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Pleiotropy, constraint, and modularity in the evolution of life histories: insights from genomic analyses

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Abstract

Multicellular organisms display an enormous range of life history strategies and present an evolutionary conundrum; despite strong natural selection, life history traits are characterized by high levels of genetic variation. To understand the evolution of life histories and the maintenance of this variation, the specific phenotypic effects of segregating alleles and the genetic networks in which they act need to be elucidated. In particular, the extent to which life history evolution is constrained by the pleiotropy of alleles contributing to life history variation is generally unknown. Here we review recent empirical results that shed light on this question, with an emphasis on studies employing genomic analyses. While genome-scale analyses are increasingly practical and affordable, they face limitations of genetic resolution and statistical power. We describe new research approaches that we believe can produce new insights and evaluate their promise and applicability to different kinds of organisms. Two approaches seem particularly promising: experiments that manipulate selection in multiple dimensions and measure phenotypic and genomic response, and analytical approaches that take into account genome-wide associations between markers and phenotypes, rather than applying a traditional marker-by-marker approach.

Keywords

population genomics; QTL; GWAS; age-specific variation; trade-off

Introduction

One of the most striking features of life on earth is the diversity of patterns in the timing and magnitude of major life events, such as maturation, reproduction, and longevity.[1–3] The patterning of these events is referred to as the *life history* (LH) of a species, population, or individual. LH patterns determine if, when, and how much an individual reproduces and thus directly determine an individual's Darwinian fitness; other phenotypes (e.g., physiological, morphological, and behavioral traits) affect fitness via their LH effects. Understanding how diverse LH strategies evolve is therefore crucial to understanding the evolution of adaptive traits in general.

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The vast diversity of LH patterns that we observe in nature originated as genetic variation that segregated within single populations. Moreover, the high levels of heritable variation for LH traits measured in contemporary populations suggest that these populations harbor many segregating alleles that differ in their effects on rates and magnitudes of growth, maturation, reproduction, and somatic maintenance.[4–7] Like all segregating variation, these alleles arose by mutation (including mutations introduced by hybridization or duplication events), and their frequencies have been shaped over evolutionary time by the combined action of natural selection, genetic drift, and migration. However, the specific phenotypic effects of these alleles and the genetic networks in which they act matter a great deal to our understanding of LH evolution, and we are largely ignorant of these effects. In addition, recent analyses indicate that LH patterns predict overall genetic diversity across animal taxa,[8] suggesting that LH strategies that evolve in response to long-term environmental trends have profound consequences for the ability of species to respond to short-term environmental change.[9] Thus, understanding the genetic and molecular bases of LH evolution and any constraints that these impose enables better prediction of species resilience in the face of accelerating environmental perturbation.

A critical feature of alleles affecting LH traits is the extent and nature of pleiotropy they exhibit. Pleiotropic alleles can underlie correlated responses to selection, which cause traits to evolve that are not the direct targets of selection (e.g., see Refs. 10–13). In particular, these loci can produce trade-offs (patterns of negatively correlated fitness effects) among LH traits and thus act as evolutionary constraints. This pleiotropy can result from alleles affecting multiple LH traits or alleles influencing the same trait expressed in different life stages. An allele causing earlier maturation, but at a cost of decreased future brood size, would be an example of the former, while an allele that increased brood size in young animals or in one environment but decreased it in older animals or in an alternative environment would be an example of the latter. Both of these are examples of negative or antagonistic pleiotropy, where the expected effects on fitness are opposite in direction, all else being equal; these are the kinds of pleiotropic effects that can impose constraints on the response to selection. Pleiotropy among LH traits can also be positive, where the expected marginal effects on fitness are all in the same direction (either all increasing or all decreasing). An allele contributing to both rapid maturation and to large brood size exhibits positive pleiotropy. Alleles with positive pleiotropy should experience strong directional selection, so we might expect that few such alleles would be found segregating within populations. However, mutation and gene flow can promote polymorphism in such alleles,[14–18] as can environmental variation that changes the direction of the expected fitness effects of alleles.[16,19–21] Finally, alleles affecting LH traits might have limited pleiotropy, such that they affect only a single LH trait or a subset of age or stage classes. Deleterious alleles with delayed age of onset of effects are an example of this kind of allele that has been widely discussed (see below and Refs. 22–24 for recent reviews).

Here, we focus on pleiotropy (or lack thereof) of alleles expressed in a single individual or a single genotype because this is the kind of pleiotropy that is generally assumed in models of LH evolution. Correlated effects of alleles across environments or in different individuals (e.g., genotype–sex interaction) have been described as types of pleiotropy, and they can certainly influence the evolution of LH traits (e.g., Ref. 25). However, in the genome-level

analyses that are the focus of this review, most studies on LH traits focus on within-genotype effects. We include studies in which the same genotypes have been investigated under multiple environmental conditions. These data are mainly available for plants or for organisms that can be cloned and the clones reared across multiple environments.

We also focus primarily on data from natural populations, rather than from domesticated plants and animals, since our main interest is in understanding LH evolution in nature. In this context, we know surprisingly little about the genes or molecular pathways that contribute to LH variation within and between natural populations. For example, we do not know the extent to which life history divergence is guided or constrained by pleiotropic loci, whether alleles of small or large effect are primarily responsible, whether the extent of pleiotropy itself varies across ages or life stages, or whether genes shown to regulate LH traits in genetic screens in model organisms are the major contributors to natural variation in LH patterns.[25–27] We also have limited mechanistic understanding of how natural variation in LH traits is regulated. In recent years, technological and analytical breakthroughs have dramatically increased the ability to assess variation at the DNA sequence level and to attribute phenotypic variation to specific regions of the genome.[28–32] The availability of fast and affordable high-throughput DNA sequencing, genome-wide reduced-complexity genotyping, and (in at least a few cases) high-throughput phenotyping have the potential to revolutionize our understanding of the genetic basis of phenotypic diversity that segregates within populations—the raw material for evolution. In this review, we summarize recent advances in this area, focusing specifically on LH traits. We also outline new experimental approaches that can be leveraged in this effort.

Pleiotropy, trade-offs, and constraints

Much of LH theory is founded on the idea that selection favors strategies of age- (or stage-) specific growth, development, and reproduction that maximize fitness in different ecological settings.[1,3,5,33] Classical LH theory is also based on the idea that, because resources available to an individual are limited, allocating these resources to one LH trait comes at a cost to others.[1,3,34–38] Under this view, variation in LH strategies within or between populations reflects differences in allocating energy to the competing demands of growth, development, reproduction, maintenance, and repair. Given a fixed energy budget, differences in allocation among individuals within a species will result in negative correlations, or trade-offs, among traits at the individual level, while differences among populations or species result in negative correlations at these higher levels of organization. The existence of these negative correlations has been taken as evidence for LH trade-offs and for the idea that these correlations arise from competing energy demands.[1,3,34]

While this energy-allocation view of LH trade-offs has long been accepted, recent mechanistic studies suggest that some trade-offs result from competition for resources while others do not. For example, the trade-off between immunity and reproduction appears to be mediated by endocrine and metabolic signaling pathways that alter energy allocation between these competing demands.[39] Trade-offs between reproduction and longevity also appear to be regulated by molecular signals, such as those from the germline,[40] that are induced by specific environmental or physiological states. In this case, however, the trade-

off may not result directly from competition for resources, because experimental manipulation of signaling pathways (e.g., through mutagenesis or tissue ablation) can decouple reproduction and longevity.[41–43] These results do not mean that energy allocation has no impact on LH traits and the trade-offs among them. Instead, alterations of signaling in response to environmental conditions (e.g., nutrient levels, infection) can induce physiological changes that produce LH trade-offs as by-products of the signal itself (reviewed in Ref. 44).

Whether we view correlations among LH traits as arising mechanistically from energy constraints or indirectly by activation or alteration of signaling pathways, these correlations arise from both genetic and environmental variation.[34,45–49] Genetic variation and covariation underlie the heritable component of LH correlations. That is, it is the sign and magnitude of genetic covariation that ultimately shape evolutionary trajectories, so most discussion of the evolutionary consequences of LH correlations focuses on these genetic parameters.[4,50,51] Genetic covariation occurs when segregating alleles have different effects on multiple LH traits (i.e., when they exhibit pleiotropic LH effects). More specifically, genetically based correlation among LH traits that are negative with respect to their effects on fitness (and therefore lead to LH trade-offs) are said to exhibit antagonistic pleiotropy.

The extent and evolutionary effects of pleiotropy in general have been the subjects of much recent debate.[25,52–64] This debate centers on apparently conflicting results of gene knockout, gene mapping, and quantitative genetic studies. Gene knockout and QTL mapping experiments have been interpreted to suggest that pleiotropy is limited, with specific genes and QTL having effects on only one or a very few measured traits.[57,59,61,65] However, others have argued that QTL and gene knockout studies will always underestimate pleiotropy, owing to limited power and high false negative rates,[25,60] and that multivariate quantitative genetic analyses that consider many traits simultaneously support the idea that pleiotropy is pervasive[25,55,58,64,66] (but see Ref. 67). The consequences of pleiotropy for evolution (i.e., its effects on evolvability) have also been debated.[21,59,68,69] Several authors have proposed that highly multivariate phenomic data, analyzed in a formal multivariate framework, are necessary to determine both the prevalence of pleiotropy and the extent to which it constrains evolution.[66,70] In recent years, this kind of highly multivariate data has been generated for morphological and gene expression phenotypes,[62,66,67,70–72] but not for LH traits. Therefore, even if empirical support for pervasive pleiotropy in the investigated phenotypes was unequivocal, it is not clear how pleiotropy in these underlying traits translates into genetic correlations that can constrain the evolution of LH phenotypes.

Unfortunately, highly multivariate phenomic data for LH traits are generally lacking, and would be challenging to acquire. The genetic basis of LH variation and covariation has traditionally been measured for only a few traits at a time, and by one of three different methods. First, common-garden experiments in which different genotypes, populations, or species are reared in the same environment have been used to demonstrate whether LH correlations are heritable. LH trade-offs that persist in a common environment for multiple generations are inferred to be heritable and genetically based.[1,73–75] Second, quantitative genetic studies use resemblance among relatives to partition LH variation and covariation into genetic and nongenetic components.[1,3,76–86] Third, experimental evolution and artificial

selection studies reveal underlying genetic correlations when traits that are not the direct target of selection exhibit a correlated response (e.g., Refs. 1, 87, and 88–104). All of these approaches have revealed that genetic correlations among LH traits are common but not universal. For example, experimental evolution studies that target a single trait and then measure correlated response to selection in other traits provide a way to assess both the existence of pleiotropy and its evolutionary consequences. Many such experiments focusing on LH traits have been conducted since the seminal studies of Rose and Charlesworth.[10,89] Correlated responses to selection are very common in these experiments (see references above), but are not always observed.[42,105–107]

The previous paragraph might suggest that genetic correlations and pleiotropy among LH traits have been thoroughly investigated, at least at the empirical level. Indeed, these studies illustrate the potential importance of pleiotropy and genetic correlations in LH evolution.[42] However, these inferences are indirect and subject to several caveats. For example, genetic correlations and correlated responses to selection can be driven by physical linkage instead of pleiotropy.[50,108] Even when pleiotropy is implicated, the relationship between pleiotropy and the genetic correlations that influence evolutionary trajectories is quite indirect.[69] The magnitude and sign of correlations can also be highly sensitive to both environmental and genetic background effects. For example, genetic correlations can be dramatically altered by both inbreeding and environmental stress (as might occur if the organism is poorly adapted to the environment used in experiments).[45,109–111] Genetic correlations can also be sensitive to environmental variation that is within the normal range experienced by a population.[85,112,113] Because the evolutionary response to selection in a suite of correlated traits depends on genetic variation and covariation,[4,50,51,114] this sensitivity means that spatial or temporal fluctuation in genetic correlations in nature could substantially influence the evolution and maintenance of variation for LH traits.[58,112]

One way to address the limitations of quantitative genetic approaches is to identify the actual genes and molecular pathways that underlie variation and covariation in LH traits. Identifying the genes affecting traits is probably the only way to distinguish pleiotropy from linkage, and gene-mapping experiments in organisms with low average levels of linkage disequilibrium (LD) have been deployed for this purpose.[115] In addition, while the environmental sensitivity of LH pleiotropy can be investigated in laboratory or greenhouse settings (see below), these experiments are probably best viewed as demonstrating the potential importance of this variability. In contrast, gene-mapping studies in natural populations (including reciprocal-transplant scenarios) can evaluate the importance of environmental variation in pleiotropy under evolutionarily relevant conditions. In the remainder of this section, we review studies that have taken these approaches to understanding LH variation and pleiotropy.

Genome-enabled studies of LH variation, such as quantitative trait locus (QTL) mapping and transcriptomics, have been reviewed elsewhere.[116,117] Briefly, while the results of many such studies are consistent with the existence of segregating alleles with pleiotropic effects on LH traits, these approaches cannot by themselves identify individual loci and their effects. These studies can identify candidate genes, followed by detailed investigation of those candidates.[118–123] In a recent example of this strategy, Page *et al.*[122] fine-mapped a

QTL for metamorphic timing in *Ambystoma* salamanders to an approximately 2-cM region containing only a few coding genes. Pleiotropic effects of this region were confirmed by assessing three traits: probability of metamorphosing, timing of metamorphosis, and growth rate. Alternative genotypes were shown to affect the probability of metamorphosing and metamorphic timing, but not growth rate. Another approach is to identify a priori candidate genes from functional analyses. To date, this functional approach has mainly been used in model species like *Arabidopsis thaliana* and *Drosophila melanogaster,*[120,124,125] but investigations of *PGI* and other genes in lepidopterans are notable exceptions.[119,126]

Population-genomic and genome-wide association studies (GWAS) have been increasingly applied to LH traits in recent years, both in natural populations and in laboratory studies of genotypes derived from nature. Many of these studies have implicated specific genes and genome regions in pleiotropic LH effects (Table 1). Examples include a study by Prunier *et al.*[127] that identified candidate SNPs associated with within-population variation in bud-set timing and growth rate in black spruce (*Pinea mariana*) using a combination of QTL mapping, outlier analysis, and bulk-segregant analysis. The authors then applied GWAS to link those candidates with LH variation across 30 different populations. A high proportion of candidate SNPs were associated with variation in both traits in the GWAS, suggesting extensive pleiotropy.

Anderson and colleagues developed a novel modification of QTL analysis to detect LH variation and covariation in recombinant inbred lines (RILs) of the perennial *Boechera stricta* grown under natural conditions at two different sites within the native range of the species.[128,129] The approach evaluates allele frequency changes after episodes of viability and fecundity selection at a genome-wide scale. This analysis produced estimates of viability and fecundity selection coefficients on individuals, and the authors used these estimates as trait values in QTL analyses. Pleiotropy was indicated if marker loci were significantly associated with more than one selection coefficient. The investigators found fitness trade-offs across environments at one QTL containing a known flowering-time locus (*nFT*) and no trade-offs within environments. However, patterns of selection coefficients evaluated across all markers simultaneously indicated that genome regions causing high fecundity in one year reduced subsequent over-winter survival, suggesting that trade-offs were highly polygenic and therefore difficult to detect at the level of individual loci. These results support the claim that studies focused on individual markers are generally underpowered to detect pleiotropic effects.[25,60]

In a few cases, mainly in model organisms, studies have progressed from identifying QTLs (or significant GWAS effects) to characterizing pleiotropic effects at specific genes and alleles. One of the first such studies characterized molecular variation at a gene, *Catsup*, that was initially identified as affecting longevity in *D. melanogaster* using RILs.[108] Remarkably, 28 different SNPs and five indels were identified in this single locus. These different variants exhibited low LD, and different sites were independently associated with variation in different traits (e.g., longevity and locomotor behavior). These results suggest that the high genetic correlations among traits observed in the RIL population were driven by pleiotropy at the level of the gene locus, but the causal polymorphisms for different traits were independent. In addition, the low LD suggested that different sites within the gene were

evolving independently. This was a truly surprising and important result, but one potential caveat is that long-range LD with other (unknown) causal loci can also produce this pattern.[130]

In *A. thaliana*, Scarcelli *et al.*[124] built on previous studies that identified loci affecting flowering time. By intercrossing 19 different accessions and measuring traits and genotypes after five generations of intermixing, these authors found that alternative genotypes at two loci, Frigida (*FRI*) and Flowering-Locus *C* (*FLC*), had antagonistic effects on flowering time and plant architecture traits that determine fruit production. In addition, the magnitude but not the direction of these effects varied seasonally. Similarly, a study of 40 *A. thaliana* accessions containing 20 different haplotypes at the *FLC* locus found that alternative alleles were associated either with early flowering and high seed set or with late flowering and low seed set under two different seasonal conditions.[131] While compelling, one feature shared by these *Arabidopsis* studies is that they investigated variation that occurred between, not within, populations. We also note that model organism studies often make use of common collections of strains or genotypes for GWAS because a great deal of effort is required to produce these strains and because a common collection provides a useful resource for replicating previous experiments. A potential inferential problem with this approach, however, is that the creation of the mapping strains represents a single sample of the genetic variation available in the population or species. Inference is therefore limited to one particular sample of genotypes, and any long-range LD that creates spurious associations between SNPs and phenotype are then replicated across different studies and different labs that use the same genotypes.

Most of the studies described above investigated among-population or among-strain variation (but see Ref. 127). Fewer studies have identified and characterized alleles with pleiotropic LH effects that segregate within populations. One of the best-documented examples is that of the insulin-like receptor gene (*InR)* of *D. melanogaster*. Paaby *et al.*[125] reported pleiotropic effects of an insertion–deletion polymorphism in the first exon of this gene. The polymorphism produces two versions of the *InR* protein, a long and short form, that differ in two amino acids. Although both forms segregate in most populations, the short form is more prevalent in northern populations and the long form is at higher frequency in southern populations in North America. Flies with the short allele (but with a mostly randomized genetic background) had improved survival in colder temperatures, greater starvation resistance, longer time to eclosion, reduced fecundity, and longer life span compared with flies with the alternative allele. These alternative alleles were also associated with variation in insulin-like (IIS) signaling, with the longer allele having increased signaling, and phenotypic effects of changed IIS signaling were confirmed experimentally using RNA interference (RNAi). Another potential example of a segregating allele with pleiotropic LH effects is couch potato (*cpo*), which is also associated with latitudinal variation in diapause propensity and other LH traits in North American *D. melanogaster* populations.[132,133] This polymorphism also segregates within populations and exhibits latitudinal variation in Australian populations of *D. melanogaster*. However, neither the phenotypic cline nor the phenotypic effects of *cpo* alleles on diapause were replicated in a study of these populations,[134] and the allele frequency cline in Australia was shown to be

confounded by the tight linkage between *cpo* and the cosmopolitan inversion polymorphism *In3R(P)*.

We know of only two cases where a gene-specific LH trade-off segregating within a population has been identified in a free-living organism. In Soay sheep (*Ovis aries*), covariation between male LH traits and a sexually-selected ornament was attributed to a single gene (relaxin-like receptor 2 (*RXFP2*)).[121] Males homozygous for one allele (conferring large horn size) had high reproductive success and low survival, while homozygotes for the alternative small-horn allele had low reproductive success and high survival. Heterozygotes at this locus had higher overall fitness than either homozygote, generating overdominance and thereby accounting for the maintenance of both alleles at intermediate frequencies within a single population.

The other case implicating a single locus in LH trade-offs in a natural population also involves apparently strong sexual selection. In stick insects (*Timema cristinae*), melanism segregates within populations and has been associated with a single major-effect locus.[123] Melanic individuals of both sexes exhibited higher mating success than non-melanic individuals in laboratory tests, and were also more likely to disperse in mesocosm experiments. Melanic and non-melanic individuals also differed in immunity and crypsis, but the specific source of balancing selection that maintains the polymorphism has not been definitively identified in this case.

Genetic modularity of life histories: the case for evolutionary independence

The scope for evolutionary independence of different LH traits depends on the availability of genetic variation that is not highly pleiotropic. This idea has been described as *modularity*, where a gene contributes to a limited number of phenotypes rather than to many (or most) phenotypes.[106,135] The modularity concept is important not only for understanding LH evolution, but more generally for uncovering whether and to what degree evolutionary outcomes are constrained by the genetic architecture of traits.[59,68,135] Studies suggesting genetic independence or modularity of LH traits include those that fail to find genetic correlations among traits (e.g., Refs. 77, 112, 136, and 137) that document age-specific differences in genetic variation of LH traits (e.g., Refs. 138, and 139–147), and that implicate different genome regions in influencing different traits or the same trait expressed at different ages.[65,137,148–151]

Within the LH literature, evolutionary independence has been most discussed in the context of age-specific variation and the evolution of senescence. Different versions of the evolutionary theory of senescence assume either that allelic effects exhibit trade-offs across age classes (the antagonistic pleiotropy theory) or that allelic effects can be independent across age classes (the mutation accumulation theory). One of the first considerations of this issue was Haldane's famous attempt to explain the persistence and high frequency of Huntington disease in humans,[152] which is caused by a dominant lethal allele. Dominant lethals should be extremely rare, but Haldane reasoned that late-onset human diseases would experience little natural selection; in premodern societies, most individuals would die for reasons unrelated to senescence before symptoms of late-onset disease were expressed.

Medawar, Williams, Hamilton, and others later generalized this reasoning as the key to understanding why organisms with age-structured populations generally exhibit senescence.[153–157] This argument assumed that alleles causing late-onset disease have no fitness consequences (either beneficial or deleterious) early in life, so that their evolutionary dynamics are governed mainly by the rate of recurrent mutation and genetic drift. This mutation–selection–drift equilibrium model came to be known as the mutation accumulation theory. We now know that Huntington disease and some other late-onset pathologies are associated with the accumulation of abnormal proteins that resist degradation by normal cellular pathways.[158,159] This slow accumulation of toxic substances suggests a biochemical basis for alleles that have delayed age of onset of effects and that lack early-age phenotypes (see below). The alternative version of the general theory of senescence postulates that alleles with beneficial effects on early-age LH traits have deleterious effects on late-life fitness. These antagonistically pleiotropic alleles are favored by natural selection because of their early-life beneficial effects, but selection against their late-life deleterious effects is weak, for the reason Haldane suggested.[155,160] Therefore, these alternative versions of the evolutionary theory of senescence are a special case of the general question of the relative importance of pleiotropy and evolutionary independence in LH evolution.

A large number of studies indicate that quantitative genetic variation for LH traits can be age specific.[77,138–140,142–147,151,161–166] A general, though not universal, pattern in these studies is that genetic variation for LH traits increases with age. A smaller number of published studies have failed to find age specificity, or have found the reverse pattern, with variance decreasing with age.[167–171]

Recently, transcriptomic and genomic approaches have been applied to the same question: to what extent is genetic variation in LH traits age specific? Several investigations support a pattern of genetic independence across age classes. Using transcript profiling, Viñuela *et al.*[172] found that gene expression became more polygenic with age in *Caenorhabditis elegans*. Similarly, Felix *et al.*[173] reported that heritable variation in gene expression phenotypes increased dramatically with age in *D. melanogaster* (Fig. 1A). QTL and GWAS techniques have also identified seemingly modular age-specific allelic effects in a wide range of organisms.[151,174–181] These studies have all reported that the polymorphisms contributing to trait variation are different at different ages. In one of these cases, Durham and colleagues used the *Drosophila* Genetic Reference panel (a set of inbred lines derived from a single natural population), to identify loci affecting age-specific fecundity and life span.[151] More than 1000 single-nucleotide polymorphisms (SNPs) were associated with age-specific variation in fecundity, and 52 SNPs were associated with variation in life span. However, only one SNP appeared to have pleiotropic effects on early- and late-age fecundity, no SNPs were associated with both fecundity and life span, and the number of SNPs associated with fecundity increased dramatically with age (Fig. 1B).

Evidence also exists for genetic independence of LH traits expressed at the same age, such as offspring size and mass, which are often invoked as classic examples of traits that trade off within species (Table 1). Using a combination of chromosome partitioning, QTL mapping, and GWAS approaches, Santure *et al.*[137] investigated clutch size and egg mass in a free-living great tit (*Parus major*) population. On the basis of these analyses, the

investigators concluded that variation in both traits was underlain by many loci of small effect, and they found no evidence that any region of the genome contributed significantly to both traits.

One interpretation of these studies is that LH phenotypes can be highly modular.[148,151] For example, age- or trait-specific genetic variation could result from strictly age- or trait-specific allelic effects, as suggested by the results of Carbone *et al.*[108] However, there are reasons to be skeptical of the claim that studies showing age- or trait specificity of genetic variation also implicate high genetic modularity of LH traits. First, even large genetic experiments are generally unable to discriminate true negative results from false negatives. This concern is particularly relevant to analyses in which many hypotheses are tested and strong statistical control of the false positive rate is implemented, which tends to generate very high false negative rates.[182] Genomic analyses that test thousands to millions of associations between sequence variants and phenotypes are therefore especially susceptible to this problem. For example, in any genome-scale analysis of LH variation, there are likely many loci that have real effects on the measured traits, but only some of these effects will be detected in any given analysis. Some studies have attempted to account for this possibility. For example, Durham *et al.*[151] ranked SNPs affecting age-specific female fecundity in *D. melanogaster* at each age, regardless of whether or not SNPs were deemed formally significant. In doing so, the authors found little to no overlap in candidate SNPs across ages, supporting the interpretation of substantial genetic independence across age classes for this trait. This procedure does not completely solve the false negatives problem, however, because the results still relied on statistical estimates and moderate sample size.

In addition to these purely statistical concerns, theoretical and biological considerations suggest caution in interpreting studies that implicate high modularity of LH traits. As noted above, genetic correlations can be dependent on the environment, and it is not always possible to estimate these correlations in salient environments.[85] Even more fundamentally, models of multitrait systems indicate that, even when there are strict functional constraints among a set of traits (e.g., a direct trade-off between survival and fecundity), these constraints will not generate negative genetic correlations between all pairs of traits.[4,51] That is, when more than two traits are considered, relationships between genetic correlations and the underlying constraints are indirect, although at least one negative genetic correlation between traits is expected in such systems.

In the case of age-specific variation, there are also other explanations for patterns that seemingly support modularity. The genetic variance of any trait depends on the number of segregating loci affecting the trait, the allele frequencies at those loci, and the magnitude of allelic effects.[99,183] Consequently, a change in genetic variance with age might be driven by allelic effects that differ consistently in magnitude in young compared with old individuals. This change in magnitude of allelic effects need not reflect any underlying change in molecular or physiological processes, however. We envision two different scenarios. First, imagine an allele that affects the rate of accumulation of some kind of cellular waste product or toxic substance. Again, Huntington disease provides a useful example, since the pathology has been attributed to accumulation of a nondegradable protein in neural tissue. An allele that slightly increases the rate of accumulation might be completely benign in

young individuals when levels are very low, but the phenotypic effects of the allele would tend to increase with age and continued accumulation. This kind of cumulative or threshold effect could produce age-specific patterns of genetic variation because the allelic effects on LH traits would be small or nonexistent in young animals but would increase with age.[24, 77]

Second, allelic effects and the genetic variation they produce could be highly age dependent, even in the absence of any age-dependent molecular or physiological processes. By definition, senescent individuals are frailer than young individuals that have not yet experienced any senescent decline. One consequence of this frailty, whatever its underlying cause, might be an increased sensitivity to allelic variation. In this scenario, even if the same segregating alleles affect a LH trait expressed in young and old individuals, old individuals are more sensitive to the effects of these alleles. Under these conditions, the observed genetic variation in the LH trait will increase with age because the average effects of the segregating alleles increase with age, not because different alleles affect the trait at different ages.[139] Also, under this scenario, the power of QTL and GWAS experiments to detect associations between genotype and phenotype will be higher at old ages, consistent with many of the genomic analyses described above.

A way forward?

How important is pleiotropy in guiding and/or constraining the evolution of LH traits, and how can we assess this question experimentally? Above, we outline many of the challenges involved. In this final section, we describe a few techniques and approaches that we believe will enable further progress (see also Table 2). The advent of low-cost high-throughput sequencing technologies, along with advances in analyzing these data, has made it feasible to identify candidate polymorphisms for LH variation and to characterize molecular pathways that are regulated by these polymorphisms. The development of targeted mutagenesis, allele replacement (e.g., CRISPR-Cas9), and other genomic engineering approaches provides the ability to assess causality and pleiotropic effects of candidate LH loci.[184,185] To date, these methods have mainly been used in laboratory settings, so their relevance to natural variation can be questioned. However, these methods should become available in a much larger number of species than previous allele-specific replacement techniques.[186] Systems in which replicated genotypes can be grown under naturalistic conditions (e.g., many plant species) could take advantage of these techniques.

One issue that cannot be addressed through gene manipulation is the extent to which pleiotropy can and does constrain the evolution of LH phenotypes. In light of the difficulty of obtaining highly multivariate LH data in most species, the most practical way to address this issue is to manipulate selection itself and ask to what extent response to selection is affected by non-independence among traits. One method, reviewed recently by Paaby and Rockman,[25] is to compare the estimated heritability of a trait to the observed response to artificial selection on the trait and attribute any difference to the effects of pleiotropy. We propose a complementary approach that should also be useful and potentially more informative. If pleiotropy constrains LH evolution, then selecting on multiple LH traits in different replicate populations should reveal (1) negatively correlated responses in some pairs of traits, and (2) that variants responding to selection for trait 1 also contribute to the

response to selection for trait 2, but the direction of allele frequency change will be reversed. One limitation of such an experiment is that a large number of SNPs are typically identified as significantly diverging in evolve-and-resequence" experiments, and clusters of significant SNPs occur in physically adjacent genome locations, suggesting that many of the SNPs are evolving as the result of genetic hitchhiking and long-range LD.[31] These phenomena can make it difficult to sort the causal polymorphisms underlying trait divergence from those that are merely linked to the direct targets of selection. Hitchhiking and long-distance LD in these experiments are likely due to small original starting population sizes, low initial allele frequencies of targets of selection in the base population,[187,188] and, in the case of *D. melanogaster*, LD generated by segregating chromosomal inversions. However, simulation models suggest that experimental design features can ameliorate these problems by using larger founder and selected populations and a larger number of experimental replicates than have been used in previous studies; leveraging founder haplotype information and periodic monitoring of changes in allele frequency during the selection process also improve the power and precision of these experiments.[31,189]

Of course, one danger in this approach is that a limited number of LH phenotypes could be measured in such an experiment, so traits that participate in constraints might be overlooked. This approach is therefore best deployed in cases where there are strong empirical or theoretical motivations for trait selection. An example of a well-founded question that could be addressed using this approach is whether the same LH traits expressed at different ages or life stages are under independent genetic control.[151,190,191] A check on whether unmeasured traits impose constraints would be to compare the estimated G matrix of the measured traits to the realized response to selection on each trait, a slight modification of the approach suggested by Paaby and Rockman.[25] If the estimated G matrix consistently under-predicts the response to selection, then pleiotropic constraints imposed by unmeasured traits are implicated.

Finally, analysis of many genetic markers simultaneously, rather than marker by marker (as in Refs. 129, 137, and 192), is an important advance in understanding LH pleiotropy and evolutionary constraint. In addition to innovative analytical approaches, these studies were all conducted in free-living populations or in synthetic populations observed under naturalistic conditions. Such studies have inherent limitations, including the need to assess LH phenotypes for large numbers of individuals in nature, which is not practical in many species. When feasible, however, these studies provide unique insight into the genetic architecture and evolvability of LH traits and they automatically assess these parameters under a relevant range of environmental conditions. When combined with detailed genetic dissection of LH variation and pleiotropy in suitable organisms, these insights will improve our understanding of the diversity of LH patterns that have evolved and the sustainability of this diversity in a changing world.

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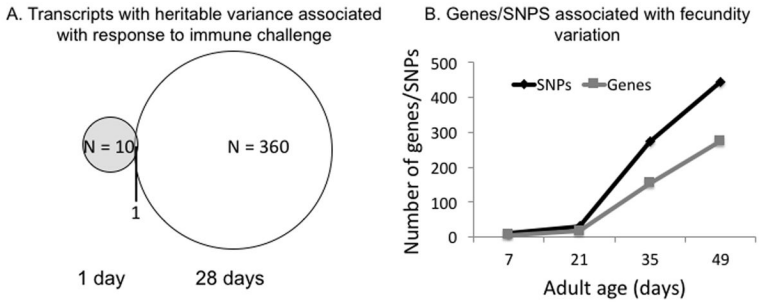

Figure 1.

Recent genomic analyses suggest that genetic regulation of LH traits becomes more polygenic with age; however, it is currently not clear whether these trends can be accounted for by increased power to detect effects at late ages (see text). (A) Number of transcripts significantly associated with genotypic variation for bacterial infection clearance ability in *D. melanogaster* at one and four weeks posteclosion. Only one transcript was associated with genetic variation at both ages. Genotypes were recombinant inbred lines derived from a single natural population (See Ref. 173). (B) Number of genes and SNPs significantly associated with age-specific fecundity in a genome-wide association study of *D. melanogaster* DGRP lines (see Ref. 151).

Table 1

Genome-scale investigations of life history pleiotropy[a]

Species	Traits	LH pleiotropy	Study type	Loci implicated	Reference
Within-environment pleiotropy					
Timema cristinae	Mating success, dispersal, resistance to pathogen	Positive	GWAS	Single locus with modifier	123
Boechera stricta	Flowering phenology, viability, fecundity, survival	Negative	RIL	Multiple QTL regions	129
Parus major	Clutch size, egg mass	Limited	QTL outbred	Highly multigenic	137
Picea glauca	Phenology, growth rate	Negative	QTL	Multiple QTL regions	193
Drosophila melanogaster	Larval viability, survival of infection	Negative and positive	Candidate gene based on previous GWAS	Single locus (*Ddc*)	194
Melitaea cinxia	Egg maturation dispersal, population growth rate	Positive	Candidate gene, microarray	Two loci (*Pgi*, *Sdhd*)	126
Ovis aries	Reproductive success, survival, horn size	Negative	Candidate gene based on previous QTL	One locus (*RXFP2*)	121
Between-environment or between-population pleiotropy					
Saccharomyces cerevisiae	Population growth parameters	Limited	QTL	Four loci (*RIM15, PUT4, DAL1, and DAL4*)	65
Boechera stricta	Flowering phenology, viability, fecundity	Negative	RIL	Multiple QTL regions	129
Picea mariana	Phenology, growth rate	Negative	QTL and GWAS	Multiple QTL	127,195
Arabidopsis thaliana	Flowering time, fruit production traits	Negative	Candidate gene based on previous GWAS	Two loci	124
Drosophila melanogaster	Stress tolerance, early and late fecundity, development rate	Negative and positive	Candidate gene with genetic background controlled	Single locus (*InR*)	125
Ambystoma tigrinum	Probability of metamorphosing, metamorphic timing, growth rate	Negative	QTL	Single locus (*met*)	122
Drosophila montana	Development rate, reproductive diapause, cold tolerance, body weight	Negative	QTL	Multiple QTL	196
Saccharomyces cerevisiae[b]	Population growth in different environments	Negative	QTL	Multiple QTL	197
Mimulus guttatus	Flowering time, vegetative allocation	Positive and negative	QTL, BSA	Multiple QTL	198

QTL, quantitative trait locus mapping; RIL, recombinant inbred lines; GWAS, genome-wide association study; BSA, bulk-segregant analysis

[a]QTL analyses were only included if they were not previously reviewed in Refs. 113 or 114.

[b]Cross between lab strain and natural isolate

Table 2

Genome-enabled approaches to investigate life history pleiotropy

Approach	Applicable species	Advantages	Limitations
QTL, GWAS in natural populations	Large populations that can be characterized in nature	Environmental relevance, fitness components as LH traits	Need relatedness and detailed LH data, selection itself cannot be manipulated, genotype–environment correlations
QTL of synthetic populations in natural conditions	Progeny of controlled crosses can be observed in nature	Environmental relevance, fitness components as LH traits	QTLs contain large genome regions, gene scale is difficult
Evolve and resequence	Short generation time, easy to maintain in large numbers	Selection can be manipulated, causality can be confirmed in some species	Environmental relevance questionable, limited to species with specific LH
Candidate gene with controlled genetic background	Any in which genetic background effects can be controlled	Causality directly inferred, many phenotypes measured simultaneously	Most informative if combined with transgenics, relevance to natural variation questionable
Comparative genomics	Any	No taxonomic or LH bias, large differences between taxa	Inference about history of selection and role of constraints is indirect