Genetic Tools for Studying Adaptation and the Evolution of Behavior

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ABSTRACT: The rapid expansion of genomic and molecular genetic techniques in model organisms, and the application of these techniques to organisms that are less well studied genetically, make it possible to understand the genetic control of many behavioral phenotypes. However, many behavioral ecologists are uncertain about the value of including a genetic component in their studies. In this article, we review how genetic analyses of behavior are central to topics ranging from understanding past selection and predicting future evolution to explaining the neural and hormonal control of behavior. Furthermore, we review both new and old techniques for studying evolutionary behavior genetics and highlight how the choice of approach depends on both the question and the organism. Topics discussed include genetic architecture, detecting the past history of selection, and genotype-by-environment interactions. We show how these questions are being addressed with techniques including statistical genetics, QTL analyses, transgenic analyses, and microarrays. Many of the techniques were first applied to the behavior of genetic model organisms such as laboratory mice and flies. Two recent developments serve to expand the relevance of such studies to behavioral ecology. The first is to use model organisms for studies of the genetic basis of evolutionarily relevant behavior and the second is to apply methods developed in model genetic systems to species that have not previously been examined genetically. These conceptual advances, along with the rapid diversification of genetic tools and the recognition of widespread genetic homology, suggest a bright outlook for evolutionary genetic studies. This review provides access to tools through references to the recent literature and shows the great promise for evolutionary behavioral genetics.

Keywords: behavior genetics, G × E, genetic architecture, linkage, molecular techniques, QTL, quantitative genetics.

Behavior, like other complex traits, shows extreme phenotypic variation and flexibility and integrates multiple levels of inputs. It shares these attributes with developmental and life-historical characters (Price and Schluter 1991; Merilä and Sheldon 1999, 2000; Kruuk et al. 2000; Raff 2001; Arthur 2002). Unlike the majority of developmental and life-history characters, behavior has the additional complexity of being both subject to selection and a major agent of selection within the same species. Evolutionary biologists are therefore often attracted to the study of behavior due to its complexity and variability, but these characteristics present a major challenge to understanding the evolution of behavior.

The study of the genetic basis of phenotypic variation in developmental and life-history characters has been the key to a deeper understanding of the constraints and forces shaping the evolution of these complex characters (Merilä and Sheldon 1999, 2000; Kruuk et al. 2000; Raff 2001; Arthur 2002). Behavior that is studied in an ecological or evolutionary context has been the subject of far fewer genetic analyses than development or life history. This may be due to the lack of genetically tractable natural systems (Wolf 2001) and the behavioral ecologist's focus on field studies, in which genetic analyses can be extremely difficult. In addition, behavioral ecologists often see genetic analyses as unnecessary or irrelevant to understanding be-

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havioral evolution (i.e., "the phenotypic gambit"; Grafen 1984). However, as we describe in this review and as shown by the authors of the articles in this issue, it is increasingly possible to use genetic approaches to answer long-standing questions concerning the adaptive significance and evolution of behavior in natural systems.

In this review, we consider questions that could be addressed with three types of genetic tools for studying the evolution of behavior. First, we discuss genetic questions that can be addressed using techniques that are available for most organisms, that is, questions that do not depend on the latest genomic technology. Second, we address molecular issues directly, describing the attempts to integrate analyses that begin with phenotypes and aim to understand the molecular genetic control of specific traits (or "top-down" approaches) and the related attempts to understand how specific genetic pathways lead to phenotypic outcomes (or "bottom-up" approaches). A major challenge for the future will be integrating these top-down and bottom-up approaches. Finally, one of the most complex aspects of behavior is the role of genotype-by-environment interactions, especially when the environment is the behavior of other individuals. We review new directions in modeling efforts and new empirical studies that are designed to address this emerging topic.

Genetic Questions That Can Be Addressed with Crossing Studies or Quantitative Genetic Approaches

Not all questions in the evolution of behavior require the latest molecular methods. The most fundamental question is, Is there evidence for a genetic influence on the behavioral trait? Another question is whether genes affecting related behaviors are physically linked, which would facilitate their coevolution. A more demanding set of questions includes the magnitude of genetic influences on a trait and the degree to which traits are genetically correlated; these questions help in the development and testing of evolutionary models. Finally, behavioral ecology is particularly concerned with identifying the adaptive function of behaviors and sometimes assumes that behaviors are under strong selection; genetic analyses can be valuable in understanding past selection.

Is There a Genetic Influence?

Identifying a heritable influence is the starting point for all other genetic analyses. The simplest approach is to ask whether a behavioral variant runs in families, which may be feasible in field studies of at least two generations of marked individuals, even if the number of families is too small for quantitative estimates of genetic variables (see below). Familial resemblance can be caused by nongenetic factors, such as a common environment (Falconer and Mackay 1996), but without a familial resemblance there is unlikely to be a genetic influence. We repeat an earlier statement (Arnold 1994; Dingle 1994; Lynch 1999) that it may not always be necessary or even desirable to estimate the heritability of a trait. In many cases, knowing that a particular variant of a behavior runs in families provides powerful information that can be used in evolutionary arguments as easily as can heritabilities. This means that those who study behavioral ecology can answer significant genetic questions without resorting to the latest molecular or statistical methods.

If the animals can be bred experimentally, then reciprocal crosses may reveal a genetic influence; linkage to sex chromosomes can often be identified by simple crosses (Reinhold 1998; Ritchie 2000). Another important form of experimental breeding is artificial selection; traits that change in response to selection do so because they are under genetic control (Dingle 1994; Hoffmann 1994; Lynch 1994). These studies can be simple to conduct. The information that behavioral variation is under genetic control is still so uncommon in the literature that studies in new taxa or of new categories of behavior are still a valuable contribution to understanding behavioral diversity.

Coevolution and Sex Linkage

The topics of coevolution and sex linkage are associated because both may be affected by physical linkage, that is, the close association of loci on the same chromosome. Animal communication contains a major coevolutionary question: To what extent is genetically based variation in signals genetically correlated with variation in receiver properties? The second question is whether traits that are sex linked evolve in different ways from traits controlled by autosomal genes.

Communication. Genetic correlations are the basis of Fisher's (1958) famous model for the evolution of exaggerated characters by mate choice (Lande 1981); genetic variation in the male signal is correlated with genetic variation in female preferences, which can lead to an evolutionary "runaway" if the signal or preference is altered. However, recombination each generation will tend to break down genetic correlations that are maintained by mate preference. Physical linkage of genes, both by close position on one chromosome or linkage to areas of reduced recombination such as sex chromosomes and in-

versions, can reduce recombination and thus make the conditions for evolution less stringent (Trickett and Butlin 1994). One such case of behavior associated with linkage within an inversion is found in the seaweed fly, *Coelopa frigida* (table 1; Gilburn and Day 1999). In general, linkage of any sets of genes whose fates are evolutionarily coupled (such as any aspect of signal receiver and sender phenotypes) to regions of reduced recombination could help maintain such genetic correlations (Lindholm and Breden 2002, in this issue).

Sex Linkage. Studies examining the linkage of genes controlling behavior to sex chromosomes have concentrated on reproductive isolation and sexual selection, mostly in invertebrates. Ewing (1969) suggested that reproductive behaviors might be disproportionately linked to sex chromosomes. However, in a recent review Ritchie and Phillips (1998, p. 302) concluded "there is little convincing evidence that sex-linked genes commonly provide a disproportionate effect except in the Lepidoptera and perhaps the Orthoptera." In contrast, a broad review of studies that included comparisons between reciprocal F_1 crosses (Reinhold 1998) suggested a strong effect of the X chromosome on characters involved in sexual selection, many of which were either mating or signaling behaviors. Examples of linkage of behavior to sex chromosomes are provided in table 1. In addition, behaviors linked specifically to the heterogametic sex chromosome (Y or W) are discussed by Lindholm and Breden (2002, in this issue). Studies from a broad range of organisms are necessary before it will be possible to know whether mating behavior is disproportionately linked to sex chromosomes. Identification of such regions can be accomplished by polytene chromosome preparations (Strickberger 1962) or in situ hybridization (Nanda et al. 1990). In some cases, linkage to inversions can be easily assayed by allozyme markers (Gilburn and Day 1994).

Goals of Quantitative Genetic Studies

Quantitative genetic methods (also known as biometrical genetics or statistical genetics) use the resemblance among relatives due to shared genotypes to study inheritance (Arnold 1994; Falconer and Mackay 1996; Lynch and Walsh 1998). These measures estimate the proportion of phenotypic variation contributed by the genetic effects of additivity, dominance, and the various forms of epistasis. The resulting estimates of genetic variances and covariances (or heritabilities and genetic correlations) can be used to address evolutionary genetic questions (Arnold 1992; Arnold et al. 2001). These parameters allow one to predict (see Deng et al. 1999) or possibly reconstruct the evolutionary response to selection (Lande 1979; Schluter 2000), as well as to characterize constraints on multivariate evolution (Cheverud 1984; Wagner 1988). Quantitative genetic analyses have been used to examine a wide variety of behavioral questions (Boake 1994) and have led to many insights into behavioral evolution. Importantly, many questions about the evolution of behavior are themselves genetic in nature, which requires a genetic approach. Examples include the dynamics of evolution by mate choice (Fisher 1958) and the role of kin effects in the evolution of sociality (Hamilton 1964*a*, 1964*b*), both of which have developed into areas that contain large numbers of genetic models and genetically based tests.

Quantitative genetic analyses are valuable for many questions about the role of inherited influences on behavior. These questions include the relative importance of genetic and nongenetic influences on behavioral variation; the genetic correlational structure of suites of behaviors; the interaction of genes with the environment; and the influence of inbreeding, outbreeding, and genetic drift on genetic variation. Quantitative genetic analyses also provide an important starting point for other genetic analyses. For example, embarking on a genetic mapping study of a trait is only worthwhile if heritable variation can be detected.

Table 1: Examples of cases where genetic influences on behavior have been associated with X chromosomes or inversions

Behavior	Species	Type of effect	Citation
Mate choice	*Coelopa frigida* (affil.)	Linkage between genes for male size and female willingness to mate are within an inversion	Gilburn and Day 1999
Behavioral isolation: cuticular hydrocarbon profile, courtship wing vibration, and female preferences	*Drosophila pseudoobscura*, *Drosophila persimilis*	Traits map to inversions on the X and second chromosomes	Noor et al. 2001
Phototaxis	*Drosophila melanogaster*	Suggested linkage to an inversion	Markow 1975
Dispersal	*Drosophila subobscura*	Suggested linkage to an inversion	Gosteli 1991
Preference for song type	*Ephippiger ephippiger*	X chromosomal effect	Ritchie 2000

Estimating Quantitative Genetic Parameters in the Field

Behavioral ecologists who wish to study inheritance in free-living natural populations, which is critical to ensuring that the traits examined are ecologically relevant, have the problem of not being able to control matings. Quantitative genetic information can be obtained from such populations in at least three ways. First, purely phenotypic measures can be used to estimate genetic correlations in natural populations (Lynch 1999; Ferguson and Fairbairn 2001), and thus some quantitative genetic information can be gleaned even in the absence of information on relatedness. Second, behavioral ecologists sometimes study populations for many generations and have data from which pedigrees can be determined. Alternatively, molecular information can be used to reconstruct pedigrees. Pedigree-centered approaches have been successfully applied to morphological (Cheverud and Dittus 1992; Milner et al. 2000) and life-history characters (Kruuk et al. 2000; Merilä and Sheldon 2000) and should also work for behavior. Cross-fostering has been used successfully for studies of the genetics of bird behavior in natural populations (e.g., van Noordwijk 1984; Kölliker et al. 2000); this involves knowledge of pedigrees plus it controls for the effects of rearing environments. Finally, Ritland (1996, 2000*a*, 2000*b*) developed an approach that makes use of highly variable genetic markers to determine pairwise relatedness in natural populations (Lynch and Walsh 1998; Lynch and Ritland 1999; Thomas and Hill 2000). The marker data are then combined with quantification of phenotypic similarity to estimate quantitative genetic parameters (Ritland 1996, 2000*a*, 2000*b*; Lynch and Walsh 1998; Mousseau et al. 2000; Thomas et al. 2000).

Estimating Quantitative Genetic Parameters in the Laboratory

Laboratory studies allow analyses of additional levels of genetic questions, such as the role of genetic dominance, maternal effects, epistasis, paternal effects, or the extent of genotype-by-environment interaction (maternal effects and genotype-by-environment interactions are described later). These analyses require large sample sizes, complex breeding designs, and controlled environments (Falconer and Mackay 1996; Sokolowski 2001). The requirement of large sample sizes means that the behavioral traits to be studied need to be easy to measure (the approach taken by Williams et al. 2001) or else an army of assistants must be recruited and trained (Meffert et al. 2002, in this issue). Another possibility is to use pedigree information where available (Lynch and Walsh 1998). The breeding designs can extend beyond full- or half-sib crosses to diallels or multienvironment rearing schemes (de Belle and Sokolowski 1987; Via and Hawthorne 2002), which are necessary to take full advantage of the available genetic statistical tools to tease out potentially subtle genetic factors such as epistasis and indirect genetic effects.

The laboratory also allows greater control of environmental influences on a trait, although the laboratory environment usually differs from the one in which the species evolved (Hoffmann 2000). In the laboratory, it is possible to rear members of a family independently, or at least in a split-brood design, so that genetic and environmental factors are not confounded statistically. Recent models and empirical studies of social effects on behavior (Moore et al. 1997, 1998, 2002, in this issue) show clearly that social effects need to be considered and controlled.

Quantitative genetic analyses are valuable in cases such as evaluating evolutionary models or when so little information about the genetics of particular characters is available that even a qualitative picture is valuable. For example, major questions persist in cases where certain kinds of behavior co-occur, such as aggression and fear in spiders (Riechert and Hedrick 1993) or sending and receiving in signaling systems (Boake 1991). Is such co-occurring variation maintained by physical linkage, or can recombination break them apart? Is the magnitude of additive genetic variation consistent with rapid or slow evolution of these traits? For many other kinds of behavioral traits that have been extensively studied at the phenotypic level, such as parental care or social dominance, few genetic data exist (see reviews in Moore et al. 2002, in this issue; Peripato and Cheverud 2002, in this issue).

Quantitative Genetics and the Ghost of Selection Past

One of the most difficult but important tasks in trying to understand the evolution of traits concerns distinguishing traits subject to strong selection from neutral traits. The direct approach is to use variation in fitness and behavior to measure selection (Lande and Arnold 1983), but this has seldom been accomplished with behavioral traits. Can identifiable fingerprints of selection be found in the genetic control of behavior, and can they tell us whether selection is neutral, directional, or stabilizing (e.g., Merilä and Sheldon 1999)? Below, we review the relationship between the magnitude of additive genetic variation and selection and the evidence provided by the direction of dominance. In the section on molecular methods, we will revisit the topic of past selection.

A prediction following from Fisher's fundamental theorem of natural selection (Fisher 1958) is that at equilibrium the additive genetic variation of traits closely related to fitness, such as those experiencing strong selection, should be close to zero (Robertson 1966, 1968; Turner 1969; Crow and Kimura 1970), although only additive

genetic variation in fitness itself is zero at equilibrium (Crow and Nagylaki 1976). This prediction is well supported by breeding studies (Falconer and Mackay 1996). In behavioral ecology, additive genetic variation of fitness is fundamental to "good genes" interpretations of mate choice, and initially, this "secondary theorem of natural selection" (Robertson 1968) was likewise invoked to suggest problems for sexual selection theory (Maynard Smith 1978). Spurred by this challenge, researchers have measured genetic variation in sexually selected characters. It is clear that behavior often exhibits a high level of additive genetic variation and thus does not conform well to the expectation for traits experiencing strong selection (Pomiankowski and Møller 1995; Merilä and Sheldon 1999). Recent analyses therefore attempt to explain why genetic variation may be maintained in populations (Alatalo et al. 1997; Roff 1997). For example, condition dependence of male traits chosen by females may effectively increase the number of genes with the potential to influence expression of the behavioral trait, leading to the "capture" of genetic variance (Rowe and Houle 1996; Kotiaho et al. 2001). The question for behavioral traits has now become, In which cases do we expect additive genetic variation to be low?

Another consequence of selection on genetic parameters was proposed by Fisher (1958) and elaborated by Mather and Jinks (1977). Traits that evolved under selection were predicted to show strong dominance effects. They further proposed that directional selection favoring extremes of the trait will lead to directional dominance. Thus, for example, if all the alleles increasing tail length in a peacock are dominant to those that decrease tail length, we can infer that selection favored long tails. However, if significant dominance is present but ambidirectional—as likely to decrease as increase tail length—then the trait was probably subject to strong stabilizing selection. Mather and Jinks (1977) developed crossing designs that allow analysis of the incidence, significance, and direction of dominance and other nonadditive genetic effects, with heritability being of secondary interest. Another potentially valuable technique is the triple test cross (Hewitt and Fulker 1981; Lynch and Walsh 1998), which consists of crossing wild-caught individuals with laboratory strains and allows direct measurement and comparison of genetic architecture in the field and laboratory. This approach has shown that both laboratory and wild-caught rats have similar genetic architecture for escape avoidance conditioning (Hewitt et al. 1981; Hewitt and Fulker 1983).

One limitation of the literature on the significance of dominance effects and behavior is that most of the research was conducted by behavioral geneticists on laboratory strains (Lynch 1994). Two exceptions are Fulker's (1966) studies of *Drosophila melanogaster* and Gerlai et al.'s (1991) work with paradise fish. Lynch (1994) compared crosses of wild house mouse populations to crosses of inbred strains and found similar patterns of directional dominance, suggesting that differences between natural populations resulted from selection. Unfortunately, little or no work has directly addressed whether the direction of dominance effects actually tells us the evolutionary history of a trait. The observation that generally advantageous traits are indeed more likely to show directional dominance is reassuring (see Crnokrack and Roff 1995), but more tests are needed in which selection on the trait is inferred independently and compared with the genetic architecture of the trait.

Integrating Quantitative Genetic and Molecular Methods to Address Evolutionary Questions

Studies that use molecular methods may begin with the phenotype or phenotypic variation and attempt to identify the genes that affect the phenotype. Alternatively, they may begin with an analysis of mutants of a gene that are known to affect a trait. These two approaches are called top down and bottom up, respectively (Takahashi et al. 1994; Bucan and Abel 2002). At the moment, there appears to be a gap between the two approaches, represented by the large question mark in figure 1. Below, we describe the two approaches in relation to behavioral studies and propose ways to bridge the gap.

Molecular methods can be used in behavioral genetics to address questions about the nature of selection on traits, their future evolution, and the nature of complexity. Some questions are more appropriate for analysis by one method or the other while a few, such as understanding past selection, can be addressed through either a top-down or bottom-up approach.

Top-Down Approaches

Statistical quantitative genetic methods provide initial evidence for genetic influences on behavior (e.g., Shaw 1996, 2000) that are the starting point for the top-down procedures such as QTL (quantitative trait locus) studies (e.g., Shaw and Parsons 2002). A top-down approach is used to ask about genetic architecture: the number of genes affecting a trait, their approximate chromosomal locations, their linkage groups, the relative strengths and direction of effects, and their interactions. Genetic architecture has been shown to be important in models of founder-effect speciation (Gavrilets and Hastings 1996), female mating preferences and speciation (Kondrashov and Kondrashov 1999; Gavrilets 2000; Shaw and Parsons 2002), and in other evolutionary studies (Orr 1998*b*; Goodnight 2000). Quantitative trait locus analysis, which combines quantitative genetic approaches with genome-wide mapping, is espe-

Top Down ("forward genetics")		
Level of Analysis	**Approach**	**Data**
Existence/Magnitude of genetic variation, potential constraints	Statistical Genetics -Breeding Studies -Selection -Ritland Approach	Behavioral phenotypes plus information on relatedness
Genetic architecture	QTL -Interval mapping -Divergent populations, inbred lines, etc.	Behavioral phenotypes plus genetic map
?		
Gene expression and interaction	Genomics and Proteomics -microarrays -EST libraries	Expressed genes, DNA sequences
Effects of single genes	Analysis of mutations -Knock outs -RNAi -mutant screens -transgenics	DNA sequences, mutant phenotypes
Bottom Up ("reverse genetics")		

Figure 1: A comparison of top-down and bottom-up approaches, with examples of the methods applicable to each level of analysis. Top-down approaches to genetics start with phenotypic variation and attempt to understand its inheritance or genetic architecture. Bottom-up approaches start with genes and investigate how their expression affects the average phenotype. The question mark refers to the current need to develop ways to integrate these two approaches.

cially useful for elucidating genetic architecture (see Lynch and Walsh 1998; Sokolowski and Wahlsten 2001; Walsh 2001; Barton and Keightley 2002).

In a QTL analysis, the genotypes of many polymorphic markers per chromosome (generally molecular) are correlated with phenotypic values, producing a profile describing the likelihood that specific genomic regions contain genes that affect the trait in question (see Liu 1997). Furthermore, additive, dominance, and epistatic effects of loci can be estimated independently. Quantitative trait locus analyses are simplest when conducted using inbred line crosses or recombinant inbred lines. As of this writing, training in the statistical methods of QTL studies is offered by the leaders in developing this field through summer institutes at North Carolina State University and as an online course (http://statgen.ncsu.edu/statgen/index.html).

The value of QTL analyses for studies of behavior is exemplified by a study of host preferences in aphids (Hawthorne and Via 2001; Via and Hawthorne 2002; fig. 2). The authors showed that QTL controlling willingness to eat a host species and fecundity on the host are clustered in the genomes of two races of pea aphids adapted to different habitats. The authors suggest that this type of genetic architecture, in which physical linkage could reinforce genetic correlations, might characterize cases of rapid reproductive isolation between races adapted to divergent habitats. Thus, their results show how host preferences could lead to speciation, an idea that has received considerable attention since it was advocated by Bush (Bush 1969; Howard and Berlocher 1998). The list of QTL studies of behavior is expanding rapidly and includes alarm pheromones and foraging behavior of honeybees (Hunt et al. 1995, 1999); *Drosophila melanogaster* chemosensory behavior (Anholt and Mackay 2001), sexual isolation (Macdonald and Goldstein 1999; Williams et al. 2001), and courtship song (Gleason et al. 2002); mating preferences, calling, and speciation in a Hawaiian cricket (Shaw and Parsons 2002); and mouse anxiety (Turri et al. 2001), aggression (Brodkin et al. 2002), and parental care (Peripato and Cheverud 2002, in this issue).

Quantitative trait locus analyses provide information about the approximate genomic location of genetic factors

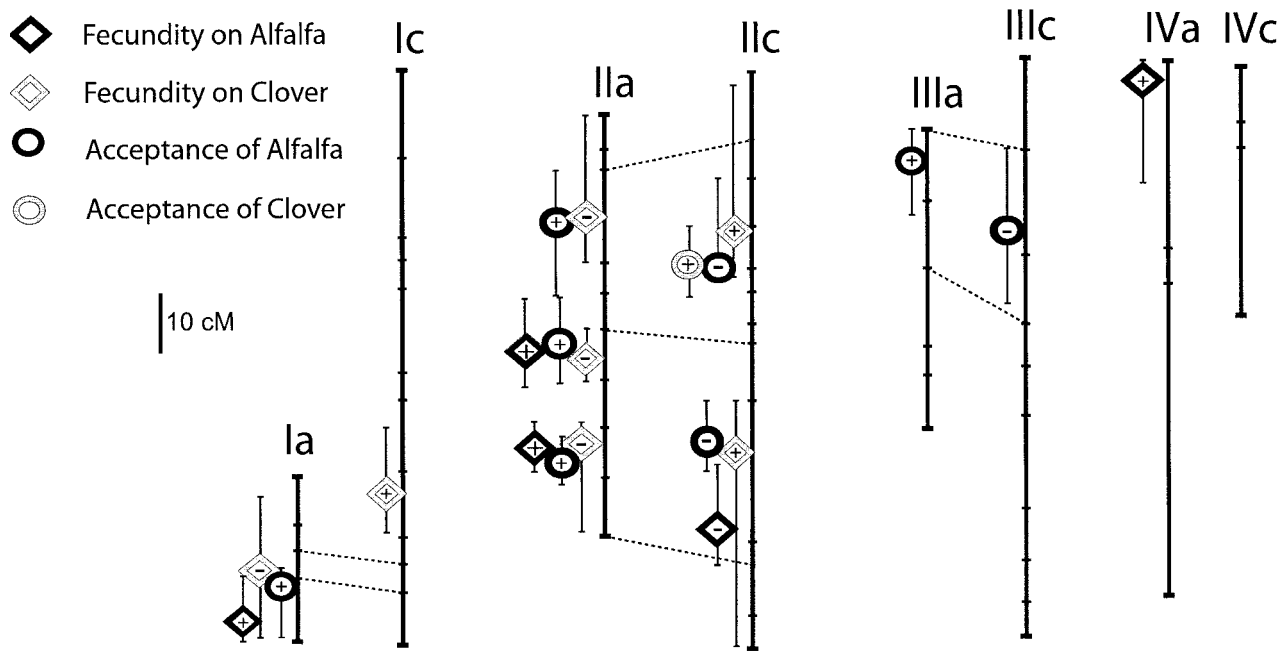

Figure 2: Quantitative trait loci (QTL) for fecundity on alfalfa and clover and acceptance of alfalfa and clover mapped onto chromosomes from two races of pea aphid (*Acyrthosiphon pisum pisum*). Chromosomes, labeled I–IV *a* and *c*, are from races specialized on alfalfa and clover, respectively. Horizontal tick marks indicate markers used to locate QTL within races; those connected between chromosomes from the two races (*a* and *c*) indicate codominant markers that were mapped in both groups. This study showed significant clustering of the QTL controlling these characters; such physical linkage could reinforce genetic correlations between these characters, ultimately enhancing reproductive isolation (modified with permission from Hawthorne and Via 2001; reprinted by permission from *Nature*, copyright [2001] Macmillan Publishers Ltd.).

that affect a trait, but the technique does not yet provide exact locations. Attempts to use QTL data to find actual genes are in their infancy, with the best example coming from a crop species (Frary et al. 2000). Quantitative trait locus data can lead to identification of candidate loci: previously identified genes that are located in the region of the QTL (see Rikke and Johnson 1998; McGuffin et al. 2001; Peripato and Cheverud 2002, in this issue). A newer approach to identifying the actual genes rather than just large chromosome segments is to couple QTL studies with microarray comparisons between lines or strains (Phillips and Belknap 2002). Progress in using QTL to identify the genes that affect behavior is therefore likely to be greatest in species that have had their genomes sequenced. Another major limitation is that each QTL study requires the cross of genetically distinct inbred lines, populations, or species. Each study provides data for a single cross and therefore for a single genetic background. Inferences about gene interactions (epistasis, genetic correlations, linkage) are specific to that particular cross. The studies are time consuming and labor intensive but until they are repeated in independent crosses with different genetic backgrounds, the generality of the results is unknown.

Bottom-Up Approaches

The bottom-up approach (Takahashi et al. 1994; Bucan and Abel 2002) focuses on genes first and refers to analyses of hierarchies of genetic control. This approach (sometimes called reverse genetics) is used by neuroethologists and behavior geneticists who wish to understand the physiological mechanisms that underlie behavior. As we suggest in figure 2, integrating this approach with approaches that emphasize phenotypic variation has scarcely begun. Below, we describe several bottom-up methods that have been used in behavioral studies.

Studies of Mutants. Spontaneous or induced mutations have been a key to identifying different classes of genes that affect behavior. Some mutations lead to major dysfunction rather than to the kind of phenotypic variation that is seen in natural populations; these "master genes" often control regulatory cascades during development (Baker et al. 2001). Analyses of mutants were used to identify a single master gene that determines the fundamental trait of the sex of an individual and thus permits the genetic analysis of all sex-specific behavior (e.g., Tomp-

kins 1984; Baker et al. 2001). Such genes can be studied to explore how the control of switches at different levels of the hierarchy of genetic control can lead to alternative behavioral expression (Bucan and Abel 2002).

Other genes, when mutated, cause smaller disruptions of behavior that resemble natural variation. Early examples were studies of courtship mutations that arose spontaneously in natural populations (Ewing and Manning 1967). Genetic influences on behavioral phenomena that have been well characterized in the laboratory with flies and rodents include foraging strategies (Sokolowski 1980; Osborne et al. 1997), circadian rhythms (Dunlap 1999), learning and memory (Dubnau and Tully 1998; Mayford and Kandel 1999), affiliative behavior (Young et al. 1999), and sexual behavior (Pfaff 1999). Remarkably, in fire ants, queen recognition (and therefore acceptance) is regulated by a pheromone-binding protein influenced by a single locus (Krieger and Ross 2002).

The analysis of mutants has long been a valuable source of information about genetic influences on behavior (Tompkins 1984; Greenspan 2001). However, screening for mutants is laborious and applicable to only a few species. It is also important to note that the act of identifying a behavioral effect of a mutated gene is not the same as finding a gene that contributes to variation in behavior in natural or captive populations. The genes of major effect that are usually identified in mutagenesis studies (e.g., master control genes) are often under extremely strong selection and as a result are rarely variable in real populations. Since evolutionary and ecological studies are generally focused on questions related to naturally occurring variation, results from mutagenesis studies may not be applicable. In this regard, long-term behavioral studies in which the adaptive significance of phenotypic variation is well understood can provide an important complement to mutagenesis studies of behavioral mutants in laboratory organisms. The function of mutants may also depend on genetic background, which is rarely varied, and may be compensated for by other systems (Phillips and Belknap 2002). The reviews of this topic (e.g., Baker et al. 2001; Sokolowski 2001; Bucan and Abel 2002; Phillips and Belknap 2002) illustrate the many steps that are needed to investigate the true function of a gene that influences behavior.

Studies of Gene Expression. Developmental changes in behavior are beginning to be related to changes in gene expression in the brain (Robinson 1999, 2002, in this issue). Emerging from these studies is the idea that information acquired by the nervous system on environmental and social conditions can induce changes in gene expression that in turn adaptively modify the structure and function of the nervous system (Robinson 1999; Clayton 2000).

Behavior can change on such a short time scale that the involvement of different genes through rapid changes in expression is difficult to investigate. New techniques may speed identification of genetic influences on behavior. Gene silencing by injecting double-stranded RNA, also known as RNAi (RNA interference), is relatively untested in animals outside of *D. melanogaster* and *Caenorhabditis elegans* but could be applied to other species (Caplen 2002; Schmid et al. 2002). Brain activities have also been studied by targeted mutations, which involves inducing or preventing the expression of genes that are known to have particular cellular functions. This method has revealed how genetic pathways affect aspects of memory (Steele et al. 1998) and is useful for investigating behavioral changes on a shorter time scale than development.

Profiling gene expression with DNA microarrays is a promising way to link phenotypic variation to underlying genetic factors (Brown and Botstein 1999; Walsh 2001; Gibson 2002). Microarrays are used to study gene expression by hybridizing the DNA from specific genes to cDNA produced by reverse transcription of mRNA taken from a specific tissue or cell type or at a specific stage of development. Variation can be surveyed with microarrays at almost any level—different species, populations, strains, lines, sexes, or individuals (Gibson 2002). The application of this technology to behavior studies is still narrow, but successes include detection of sex-specific or sex-limited gene expression leading to sexual behavior in *C. elegans* (Jiang et al. 2001), genetics of courtship and spermatogenesis in *D. melanogaster* (Labourier et al. 2002), and differences in behaviorally active (exercising) or inactive (quiescent) rats (Irwin 2001). Microarray technology can now be applied only to model genetic organisms, but we anticipate that evolutionary biologists will be able to adapt this procedure to other organisms within a few years (Walsh 2001; Gibson 2002). Microarray technology should permit rapid screening of a wide range of genes to determine the identities and interactions of those involved in a particular type of behavior.

Genetic Homology. Some very exciting developments for behavioral genetics are a result of the growing understanding of the ubiquity of gene homology across taxa. A well-publicized example is the report that a homologous gene specifies the location of eyes in different phyla (Halder et al. 1995). Similarly, homology of genes involved in clocks is found in taxa as diverse as *Neurospora*, *Drosophila*, and mammals (Lakin-Thomas 2000). A recent study of homology between genes that affect behavior shows that the well-characterized *foraging* gene in *D. melanogaster* is also associated with age-related changes in honeybee foraging behavior (Ben-Shahar et al. 2002). Growing evidence for gene homology suggests that it may soon be feasible to

conduct some kinds of genetic analyses on species that are now poorly characterized genetically.

An example of using gene homology and species comparisons to understand behavior is the study of social attachment in mammals (Young et al. 1999; Insel and Young 2001). Monogamous prairie voles (*Microtus ochrogaster*) show biparental care and extended pair bonds, whereas montane voles (*Microtus montanus*) are polygamous, and only females care for young. The social differences between these two appears to relate to genetic differences; the vasopressin V1A receptor gene of prairie voles has a 460-bp microsatellite insertion that the montane voles lack (Young et al. 1999; Insel and Young 2001). Dramatic evidence for the role of this genetic difference is shown by Young et al.'s (1999) creation of transgenic mice using the prairie vole V1A-receptor gene. Normal mice are solitary, but transgenic mice show significantly more tolerance of conspecifics, a prerequisite for a long-term pair bond (Young et al. 1999; Insel and Young 2001).

Molecular Quantitative Genetics and the Ghost of Selection Past

Orr (1998*a*, 1998*b*) suggested using QTL to identify selection. His logic resembles that for the distribution of dominance effects described above but concentrates on the distribution of the effects of QTL. Imagine that two bird species A and B differ in tail length as a result of the appearance of very strong female preference for long tails in species B. Alleles at QTL can be associated with either increasing or decreasing tail length. Any alleles that arose or were present in both species that increase tail length would be under strong directional selection and would tend toward fixation in B. Species A, however, would continue to tend toward males having intermediate tail length. Thus, contemporary studies would find a preponderance of positive alleles in species B and no pattern of effects (i.e., both positive and negative effects) in A. If, however, the trait had diverged due to drift, the distribution of effects in both species should be random. Only a few QTL studies have applied this test because in most studies too few loci have been detected to provide sufficient statistical power. Macdonald and Goldstein (1999) examined the distribution of QTL influencing genitalia shape in *D. melanogaster*, a trait that is usually thought to reflect sexual selection on males (Eberhard 1985). They found 11 QTL that influence one multivariate component of genital lobe difference. All varied in the same direction; substitution of alleles from the related species *Drosophila sechellia* made the genitalia more *sechellia*-like. Shaw and Parsons (2002) present preliminary data consistent with directional selection (preponderance of positive effects) on alleles of small effect in Hawaiian crickets, suggesting that speciation in these crickets reflects directional sexual selection.

One limitation to Orr's test is that the clear-cut differences are unlikely to be exclusively due to positive selection on one diverging species. Both species are likely to experience selection. Also, many behavioral traits may be subject to strong stabilizing selection, while only directional selection can be tested with the Orr model. Thus, some traits that would be most interesting to the behavioral biologist would appear selectively neutral to the evolutionary geneticist. Finally, because the Orr test relies on identifying enough loci to statistically distinguish between two hypotheses, it cannot be applied in many studies.

Nucleotide sequence variation underlying phenotypic variation in behavior can also be used to infer past selection. The tests that detect the effects of selection on nucleotide sequence divergence rely on contrasts between the rates of synonymous and nonsynonymous nucleotide substitutions (Yang and Bielawski 2000). If the rate of nonsynonymous substitution exceeds the neutral rate (measured as the rate of synonymous substitutions), then positive selection for divergence (diversifying selection) can be inferred. If the rate is significantly less, purifying selection can be inferred. Analogous tests for other phenotypic traits are based on quantitative genetic theory for evolution by drift in populations of finite size (Lande 1979; Lynch 1990). Such tests require estimates of genetic variances and covariances and have apparently not been applied to behavioral traits. The limitations of the sequence-based approach to detecting selection are discussed by Yang and Bielawski (2000).

Behaviorally relevant tests for selection at the codon level are limited by the availability of single genes or molecular sequences with clearly identified behavioral significance. A few behaviors are relatively well characterized (table 2). Possibly the best example is the *period* gene, a clock gene that was originally identified in *D. melanogaster*, which influences a wide range of circadian behaviors, including ultradian components of male courtship song. A codon-level test suggests that purifying selection has acted on this gene (Rosato et al. 1994), and field observations have suggested that the molecular polymorphism is associated with latitudinal variation (Sawyer et al. 1997). As more genes are identified, they can be similarly tested (e.g., Peixoto et al. 2000).

Integrating Top-Down and Bottom-Up Approaches in Studies of Behavior

Despite the incorporation of molecular techniques into both top-down and bottom-up approaches, a large gap still exists. The tension between those taking the top-down and bottom-up approach is not new, going back at least

Table 2: Tests of selection on behavior that suggest the involvement of single genes

Behavior	Species	Type of selection inferred	Citation
Clock, including cycling of male courtship signals	*Drosophila melanogaster*	Purifying	Rosato et al. 1994; Sawyer et al. 1997
Alternative larval foraging strategies	*D. melanogaster*	Stabilizing	Osborne et al. 1997
Cuticular hydrocarbons	*D. melanogaster*	Directional	Tsaur et al. 2001
Sperm-egg recognition	Sea urchins, genus *Echinometra*	Diversifying	Metz and Palumbi 1996
Gp-9 pheromone binding protein	*Solenopsis invicta*	Diversifying	Krieger and Ross 2002

as far as the debate between Mendelians and biometricians at the beginning of the last century. We see this integration as one of the major challenges in evolutionary genetics and one in which behavioral biologists may play a significant role by ensuring that the crucial evolutionary issue of variation is addressed.

One starting point for the integration is to unify bottom-up and top-down approaches in a single study. This is illustrated in studies that incorporate both QTL and candidate gene approaches (Belknap et al. 2001; Walsh 2001; Peripato and Cheverud 2002, in this issue). Nevertheless, as yet no direct link between QTL regions and a gene has been reported for behavioral traits (Belknap et al. 2001).

Another path to the synthesis of top-down and bottom-up approaches is through studies of development (Lewontin 1974; West-Eberhard 1998; Stern 2000; Arthur 2002). The wealth of information on the molecular genetics of development, demonstrating the conservation of many genetic mechanisms, suggests that one of the main ways that phenotypes may evolve is by changing the expression pattern of conserved developmentally regulated genes. Most of the current "evo-devo" research is centered on interspecific comparisons (Raff 2001; Arthur 2002), but ultimately we will need to understand intraspecific variation in the genetics of development (Stern 2000; Arthur 2002).

The most complete example that extends analyses from the level of variation in natural populations to genetic effects is Sokolowski's research program on the *forager* gene in *D. melanogaster*. She first showed that two alleles of a single gene were responsible for different larval foraging strategies, staying in one place or moving several centimeters (Sokolowski 1980). Subsequent studies have ranged from cloning the gene responsible for the behavioral variation (Osborne et al. 1997) to understanding the role of the gene in adult locomotion (Pereira and Sokolowski 1993) to studying the population genetics of this polymorphism (Sokolowski et al. 1997). What is needed now is to extend these sorts of analyses to multivariate genetic effects (Phillips and Belknap 2002).

The need to integrate top-down and bottom-up approaches is especially clear for questions about behavioral plasticity. What genes function during development to establish a nervous system that rapidly responds to changing circumstances? How does specific gene activity lead to different responses from an actively behaving animal? Understanding this relationship requires more information than can be obtained by studies of mutants, microarrays, QTL, or statistical genetics alone (Greenspan 2001; Phillips and Belknap 2002). Genetic interactions and genetic backgrounds are important regulators of individual gene activity and thus have vitally important influences on the genotype-phenotype relationship (Greenspan 2001; Turri et al. 2001; Bucan and Abel 2002; Peripato and Cheverud 2002, in this issue). Further, dedicated hierarchies of gene activity are likely to be responsible for establishing the nervous system components and interactions underlying specific behavioral phenotypes (Baker et al. 2001). Thus, we need to know more than just which genes influence which behavior; we also need to have information on cascades and backgrounds, that is, genetic networks (Wilkins 2002). Providing the means to address the relationship between genetics and behavioral plasticity may well be the true value of genomics for behavior genetics.

Plasticity, Genotype-by-Environment Interactions, and the Evolution of Behavior

Behavioral genetics incorporates models that explain how different genotypes may be expressed under different environmental conditions, a question that includes both plasticity and genotype-by-environment interaction (G × E). Plasticity and G × E are related but not the same. Plasticity refers to the ability of an individual to change its behavior in different circumstances; this may or may not reflect underlying genetic differences between individuals. Variation in the ranking of phenotypes of different genotypes in different environments is referred to as a genotype-by-environment interaction.

In behavioral studies, G × E refers to differences in behavioral responses in different situations (e.g., genotype A forages best of several genotypes in a certain lighting

condition but is ranked in the middle in terms of its foraging success in another lighting condition); the critical feature is a significant interaction variance, which is often visible as a change in ranking of genotypes in different environments (Falconer and Mackay 1996). It is often possible to measure the same behavior in different environments (e.g., courtship under different nutritional states or with females of different receptivities). If the environmental range is chosen to represent naturally experienced conditions, then behavior becomes an excellent example of G × E. Incorporating a G × E view in behavioral studies may lead to new insights, such as a different approach to understanding learning (Stirling and Roff 2000). The environment in behavioral G × E can range from the commonly studied abiotic factors (climate, habitat) to the internal condition of the animal (nutrition or hormonal state) to the social environment (parental care, flocking, or courting). Although some authors have suggested that G × E for behavior makes the study of behavior genetics futile (reviewed by Greenspan 2001), with careful control, behavioral traits can be excellent candidates for investigating G × E (Meffert et al. 2002, in this issue; Moore et al. 2002, in this issue; Rauter and Moore 2002*a*; Via and Hawthorne 2002).

Social behavior is a very promising topic for application of G × E theory. A social interaction such as the behavior of kin toward an individual is both an environment to the focal individual and a trait that is potentially genetically influenced and therefore capable of evolving (in the relative). Recognition that behavioral influences are "evolving environments" and that interacting phenotypes result in indirect genetic effects (Moore et al. 1997) has led to the development of genetic models of social evolution incorporating these gene–by–social environment effects (Wade 1998; Wolf and Brodie 1998; Wolf et al. 1998; Wolf 2000). When genetic effects of this kind are included in models, some otherwise mysterious biological phenomena are explained (Cheverud and Moore 1994). Models of kin effects have stimulated a new wave of studies of parental care and other types of sociality (Hunt and Simmons 2000, 2002; Agrawal et al. 2001; Rauter and Moore 2002*a*, 2002*b*). The theoretical models considering how social environments interact with genetic influences have been extended to include social interactions among unrelated individuals (Moore et al. 1997, 1998; Wolf et al. 1998) and may help explain the evolution of traits such as communication, mating, and social dominance (e.g., Meffert 1995; Moore et al. 2002, in this issue).

Conclusion

In our review, we have tried to identify questions that can and should be addressed in behavior genetics, the suitability of different methods of genetic analysis for the study of different questions in behavioral variation and evolution, and have provided examples and entries into the literature where possible. We have tried to indicate strengths and weaknesses of different approaches. Some methods based on quantitative genetic approaches simply require measurable phenotypic variation and expected genetic relationships. Other methods are based on knowing the identity of a gene that influences a trait. Thus, not all approaches can be adopted with all species (Wolf 2001), and many of the studies we mentioned were conducted with genetic model organisms rather than with species that are well known from field studies. However, widespread genetic homology leads us to be optimistic that tools will be developed to examine the effects of the same gene in different species, addressing questions from gene function to the fitness consequences of genetic variation. Further, the rate of technological advances suggests that many methods will become applicable to organisms that have received little attention from geneticists in the past.

Another concern for behavioral ecologists is that many genetic studies are limited to the laboratory. However, many phenotypic-level laboratory studies of topics such as parental care, communication, navigation, and foraging have provided major understandings of behavior in natural populations. Here, the critical issue is for the questions and experimental conditions to be designed to allow translation between the two environments. Further studies of plasticity and G × E for behavioral traits in captive populations will help us to understand the value and limitations of laboratory studies of genetics to studies of behavioral evolution.

We see the challenge for the future to be to integrate perspectives and techniques from studies of model behavioral systems with those from model genetic systems. Genomics alone will not let you understand biology, just as a dictionary alone will not make you a writer. Nonetheless, a good dictionary (and spell checker) greatly improves writing. Molecular genetics provides powerful tools to address new and old areas of behavior genetics. In combination with more familiar studies and methods, it appears that evolutionary behavior genetics as a discipline is poised to make rapid advancements through incorporation of molecular techniques.

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