtain (i.e., mammals often take several years to reach sexual maturity, and then only produce a small number of recombinant offspring [26]). With small family sizes, the power of detecting small- to medium-effect QTLs is limited, which is corroborated by empirical data showing that QTL mapping studies generally

Glossary

Background selection: a process in which weakly deleterious mutations drift to low frequencies and are then purged from the population by negative selection, which causes decreased genetic diversity at linked loci in general and around conserved genes in particular.
Candidate gene: a gene of hypothesized relevance to the studied phenotype. This could be a gene involved in a pathway affecting a phenotype or a gene that has been implicated with the trait in previous studies. Sequencing the gene in individuals with divergent phenotypes can identify mutations which are associated with adaptive variation.
Genome-wide association study (GWAS): also known as association mapping, a trait-mapping approach where polymorphisms across the whole genome are screened for an association with a trait in multiple individuals. Statistical associations between genotype and phenotype only arise when the marker and the causative locus are in strong LD. It relies on historical recombination in the mapping population and has therefore relatively high precision.
Genomics: study of the function and structure of genomes.
Linkage disequilibrium (LD): non-random association of alleles at different loci (often, but not necessarily, in close genomic proximity).
Mendelian trait: a trait controlled by a single locus that is inherited according to Mendel's laws.
Next-generation sequencing (NGS): several different types of high-throughput DNA sequencing methods where hundreds of thousands or millions of reads (sequences) are produced simultaneously.
Omics: a study that targets everything of something. For example, genomics targets all genes in the genome, and transcriptomics targets all expressed gene in the genome.
Polygenic evolution: a process in which adaptation occurs by simultaneous selection operating on variants at many loci (perhaps tens or hundreds or more). A common scenario of polygenic evolution would be that there is a shift in the optimal phenotype for a quantitative trait that is affected by hundreds of alleles of

show a skewed distribution of effect sizes (where few large-effect loci account for most of the variation). Part of this large skew is related to the 'Beavis effect', which describes the upward bias in the estimated proportion of the genetic variance explained by mapped QTLs owing to small sample sizes [27]. Indeed, simulations suggest that the Beavis effect may even occur when sample size is as large as 1000 individuals [28]. This bias is related to the effect known as the 'Winner's Curse' in GWAS studies, which also leads to an overestimation of the effect size for significantly associated loci [29]. An additional problem with QTL detection in mapping studies is that, if a sufficiently high number of effective recombination events are lacking in the pedigree population, then linked QTLs are commonly grouped into single large QTLs [7]. The relative flexibility of the two approaches also differ because GWAS-based methods require no experimental crosses, meaning that the size of the sampling population, and thus the power of the sample relative to the genome size and number of groups, can be more easily controlled. The enhanced experimental flexibility of GWAS studies is somewhat counterbalanced by the need to apply a high number of **single-nucleotide polymorphisms** (SNPs; i.e., via the application of high-density genotyping platforms) such that genomic background levels of LD can be covered [30,31]. Some of these aforementioned problems can be minimized by combining these different approaches, as outlined in Box 1.

small effect. Polygenic adaptation might also occur from new mutations at many loci, followed by a shift in the optimal phenotype.

Quantitative trait: typically a phenotypic trait that is influenced by polygenic effects (two or more loci) and the environment, and can be measured quantitatively.

Quantitative trait locus (QTL): a chromosomal region containing one or more genes that underlie a quantitative trait.

Quantitative trait locus mapping: a trait-mapping approach that analyses co-variation between markers of the trait in a set of related individuals, e.g., an experimental cross or a pedigree.

Single-nucleotide polymorphism (SNP): nucleotide variation at a single sequence position in the genome.

Reverse-Genetics Approaches

Reverse-genetics approaches, such as genome scans, analyze the genomic pattern at the sequence level to infer evolutionary scenarios or genomic architectures of traits or phenotypes [11,32]. At the heart of this method lies the detection of outlier loci to uncover signatures of selection by identifying large allele-frequency changes along geographic clines or environmental gradients [33,34], or by identifying the diversity-reducing signatures of selective sweeps [35]. By screening the genome for markers that have extreme levels of differentiation (outlier loci) between species, populations, or phenotype classes, this method typically tests one locus at a time, or the average signal along a chromosomal interval [36], and can uncover loci that have evolved under relatively strong selection that have either swept to high frequency in the population or are under strong divergent selection among populations [37]. In the context of genotype–phenotype mapping, these methods thus differ from the forward genetics approaches by explicitly inferring the genomic architecture of traits from genome scans rather than directly associating genotypes and traits in statistical models.

Box 1. Combining Approaches To Minimize Problems

Both QTL mapping studies and GWAS procedures are well suited to detect and map *mono- or oligogenic* trait architectures that show a moderate to large effect (e.g., many diseases [76]). However, the moderate power of both approaches makes them much less suited to detect complex traits shaped by many minimal effects. One can combine different forward genetic approaches in a sequential fashion to harvest the advantages of each method, as illustrated in studies investigating the genomics of horn morphology in long-lived Soay sheep (*Ovis aries*, see Figure 1A,B in main text). First, QTLs were mapped through QTL analyses using a detailed, but wild, pedigree [77], and then the same region was confirmed with a GWAS [78]. The association study allowed the fine mapping of the trait and it was possible to identify the relaxin-like receptor 2 gene (*RXFP2*) as the QTL for horn morphology [78]. The *RXFP2* gene has two alleles, of which one is dominant and co-segregates with normal, large horns, whereas the other is a recessive allele and co-segregates with small and sometimes deformed horns. Interestingly, heterozygotes are favored by selection because one of the homozygous genotypes (the double recessive) has low mating success, whereas the other homozygote experiences low survival. Thus, in this system the combined action of sexual (mating) and natural (survival) selection causes a heterozygote advantage, thereby maintaining alternative alleles in the population.

It is also possible to overcome some of the aforementioned problems by combining forward and reverse-genetics approaches, such as in studies on parallel evolutionary divergence in limnetic–benthic whitefish ecotype pairs (Coregonus clupeaformis). First, eight growth-associated QTLs were mapped in families using AFLP markers, and subsequent genome scans in four sympatric pairs of whitefish then showed that two of the strongest QTLs were under the influence of directional selection and showed parallel reductions of gene flow over multiple populations [79]. More recent analyses developing and utilizing a large panel of SNPs in the same whitefish study system managed to provide evidence for both genetic parallelism and independent genetic routes underlying phenotype evolution among populations [80].

With the rise of high-throughput technologies, the use of genome scans has become a popular pursuit in the past 10 years; however, these outlier tests are not without drawbacks. All genome scan methods rely on the premise that it is possible to clearly distinguish between the genetic signals caused by non-neutral, selective processes and those signals caused by other processes such as drift. Non-neutral processes can be detected when an allele is advantageous and increases in frequency in the population. The rapid change in allele frequency reduces the opportunities for recombination in the genomic region where the allele resides, and leads to a similar reduction in mutations (since the most recent common ancestor), which in turn typically results in high levels of LD, long high-frequency haplotypes, a large proportion of commonly derived alleles, and a loss of genetic variation around the selected site(s). The interpretation of such patterns in genome scans is made complicated by the fact that several processes can produce similar patterns. Specifically, a similar genomic pattern can be produced by differences in recombination rates across the genome, where genetic drift and/or **background selection** create high differentiation in regions with low recombination rate [38]. Likewise, population stratification, family structure, or cryptic relatedness lead to the correlation of allele frequencies among demes, which affects outlier detection [39,40]. Furthermore, if selection is recent, then the time may not have been sufficient to result in the differentiation of alleles to be detectable with the (often modest) sample sizes [41]. Compared to the forward genetics approaches, genome scans involve, in principle, less bias towards large-effect loci because they do not target large phenotypic differences *a priori* but instead seek to identify any signatures of selection [42]. However, these differences between methods erode when GWAS compare more-or-less discrete phenotypic traits that segregate into, for example, two distinct classes, in which case GWAS and genome scans behave similarly.

Finally, candidate gene approaches are also grouped in the 'bottom-up' methods. Given that they typically target monogenes *a priori*, and as such induce a strong ascertainment bias towards large-effect loci. This is because the literature becomes biased towards a few well-known candidate genes, which may not be representative of their actual importance in evolutionary change. In a recent review cataloging the genetic hotspots of phenotypic variation [43], it was found that the majority of studies target well-known regions, for example, a sixth of all entries belonged to the *MC1R* candidate gene category.

Recent Promises Towards the Detection of Polygenic Evolution

It stands clear that the traditional forward- and reverse-genetics methods are struggling with several inherent shortcomings. The main problem lies in the lack of power and precision to detect polygenic selection [29,44]. This has led to a skewed literature that predominantly describes monogenic selection, which in turn has biased our general understanding of the genetic architecture of quantitative traits. The genetic mapping field is, however, increasingly aware of these shortcomings, and during the past few years concerted advances have been made at many levels. These advances include improved experimental design as well as improvements to phenotyping and genotyping methods and their associated statistical analyses. In this section we review these new advances and their potential benefits.

Improved Resolution and Experimental Design

Genetic maps are continuously being improved. Only a few years ago, most genetic maps were based on less than a hundred markers, but have since been replaced by dense maps with markers selected from millions of detected polymorphisms [45]. For example, whereas the three-spined stickleback *Gasterosteus aculeatus* linkage map in 2001 included a mere 227 informative markers, the improved genomic facilities of the species nowadays make it possible to screen several thousand SNPs [46]. Likewise, the list of genomically enabled species is rapidly and continuously growing, thereby new traits, genetic backgrounds, and evolutionary histories can be investigated [15,47]. The increased marker density and the more-elaborate maps, such as seen in the collared flycatcher *Ficedula albicollis* [48], make it also possible to scan highly-recombining regions of the

genome in species with very low levels of LD, thereby increasing the proportion of the genome that is actually screened and reducing the risk of missing causative loci [49].

Increased awareness of the Beavis effect [28] and the Winner's course [29] has made researchers aware of the importance of sufficiently large mapping panels, and the majority of designs nowadays use a sufficiently large sample size to minimize false positives (i.e., several hundred individuals). Indeed, the most data-rich association studies in medicine and animal and plant breeding are screening thousands of individuals for numerous polymorphisms. For example, a recent study investigating the highly polygenic human height trait used data from no less than 253 288 genotyped individuals [25].

It is well known that population stratification and hidden family structure in the mapping panel can cause false positives, and that this can only be overcome with a careful and controlled sampling design. However, despite increased awareness, population stratification is difficult to fully account for *a priori* and it continues to be a risk that is hard to address [25]. For example, in longitudinal studies environmental variation may change over time, thereby introducing phenotypic variance in the sample and affecting the estimate of effect sizes. Similarly, migration, drift, and family structures can be difficult to account for, particularly in species which are only little understood ecologically. Some recent genome-scan approaches have employed refined experimental designs that minimize, if not circumvent, problems associated with relatedness among individuals. In a recent study on host ecotypes of *Timema cristinae* stick insects (Figure 1C,D), where host plant-dependent natural selection causes differential mortality of phenotypes, selection was studied by quantifying allele frequencies before and after an episode of natural selection [50]. By sampling the same pool of individuals before and after a selective event, one can account for the issues associated with population stratification, relatedness, and so on. Gompert and colleagues [50] found large genome-wide allele-frequency changes that could mostly be attributed to random mortality. A similar approach was also utilized when assessing the extent of parallelism in the *Timema* ecotype system. To gain insights into the processes that may have led to this parallel genomic and phenotypic divergence, the two ecotypes were transplanted to different hosts in nature in a paired-blocks design, and the genotypic signatures before and after the experiment were measured. The experiment showed that several highly-divergent SNPs exhibited parallel divergence between hosts, consistent with the idea that natural selection is the driving force in creating islands of repeatable genomic divergence [51].

Improved Phenotyping

While genotypes are inherited in a discrete space, which makes them amenable to mapping studies, phenotypes span a more continuous and multidimensional space. Understanding the genotype–phenotype map therefore demands careful evaluation of the phenotypic space [52], which ultimately requires the parallel development of high-throughput and accurate methods for fast phenotyping. Advances in technology and manufacturing of digitizing equipment and video cameras have greatly increased the ease with which body landmarks and outlines can be recorded, especially in organisms where the specimen is readily projected in 2D. A prominent case where this has been successfully applied are dipteran wings. For instance, an automated wing-measuring machine has been developed for *Drosophila* that can record 100 wing parameters (that together sum up the positions of the major wing veins), allowing wings of live flies to be measured at a rate of >1 per minute [53]. Likewise, in recent years many studies have been moving on from univariate measures of phenotypic divergence to multivariate traits, for example, using geometric morphometrics [54].

Improved Relatedness Estimation

Traditionally, QTL analyses apply a relatedness matrix which is estimated with the identity-by-decent (IBD) matrix derived from pedigrees. When dense genotype maps are available, the IBD

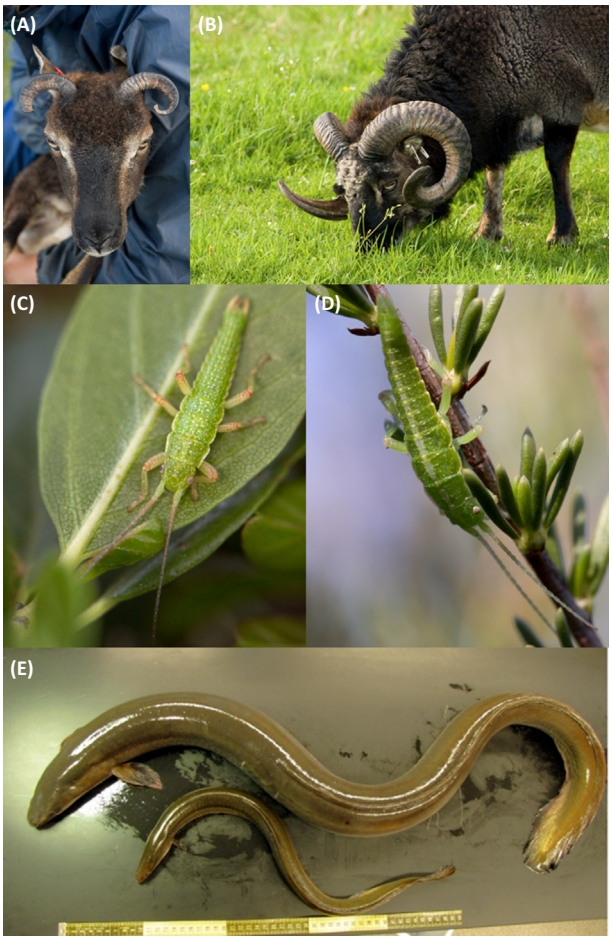

Figure 1. Examples of Studies where Different Approaches Were Combined To Improve the Mapping Resolution (A,B) or where Improved Experimental Designs Yielded a More-Powerful Way To Describe the Genomic Footprint of Natural Selection in Nature (C–E). Panel (A) shows a small horned and (B) a large-horned Soay sheep (*Ovis aries*) (photo courtesy Arpat Ozgul), where horn morphology could be successfully mapped to a local genomic region using a combination of QTL mapping and subsequent GWAS analyses [77,78]. Panel (C) shows a green and (D) a striped ecotype of the walking stick insect *Timema cristinae* (photo courtesy Aaron Comeault), a study system where genomic effects of natural selection on different phenotypes could be detected using an experimental design that quantifies allele frequencies *before* and *after* an episode of phenotypic *selection* [50,51]. Lastly, panel (E) shows two eel (*Anguilla rostrata*) ecotypes: on top is the large ecotype, representative of the rare, slow-growing, and late-maturing form that characterizes Lake Ontario/upper St. Lawrence River. Below is the small ecotype, which is found in the brackish/salt-water habitat, and is fast-growing and early-maturing (photo courtesy Guy Verreault). Using both a monogenic and a polygenic framework, Pavey *et al.* [81] were able to show that habitat-specific ecotypes from the same panmictic eel population could be best reclassified into the ecotype classes with a polygenic model, as opposed to a monogenic model.

matrix can be replaced by a genomic relationship matrix, in other words an identical-by-state (IBS) matrix estimated from genome-wide SNPs [55]. This has at least two benefits. First, an IBS matrix might better describe relatedness than an IBD matrix because the latter is compromised by the fact that the genome segregates in a finite number of chromosome chunks (linkage blocks), causing variation in genomic similarity between individuals [56]. Second, by avoiding pedigree-based methods one can more freely sample individuals, and thereby increase the sample size to avoid problems such as the Beavis effect. The power of this method remains to be determined, but recent studies show that genome wide markers often result in superior estimates of relatedness, whereas quantitative genetics estimations are only marginally improved [57,58]. Nevertheless, a clear benefit with marker- and IBS-based QTL analyses is the possibility to explore the genotype–phenotype space also in systems where reconstructing pedigrees is not practical or feasible.

Improved Analytical Frameworks To Detect and Evaluate Polygenic Signals

In addition to improved genotyping, phenotyping, and experimental designs, new statistical frameworks are continuously being developed. This has led to important recent advances in the accuracy and precision of single- and polygenetic modeling, and some of these methods are now capable of incorporating several confounding factors (e.g., environmental noise). These methods include improved mixed models, and phylogenetic and polygenic modeling, as outlined in Box 2.

Box 2. Recent Modeling Advances To Increase the Accuracy and Precision of Single- and Polygenetic Detection

Improved Mixed Models

Mixed models that incorporate confounding factors (e.g., environmental noise) increase the accuracy and precision of both single- and polygenetic models. The GEMMA software (www.xzlab.org/software.html) [82], for example, implements the Genome-Wide Efficient Mixed Model Association algorithm for a standard linear mixed model and some of its close relatives for GWAS. The program works by fitting a univariate linear mixed model (LMM) for marker association tests with a single phenotype to account for population stratification and sample structure, and for estimating the proportion of variance in phenotypes explained (PVE) by typed genotypes [83,84]. It also applies a multivariate linear mixed model (mvLMM) for testing marker associations with multiple phenotypes while simultaneously controlling for population stratification, and for estimating genetic correlations among quantitative traits [85]. Finally, Bayesian sparse linear mixed model (BSLMM) is implemented which allows estimating PVE by typed genotypes, predicting phenotypes, and identifying associated markers by jointly modeling all markers while controlling for population structure [86]. GEMMA has been widely applied recently (e.g., [87]), but some caution is warranted [88].

Phylogenetic Modeling

Another approach to separate processes shaping local versus genome-wide patterns is implemented in Saguaro (http://saguarogw.sourceforge.net) [89]. This program uses algorithms that combine hidden Markov models (HMM) with self-organizing maps (SOM) to create statistical local phylogenies for genomic regions in sequence alignments of species, subspecies, and/or populations. Then, the overall genome-wide phylogeny of the ancestral pattern is recognizable, as are deviating genomic phylogenies reflecting evolutionary forces that act locally. Depending on the study question and experimental design, the local genomic phylogenies may reveal genomic architectures and adaptations to particular environments. Saguaro does not rely on *a priori* hypotheses through implementing HMM and neural network models. Saguaro was applied recently in a study of speciation genomics in carrion and hooded crows *Corvus corone* [90].

Polygenetic Modeling

Several pathway analyses to model multiple SNPs have been developed. The Random Forest algorithm, for example, uses a tree-based ensemble machine learning tool and explicitly takes the correlation and interaction among multiple loci into account [91]. Because random forest approaches explicitly take the correlation and interaction among multiple loci into account, this method is well suited to detect polygenic signatures of selection in the genome [91]. The R package Random Forest and its associated function randomForest can be used to identify correlations as well as interactions among tens or hundreds of loci [92]. A few studies have applied this approach to study selection in natural populations, including the genomic basis of run time in Chinook salmon [93], habitat discrimination of ecotypes in the panmictic American eel ([81], see also Figure 1E in main text), anthropogenic selection in polluted environments of the European and American eel [94], and genetic architecture underlying the evolution of body shape in whitefish ecotypes [80].

Important progress is also being made to further understand whether the studied traits have a poly- or monogenic basis. One such method is to partition the cumulative additive effect sizes of multiple loci to particular regions of the genome, for example, to the different chromosomes, as applied by chromosome-partitioning methods [57,59]. The rationale is that if the focal trait is highly polygenic, large chromosomes will hold more QTLs than small chromosomes, and therefore that the proportion of variance explained by each chromosome should be proportional to the size of the chromosome. Thus, the correlation between the additive contribution and length of chromosomes can indicate the degree to which the trait is polygenic. Similarly, when an annotated reference genome is available, it is possible to estimate whether SNPs within or in close proximity to genes explain a higher proportion of the phenotypic variance than do loci located between genes [59]. Such a pattern of genomic location of SNP effect sizes would appear only if a large number of markers reflect variation at true causative loci, thus supporting a polygenetic nature of a trait, such as for example, was recently detected in two great tit *Parus major* populations [60].

Another promising methodological advancement is genome prediction, which can be used to confirm whether a detected set of QTLs, for example, from a genome scan, reflects variation at true causative loci. By genotyping individuals and evaluating to which degree their phenotypes can be predicted by their multi-locus QTL-genotypes, support for causation can be achieved [61]. Mixed-model approaches are implemented in the gBLUP (genomic best linear unbiased predictor method [62]) and TABLUP (a BLUP method that includes a trait-specific relationship

matrix [63]) methods, which utilize genomic relationship (IBS) matrices to estimate the genetic merit of an individual. These methods are of particular interest in animal and plant breeding [64], but also have the potential to revolutionize the study of trait biology in an evolutionary context.

Concluding Remarks

Theory predicts that selection on quantitative traits at the phenotypic level is accompanied by subtle allele-frequency changes in many loci that covary (a polygenic soft sweep), rather than a large, single-effect allele (a hard sweep). Thus, for quantitative traits, adaptation may be reached by an increased covariance of allelic effects rather than via large allele-frequency changes [65]. Rockman [7] suggested that this is the typical mode of evolution and that these quantitative traits are the traits of interest in evolutionary biology. That the polygenic case is the norm rather than the exception in nature is also a view held by other researchers [6]. By contrast, it stands clear that years of underpowered and biased QTL analyses have led to a strong skew in the literature in favor of large-effect loci [7,66]. The disconnect between theoretical models and empirical data is troublesome because it may bias our conclusions of the main genetic architecture of phenotypic traits and our understanding of the causal molecular targets of selection.

Indeed, quantitative genetics assumes that quantitative traits have a polygenic basis. However, this is still only a hypothesis for most traits, and we need more QTL studies with high mapping power to empirically demonstrate the polygenic nature of quantitative traits (*cf.* [25,60]). Nevertheless, when (or rather if) we demonstrate that such traits are truly polygenic, one might question whether molecular genetics analyses are at all meaningful, and potentially return to quantitative genetics frameworks. Overall, even if a tractable number of significant QTLs are detected, one might ask whether we wish to map genes explaining as little as a few percent of phenotypic variation. However, such seemingly small effect sizes can be important over long evolutionary timescales, and also in medicine, and plant and animal breeding, where they can carry significant commercial value. Thus, functional validation of low- and medium-sized QTLs should be a prioritized research aim in motivated situations.

Moreover, even though large-effect loci may be comparatively rare, it has still been suggested that they can play an important role in phenotypic evolution [67]. Several studies of large QTLs have given important insights into evolutionary biology, for example, in terms of understanding epistasis [68], heterozygote advantage [69], and sexual antagonisms [70]. Furthermore, genetic variation important for fitness may sometimes segregate as large inversions (i.e., a single segregating unit) in natural populations, as seen in the seaweed fly *Coelopa frigida* [71], in *Mimulus* monkeyflowers [72], and in white-throated sparrows *Zonotrichia albicollis* [73]. In addition, the evolutionary importance of other tightly linked regions, for example, the sex chromosomes, should not be neglected [74]. Finally, it may be argued that at least some **Mendelian traits** may have an evolutionary background through co-adapted gene complexes that have evolved via polygenic segregation, and that they for that reason are interesting to study, as has been suggested for some heritable genetic color polymorphisms [75].

Overall, much empirical work remains to be done before a more accurate understanding of genotype–phenotype associations can be gained (see Outstanding Questions). As outlined in this review, many exciting approaches have been developed over the past years that can overcome some of the problems and biases of traditional methods. This should make it possible to go beyond the mapping of monogenes and to better capture the polygenic nature of many traits. Many of these approaches are now starting to be applied in medicine and agriculture, and the field of evolutionary biology is following. While much of the progress to detect polygenes has focused on new statistical frameworks, we would also like to emphasize that continued effort must be placed on the integration of '**omics**' approaches with phenotypic data, the species' natural history, sophisticated population-level experiments, and work on fitness effects of

Outstanding Questions

Will the Bias Towards Detecting Large-Effect QTLs Persist? Genotype–phenotype mapping is currently experiencing rapid advances, but identifying the refractory small-effect QTLs requires an incomparable amount of genotyping and phenotyping effort. This will inevitably continue to lead to a bias towards mapping traits with simple segregation, such as Mendelian traits.

Can We Map Structural and other Types of Variation? There is a bias towards screening SNP variation, whereas structural variation, such as indels and inversions, has been largely neglected despite the fact that such variation can be substantial between species, populations, and even individuals. New screening methods, including single-molecule real-time sequencing, nanopore-based sequencing, and optical genome mapping, where fragments between 30 and 500 kb can be studied, hold the promise to accurately detect such variation.

Are Quantitative Genetics Approaches Sufficient for Studying Polygenic Traits? We are far from incorporating and understanding the influences of gene by gene interactions underlying variation in most traits. Future efforts need to document the importance of gene interactions because these can be responsible for the small additive effects, missing heritability, and lack of replication typically observed for human complex traits.

Will More than a Fraction of Polygenes Ever Be Functionally Understood? Gene complexes and genes in regions with limited recombination (e.g., inversions) will be difficult to study above their total multi-locus effects. Moreover, the genetic architecture and functional understanding of traits that are difficult to quantify, including most behavioral traits, may be neglected.

phenotypes in nature. Only if these approaches are integrated will we be able to understand the genetic underpinnings of phenotypic traits relevant in nature.

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