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Testing hypotheses regarding the genetics of adaptation

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Abstract

Many of the hypotheses regarding the genetics of adaptation require that one know specific details about the genetic basis of complex traits, such as the number and effects of the loci involved. Developments in molecular biology have made it possible to create relatively dense maps of markers that can potentially be used to map genes underlying specific traits. However, there are a number of reasons to doubt that such mapping will provide the level of resolution necessary to specifically address many evolutionary questions. Moreover, evolutionary change is built upon the substitution of individual mutations, many of which may now be cosegregating in the same allele. In order for this developing area not to become a mirage that traps the efforts of an entire field, the genetic dissection of adaptive traits should be conducted within a strict hypothesis-testing framework and within systems that promise a reasonable chance of identifying the specific genetic changes of interest. Continuing advances in molecular technology may lead the way here, but some form of genetic testing is likely to be forever required.

Introduction

How should we view historical developments in evolutionary genetics through the particular lens of the genetics of adaptation? Although it is perhaps a bit premature for such pronouncements, one could argue that we are entering a new era of modern evolutionary genetics. The first era, roughly from 1918–1968, was characterized by the theoretical developments in population and quantitative genetics that have laid the foundation for nearly all other work in evolutionary biology (Figure 1, see also Provine, 1971). This period began with the theoretical reconciliation of quantitative and Mendelian genetics by R.A. Fisher (1918) and rapidly expanded into the codification of population genetics theory in the 1920's and 1930's through the work of Fisher, Sewall Wright and J.B.S. Haldane. It runs on through the beginnings of ecological genetics by the likes of E.B. Ford and others and the application of population genetic principles to natural populations led by Theodosius Dobzhansky. It ends with a formalization of earlier models by Gustave Malécot and Motoo Kimura into a framework that set the stage for the utilization of the truly genetic data that was soon to follow (Lewontin, 1974). This period could be classified as theory rich and data poor. Most of the theory that we still utilize today was established before we had any knowledge of the nature of the genetic material, and in this sense these approaches are essentially purely genetic and largely devoid of functional context. Fundamental concepts of genetic entities like loci and alleles have hardly changed in population genetics theory, despite tremendous advances in our knowledge of the physical and molecular properties of genes and genomes.

The second era, from 1968 to 1998, was dominated by an explosion of data, frequently collected in the absence of a compelling theoretical context (Lewontin, 1991). In population genetics, the development of protein electrophoresis

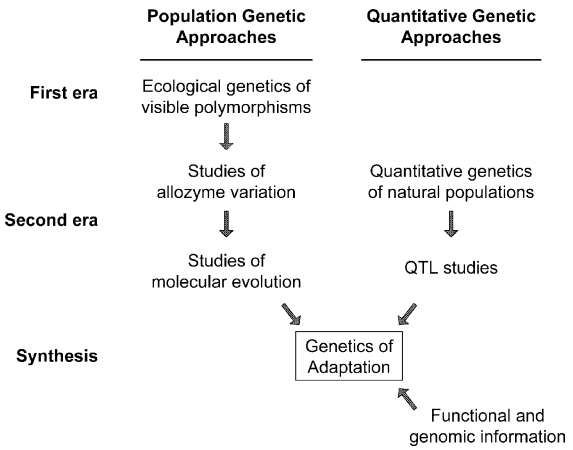

Figure 1. Transitions during the history of population and quantitative genetic approaches to studying the genetics of adaptation. Movement toward a new era of study incorporates these approaches with functional genomic information.

allowed researchers to assess levels of genetic variation in a wide variety of organisms rather than being limited to special cases of known genetic markers (e.g., Drosophila chromosomes) or obviously Mendelizing phenotypes (e.g., snail shell polymorphisms). On the quantitative genetic side of things, the theories originally developed by Fisher and greatly expanded by Wright were finally migrated from agricultural systems into a more formal theory of evolutionary quantitative genetics (e.g., Slatkin, 1970; Lande, 1976; Felsenstein, 1977). Here again, researchers could venture into natural populations to ask questions about levels of genetic variation for ecologically important traits. It seemed that no study of the evolutionary ecology of quantitative traits could be complete without an analysis of underlying genetic variation, because evolutionary change is predicated on its existence. To some extent, both the population and quantitative genetic approaches were victims of their own success. Electrophoretic studies revealed ample levels of genetic variation at most loci, while quantitative genetic studies found significant heritability for most traits. Finding genetic variation for its own sake became a hypothesis-free endeavor. Enough studies of this type have now been performed that one need not actually conduct the studies to know their probable outcome. For the most part, average heterozygosity will vary between 0.05 and 0.2 and heritability will fall somewhere between 0.2 and 0.5. Even if a particular estimate where off by a factor of two or three, would the discussion sections of these particular studies be very different? It is unlikely that they would, which is a testament both to a general lack of precision in these estimates and the lack of a broader hypothesis-testing framework for this work.

Studies of variation per se have developed on one side into much more sophisticated treatments of DNA sequence variation from a molecular evolution viewpoint and on the other side into a formal theory of evolutionary quantitative genetics that treats the entire organism as an integrated whole (Figure 1). Using sequence data, we can address very specific hypotheses regarding historical patterns of selection and rates of evolution of genes of interest, but are frequently far removed from the how, why, what, and where of the adaptive context of that selection. In contrast, in multivariate views of quantitative inheritance, we can measure how selection operates on suites of traits and how trade-offs among traits might structure and constrain the response to selection (Lande, 1988), but are limited to some extent by complexities introduced by the total dimensionality of the system (Charlesworth, 1990) and by the fact that, in order to understand how summary parameters like genetic correlations themselves evolve, we need to have much greater knowledge of the genetic systems underlying these traits (Barton & Turelli, 1989). We are caught between molecular knowledge in the absence of adaptive context and ecological context in the absence of molecular details. One view of the modern challenge to understanding the genetics of adaptation is the need to span this chasm – to be able to move freely from sequence to phenotype to ecological context and, more importantly, to be able to test specific hypotheses at each of these levels.

Are we, then, at the beginning of a self-proclaimed new era? If so, then it is an era that is sure to be dominated by genomic analysis (the 1998 date was chosen because of the publication of the first metazoan genome during this year, The C. elegans Sequencing Consortium, 1998). The hope is to use our new abilities to look at genome-wide patterns of genetic variation and gene function to investigate the genetics of adaptation from multiple perspectives. The fear is that we instead will repeat the mistakes of previous technological transitions and collect information in the absence of definitive hypothesis tests; or worse,

Table 1. Some central questions in the genetics of adaptation

- How many genes underlie specific adaptations?
- What is the distribution of their effects?
- What is the spectrum of new mutations at these genes?
- How do these genes interact with one another?
- Do genes tend to affect traits independently of one another or do genes typically have manifold effects across the whole organism (i.e., pleiotropy)?
- How does natural selection affect the distribution of effects and/or the nature of the interactions?
- Does the response to selection tend to occur more frequently through changes in gene regulation or gene structure/function?
- What is the relationship between loci that generate variation within populations and those responsible for differences among populations?
- How can we combine these insights into an understanding of the evolution of developmental systems, morphology, behavior, etc.?

over-interpret the results that we are capable of collecting right now without appreciating the limitations inherent in our current methods.

Questions and hypotheses

It is not difficult to collect a long list of questions that we would like answered regarding the genetics of adaptation (Table 1). Primary among these are the most basic, like how many genes are involved, what are the distribution of the effects of alleles at these loci, and how does standing variation and mutational input become converted by selection into the adaptive differences that we might observe today? We currently cannot answer these questions for any trait, for any organism, for any natural system. It would therefore seem that we have a long way to go before we can address even the most basic questions in what should be a central area of evolutionary genetics. Many people, of course, are trying to tackle one or another of this broad set of questions, but if we are not careful we will find ourselves in same state as those studying the allozyme variation and heritability a few decades ago: lots of information and precious little context within which to evaluate that information. We can already guess that adaptive changes are sometimes going to be caused by a few loci and sometimes by many more. Some loci are undoubtedly going to have large effects while others will have smaller effects. Sometimes standing variation will be central, other times novel mutations will be essential. Collecting the basic pieces of information underlying the genetics of adaptation is obviously going to be important, but as with earlier revolutions in evolutionary genetics, will our level of resolution be sufficiently adequate to estimate the needed underlying parameters in such a way that estimation alone will be sufficient justification for conducting the work? We can avoid these pitfalls by making sure that the work that we do is conducted within a specific hypothesis-testing framework.

The essential problem with studying adaptation in natural populations is that we have no control over the genetic system. Genomes are vast and important change can potentially be anywhere. How then are we to find the genes of interest? More humbly, how effectively can we address questions related to the genetics of adaptation without actually having our hands on the genetic changes themselves? There are multiple approaches to this problem, each of which provides varying levels of precision (Figure 2). Each major approach can be seen as logical extensions of the two major branches of evolutionary genetics, and it is in their synthesis that we will finally be in a position to address fundamental questions about the genetic basis of adaptive evolution (Figure 1).

Mapping as a paradigm

Number of genes

If we are lucky enough (or choosy enough) to study a character that readily Mendelizes, we can at least to hope to map the gene with some precision. Moreover, we have prima facie evidence that we are dealing with at least one gene of major effect. Although there are important instances of changes of this sort (e.g., Crow, 1957; Peichel, et al., 2001; Nachman, Hoekstra & D'Agostino,

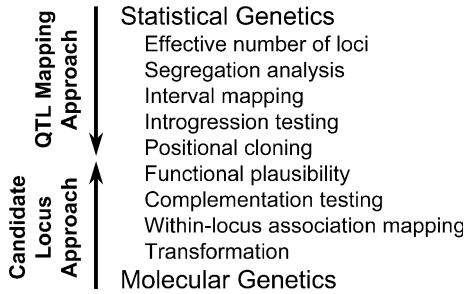

Figure 2. A hierarchical set of methods for determining the genetic basis of adaptive variation. A top-down, statistical genetics approach is built upon QTL mapping, while a bottom-up, molecular genetic approach is built upon identifying specific candidate loci. Confidence in a genetic causation increases as one moves from top to bottom.

2003), we might expect such systems to be outside the norm. Indeed, focus on these single-locus systems has resulted largely from the fact that they are more tractable than systems with more complex genetics. Once we move beyond a single locus, it is extremely difficult to estimate the number of loci affecting a trait simply by observing variation in the trait. In one of the first problems that he addressed, Sewall Wright (in Castle, 1921) derived an estimator for the *minimum effective number of loci* (the number of loci with equal effects) by assuming that two lines being crossed are uniformly divergent for the loci underlying the differences (Figure 2). Although there have been refinements of Wright's original approach (Wright, 1968; Lande, 1981), the method has so many caveats that its overall value beyond demonstrating that a trait is polygenic is questionable (Zeng, Houle & Cockerham, 1990; Zeng, 1992). A slightly more sophisticated approach that combines specific genetic models within the context of a defined pedigree, known as *complex segregation analysis*, is used frequently in human genetics (Figure 2, Khoury, Beaty & Cohen, 1993). Neither of these methods is likely to bring us very close to answering the most basic question of how many loci underlie a given adaptation, much less provide us with any hope of moving us further up the hierarchy of questions (Table 1).

Mapping

One of the more significant developments in evolutionary genetics over the last two decades has been the development of techniques aimed at mapping multiple genes underlying quantitative variation, *quantitative trait locus (QTL) mapping* (Figure 2, Mackay, 2001b). The promise here is that identifying specific regions of the genome responsible for quantitative differences between lines, populations and/or species will allow estimates of some of the fundamental parameters needed to understand the evolution of quantitative characters. While there can be no question that this is the right direction to be heading, we should be very careful not to over interpret the results obtained from such studies. Indeed, it can be argued that mapping per se gets us only slightly further along the road toward answering our fundamental questions than trying to estimate the genic effects directly from variance data.

The real problem is that QTL ('L' = loci) should have been called QTR ('R' = regions). There has been a pull toward creating a central dogma of 'one peak-one gene' in these mapping experiments. If such a one-to-one correspondence where possible, then we would indeed be well on our way to discovering the number of loci underlying specific adaptations. While the attraction of this notion is clear, our current limited experience provides reasons for caution. Mapping is based on linkage disequilibrium between markers that we can measure and QTL of unknown location (Lander & Schork, 1994). Maximizing linkage disequilibrium across the whole genome, as can be accomplished in controlled cross between two extreme populations, greatly enhances the probability that at least one of the markers will be found in association with the QTL of interest (Figure 3). This is a double-edged sword, however, since broad-scale linkage disequilibrium means that a potentially large non-informative chromosomal region surrounding the marker will also be linked to the QTL. This decreases the precision with which the location of the QTL can be identified (Figure 3).

Several decades ago, Coyne (1983) examined the genetic basis of difference in genital morphology between two Drosophila species using a single visible marker per chromosome arm. Each marker did indeed show a significant association with the morphological difference, but rather than conclude that each marker represented a single QTL, Coyne instead reasonably suggested that these differences were likely caused by a potentially large number of

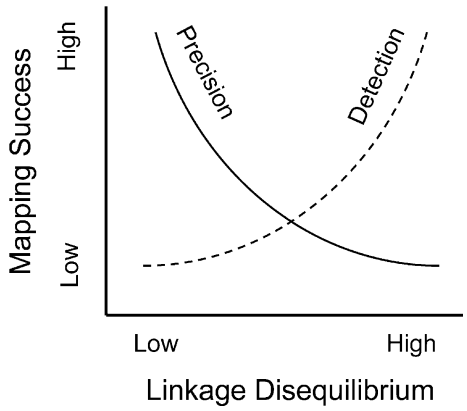

Figure 3. The trade-off between precision and detection as a function of the level of linkage disequilibrium within a population when trying to specific genes. Crosses usually generated in QTL mapping experiments have high levels of linkage disequilibrium and therefore have a large chance of detecting the underlying loci. They may have low precision for identifying where the loci are or even if there are indeed individual loci involved. In contrast, association mapping studies use outbred populations, usually with much lower levels of linkage disequilibrium. These studies require very large samples and very localized dense genetic maps in order to detect the loci involved, but they should in principle allow high precision in identifying the genes, and potentially the nucleotides, involved.

loci since his level of genetic resolution was so crude (a prediction that turned out to be correct, Zeng et al., 2000). We must be careful not to cavalierly equate regions of large effect with genes of large effect when we are in fact frequently barely a few steps beyond Coyne's level of resolution, even with the advent of large numbers of molecular markers. For example, using a high-resolution deletion mapping study of longevity in *Drosophila melanogaster* layered on top of a traditional QTL analysis, Pasyukova, Vieira & Mackay (2000) demonstrated that many of the QTL peaks obtained from a standard cross in fact housed several loci, frequently with opposing effects.

A more fundamental problem for interpreting mapping results is that we may be dealing with a scale of resolution that is simply impenetrable to traditional mapping approaches. Although in many applications of QTL analysis, such as in human health, it may be sufficient to simply identify the locus of interest, in evolutionary studies a 'locus of large effect' and a 'substitution of large effect' should not be equated (Phillips, 1999). The potential confusion is derived from typological definitions of concepts like 'locus' and 'allele' that span the last one hundred years of evolutionary genetics, but which are at odds with modern understanding of genetic change. Two very distinct 'alleles' may segregate in a cross between populations, but the alleles themselves may be the products of many substitution events over the evolutionary history of the divergence of those populations. The concept of 'locus' in theories of the genetics of adaptation may be quite different from traditional definitions of locus – we may frequently need to look for multiple changes within individual genes (e.g., Orr, 2002). The best example of this is Stam and Laurie's (1996) study of functional variation at the ADH locus in *Drosophila melanogaster*. They found that most of the difference in levels of gene expression could indeed be explained by the traditional fast/slow replacement that leads to differences in allozymes, but also that a secondary and very significant effect is generated by an epistatic interaction between two control regions within the gene that is also part of the 'allelic' difference in this case. Even high resolution QTL mapping will not allow us to detect complex changes and interactions occurring within genes. The importance of resolution at this scale is likely to depend on the general ubiquity of complex regulatory systems within genes (Davidson, 2001), but much of the future challenge of the functional genetics of adaptation lies firmly here.

Otto and Jones (2000) provide a method for extrapolating from the estimated number of QTL to the like number of 'true' QTL by assuming certain distributions of effects. Approaches such as this are surely improvements on the Wright-inspired estimators, but these methods will be strongly limited by the resolution of the map, as indicated above.

Distribution of allelic effects

Ignoring the problem of counting genes for a moment, to what extent can we expect to be able to infer the nature of the effects of the genes that we do find, especially with an eye toward estimating the distribution of effects (Orr, 1998)? There are several obstacles here as well. Because QTL are recognized statistically as genomic regions yielding a significant association with phenotypic variation, when sampling errors lead to an overestimate of the size of an effect, that effect is more likely to be classified as a QTL. This

leads to an upward bias in estimated effect sizes, which becomes especially magnified in studies with low statistical power (Beavis, 1994, 1998). Lack of resolution can also lead to misestimates of the distribution of effect sizes when more than one gene is located within a QTL region. For example, even when the actual distribution of effects is constant or uniform, it is possible to wrongly infer a negative exponential distribution of effect sizes when a large number of loci are randomly distributed throughout the genome (Bost et al., 2001). When regions of the genome below the mapping resolution threshold accumulate a number of true QTL, the single estimated effect size will be closer to the sum of those QTL than to the effect size of the individual elements (see Noor, Cunningham & Larkin, 2001). Thus, summary distributions of QTL effects are likely to be somewhat suspect in the absence a firm sense of the level of precision in the mapping itself. Finally, complications introduced from genetic interactions among loci (epistasis, Phillips, 1998) are only now starting to be explored because of complications in the analysis and issues of the scale of experiments needed to estimate such a large pool of pair-wise interactions (e.g., Kao & Zeng, 2002).

Mapping to test hypotheses

If mapping serves somewhat poorly for estimating the essential parameters of the genetics of adaptation, then what can it provide us? Apart from serving as an important step on the road toward finding the genes themselves, mapping can be used to test specific hypotheses regarding the genetics of adaptation. The essential point here is that the heart of hypothesis testing involves a comparison of some sort. Comparative mapping in well-articulated circumstances allows one to test the hypothesis of a shared genetic basis of traits across different environments or in different populations. Pleiotropy is the hypothesis of interest when looking at variation within a population, while a parallel response to selection is the focal hypothesis when comparing populations. Unfortunately, much like paternity analysis, it is easier to exonerate a particular region of the genome than to implicate a specific gene. If two QTL regions can be clearly distinguished from one another, then the hypothesis of a shared genetic basis to the traits can be rejected (although it is still possible that similar genes are involved, but with very different effect sizes). If a similar location is identified, however, the precision issue discussed above rears its head again. Is this in fact the same gene being used in each case or simply another locus that happens to be linked to the target QTL region by chance? Fortunately in a comparative context, we can take the precision of our QTL estimates into account and actually test the hypothesis of pleiotropy quantitatively (Cheverud, Routman & Irschick, 1997; Lebreton et al., 1998).

The pleiotropy can be across different traits, such as different morphological features of a flower (Juenger, Purugganan & Mackay, 2000); across time, as in growth and change in body size in mice (Vaughn et al., 1999); and/or across environments such as larval density (Leips & Mackay, 2000) or geographic differences in growing conditions (Weinig et al., 2002). Parallel mapping across multiple divergent populations has rarely been performed, but even between-population crosses can be used to test for similar regions affecting the trait of interest in different genetic backgrounds (e.g., Zeng et al., 2000).

Getting to the genes

Ultimately, tests of pleiotropy and the genetic basis of adaptive differences in general will require finding the genes themselves – indeed the nucleotide changes themselves. At its heart, mapping is a correlational approach. To move closer to causation, it is necessary to verify hypotheses generated by mapping using more conventional genetics. One of the strongest approaches in this regard is *introgression testing* in which a genomic region containing a putative QTL is backcrossed into a common background and retested for its effects (e.g., Laurie et al., 1997). Repeated backcrossing can be used to generate near-isogenic lines, a springboard for the holy grail of QTL mapping, *positional cloning* of the locus (Remington, Ungerer & Purugganan, 2001). The level of effort needed to positionally clone a QTL from mapping data alone is quite daunting. At the very least, significant genomic resources will need to be brought to bear on the problem. While this may be feasible (and justifiable) in many cases in agriculture and human health, the general level of effort needed will remain a significant issue in the evolutionary genetics of non-model species until

technology takes another leap forward. For the time being, most studies in evolutionary genetics that use QTL mapping are likely to find themselves marooned upon QTL peaks surrounded by a sea of thousands of possible genes, with little means of identifying or distinguishing among them. This is why it is crucial to choose the level of resolution appropriate (and possible) to the hypothesis being addressed. The field as a whole will gain very little if the majority of studies become stranded halfway between ideals of causal explanation.

The candidate-locus paradigm

The alternative to the top-down approach of QTL mapping is a bottom-up approach based on candidate loci (Figure 2). Here, in-depth knowledge of gene function motivates the selection of a subset of genes that can be used as targets for genetic analysis. A possible first step here is to examine *functional plausibility* by examining differences in DNA sequence among the divergent populations of interest. Since most populations are likely to differ at many nucleotide sites, this is unlikely to be a particularly fruitful exercise, although information of this sort can be used in a broader molecular evolutionary context (e.g., Jovelin, Ajie & Phillips, 2003). The advent of the ability to perform genome-wide functional analyses, such as microarrays, has greatly expanded the set of 'plausible' targets, however (Gibson, 2002). For example, Wayne and McIntyre (2002) have combined gene expression data with QTL mapping results to develop the most likely set of functional targets to pursue as candidate loci in the studies of ovariole number in *D. melanogaster*. At present, these approaches are too new to know whether gene expression differences per se will be useful indicators of underlying genetic divergence. Expression at a given locus can be different due to changes at other loci and the total variance in expression may tend to overwhelm the available signal. Nevertheless, the potential for using this and similar technologies for hypothesis building, especially in non-model organisms, is tremendous (e.g., Oleksiak, Churchill & Crawford, 2002).

The best next step beyond simple plausibility is genetics. An especially powerful approach is to use a *quantitative complementation test* to examine variation in the genetic pathway involving the candidate locus (Doebly, Stec & Gustus, 1995; Long et al., 1996; Lyman & Mackay, 1998). This is an interaction test in which a line with a mutation at a given locus is crossed with natural variants with the aim of assessing allelic variation at that specific locus (Mackay, 2001a). In reality, the response could be due to variation at the locus of interest or a locus that interacts somewhere in the same pathway as the mutation, such that variation is exposed when tested against the mutant background. This approach can be generalized on a genomic scale using deficiency mapping with a very large set of tester lines (Pasyukova, Vieira & Mackay, 2000; Steinmetz et al., 2002).

Finer scale mapping of allelic differences can be addressed using *association mapping* (Figure 2, Mackay & Langley, 1990; Long et al., 1998). Here, QTL mapping is essentially being performed within a locus. In the balance between precision and detection outlined in Figure 3, association mapping is decidedly on the side of precision. The linkage disequilibrium utilized in an association mapping study is that present in the natural population after many generations of recombination. Association mapping looks to detect the resonance signal left behind from the appearance of the unique mutation that is now the target of interest. Because every mutation arises within a unique genomic background, it is in initially in complete linkage disequilibrium with every marker in that genome. Over time recombination will break these associations down until only the closest associations remain. This is how it works in principle. In practice, the pattern of linkage disequilibrium can be non-uniform over a given genomic region. Low detection thresholds suggest that sample sizes will frequently need to be very large for this approach to work in most outbred populations. It is important to note that the studies in which this approach has been more successfully been applied have used crosses to isolate the chromosomal region of interest against a stable genetic background so as to reduce the total level of genetic variation in the system (e.g., Long et al., 1998; Long et al., 2000). Most outbred populations are likely to require samples potentially orders of magnitude larger to overcome the 'needle in the haystack' nature of the entire approach.

Interestingly, a number of the studies that have been able to work down toward the level of

individual nucleotides have found their significant associations in control regions and introns rather than in coding regions (Phillips, 1999). This makes identifying the specific changes responsible especially difficult since we currently do not understand the language that describes gene regulation in the same way that we are able to understand how changes in coding regions change gene function (Stern, 2000). This also stands in stark contrast to mapping results for human disease genes, in which a small minority of changes appear to be regulatory in nature (Botstein & Risch, 2003). Resolution of this contrast with additional data will illuminate one of the more interesting long-term questions in evolutionary genetics: evolution via regulatory changes versus structural change (Table 1).

Finally, all of the approaches for finding genes underlying complex adaptations outlined above are essentially circumstantial. Any given study is likely to need to combine a number of different approaches to properly address a causal hypothesis relating to specific gene function. One remaining approach neatly solves this problem through a strong hypothesis test in an experimental context. *Transformation* of one natural allele with another allows for a direct test of allelism while completely controlling for the effects of genetic background. Unfortunately, transformation at this level of precision is difficult even in model systems like Drosophila and *C. elegans*. Yeast is currently the most capable system from genetic manipulation standpoint (Steinmetz et al., 2002). Techniques for transformation in Drosophila have also recently taken a large step forward in the context of testing adaptive gene function (Siegal & Hartl, 1998; Greenberg et al., 2003). Non-model systems being investigated in more meaningful ecological contexts will be hard pressed to meet this standard for the time being.

Conclusions

Running completely through the cycle of causation outlined in Figure 2 is likely to be difficult in most circumstances, and 'proof' that one has actually identified a gene underlying a specific adaptation quantitative trait has only been obtained thus far in a handful of circumstances (Glazier, Nadeau & Aitman, 2002). All of the successful cases have been in either agricultural or model systems. Will finding the actual genes underlying adaptations be feasible in most natural systems? To do so will require generating sufficient genomic resources such that non-model systems essentially serve as their own models. Rapid progress in genomic technology is making this more possible all of the time, but it is important to recognize the cost of this pursuit, both financially and in terms of the large set of potentially more tractable questions that are likely to be abandoned along the way (Lewontin, 1991). Furthermore, ultimate tests of genetic causation rely on actually being able to do genetics – the ability to perform crosses to test specific hypotheses. This will not be feasible in many non-model systems. If we cannot test the hypotheses we are setting out to study, is it worth beginning the endeavor in the first place?

A central question, then, is the extent to which we actually need to identify the specific genes underlying adaptive change to in order to address the big questions in evolutionary genetics. I contend that we do. Indeed I will go further to say that we need to know the specific nucleotide changes responsible. We cannot be distracted by allelism per se but instead need to concentrate on the pattern of substitution of specific variants that have arisen via natural mutations. This will not be easy or even possible in many instances, but the very fact that we are contemplating it suggests that we are indeed entering a new era.

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