Anniversary Essay

Behavioral ecology and genomics: new directions, or just a more detailed map?

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INTRODUCTION

The genomic revolution is visible in all parts of biology, and behavioral ecology is no exception. New sequencing techniques allow us to examine the genome at an unprecedented level of detail, and methods become outdated almost as soon as they appear. The genomes of many nonmodel species, the bread and butter of behavioral ecologists, are now sequenced, permitting examination of genetic variation across a broad phylogenetic range. The ability to sequence and quantify transcribed genes, rather than simply cataloguing variation in DNA sequences, is enabling new connections between pattern and process.

As with all new fields, however, it is worth considering whether we are witnessing a true advance in our understanding, or simply a new bandwagon of cookie–cutter projects that become possible with sophisticated technology. As the saying goes, just because you can doesn't mean you should, and along with the enthusiasm for massive sequencing projects and the "-omics" of everything has come some skepticism. Are scientists becoming distracted by the "ooh, shiny" aspect of the latest techniques, without sufficient thought to the hypothesis-driven nature of the field? Some have said that we have moved from a time when we had too few data to test our ideas to one where we have an abundance of data but too few ideas.

Behavioral ecology has a unique role to play in this discussion, for several reasons. First, one of the goals of genomics, insofar as it is applied to evolutionary biology, is to elucidate the link between the genotype and the phenotype. If that is the case, nowhere is the interest in that link clearer than with behavior, where the question of how genes influence behavior is central. Second, questions about the promise of genomics are strikingly similar to the longstanding debate about the utility of mechanisms in animal behavior, whether these are endocrine, neurobiological, or immunological. Third, as we will discuss later, the social insects have been central in the use of genomics in organismal biology, as they have been for many other

important questions in our field. And finally, by using new model systems and exploring the intricate links between genes and the environment, it is possible that genomic approaches will help resolve the longstanding nature–nurture debate. Examining the genome in conjunction with, for example, developmental and physiological processes will likely provide clearer connections between genes and the environment and the ultimate phenotype that is produced.

Here we ask whether genomics is indeed showing us new kinds of questions in behavioral ecology, or whether it is simply a new kind of mechanism, illuminating more details but possibly distracting us from a more process-driven approach. We begin by briefly summarizing what we mean by a "genomics approach," and then consider in detail a few examples of the use of genomics in behavioral ecology. Although we consider progress in the field, we do not attempt to provide an overview of past and future direction of behavioral ecology in the sense of Owens (2006), though it is worth noting that Owens did pinpoint "the genetic basis of behavior" as a major challenge for the next decade.

GENOMICS AND BEHAVIOR

Genomics itself is a rapidly growing field of research, focused on understanding the structure, function, and evolution of genomes. The widespread availability of high-throughput sequencing platforms, however, has led many of us to use the term "genomics" as a shorthand for genome sequencing tools and the data they generate. Here we use genomics in a broad sense, and thus consider both how the tools used to characterize aspects of the genome as well as how study of the structure, function, and evolution of genomes themselves may aid in our understanding of behavior.

Whole genome sequences are crucial for addressing questions about the ways that genomic architecture may facilitate or constrain the evolution of phenotypic characters, including behavior. Although as we note later the cost of sequencing is plummeting, for many behavioral ecologists, obtaining a full genome sequence for their organism(s) or interest still may be prohibitively expensive, time-consuming, bioinformatically and computationally

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challenging, or just plain unnecessary. Currently, perhaps the most useful part of having access to a genome sequence for a behavioral ecologist is the ability to align and map massive numbers of expressed genes (e.g., transcriptomes) or genome-wide markers (e.g., restriction-site-associated DNA markers) onto a reference genome.

The majority of examples we will discuss in this paper utilize whole genome, transcriptome, or genome-wide marker sequencing methods to study the evolution of behaviors and/or other phenotypic traits that have important associations with and implications for the evolution of behavior in wild populations. Transcriptome sequencing, often called RNAseq, both sequences and quantifies the portions of the genome being transcribed under conditions of interest (Wang et al. 2009; De Wit et al. 2012). Such information can be used to identify differentially expressed genes and the mutations underlying variation in gene expression and can lead to the identification of functionally important genes or suites of genes that function together ("modules") to produce adaptive behaviors. Genome-wide marker sequencing methods, on the other hand, produce data in the form of sequence variants alone (Davey et al. 2011). Without a genome sequence, such data sets can still provide massive amounts of information regarding single nucleotide polymorphisms (SNPs) and insertions and deletions at thousands of anonymous loci, which can be investigated for associations with any phenotype of interest. In combination with a genome sequence, on the other hand, many of these genetic polymorphisms can be mapped to genes or regulatory regions that are likely to be associated with the phenotype of interest. Essentially, it can be seen as a rapid method for creating a quantitative trait locus map, where the actual nucleotide polymorphism is known.

LOST IN THE MAP

Several criticisms of the wholehearted embrace of a genomics approach to evolutionary biology have emerged over the last few years. Travisano and Shaw (2013) argue that obtaining genetic details of pattern turn out to obscure function and process. They suggest that trying to link genes to a given phenotype is not necessarily a critical, or even laudable, goal. In trying to achieve it, scientists can become "lost in the map," with a flood of sequence data that distracts from the original point of understanding the process of evolution. They briefly review several examples in which a greater knowledge of the genes associated with a particular trait have not aided progress in the field, including microbial genes associated with cellular processes. Because a large number of traits are influenced by many genes of small effect, cataloguing the numerous contributors to those traits is not particularly productive (Travisano and Shaw 2013).

Rockman (2012) concurs that the search for "readily discoverable large-effect alleles" is not likely to bear much fruit, and that phenotypic effect size is important, with the low-hanging fruit of such large-effect alleles already discovered and the effort required to assign functions to the vast array of alleles of small effect not necessarily worthwhile. He is critical of the QTN, or Quantitative Trait Nucleotide, approach, analogous to the more established Quantitative Trait Locus view, asking, "[W]hat is the question to which QTNs are the answer?" On the other hand, Sumner (2014) takes a more optimistic view on applying genomics to social behavior in particular, suggesting that "Sociogenomics is a new field. New fields need new hypotheses."

We are skeptical about this last point. Although we agree that the fire hose of genetic data now available through genomics makes new questions possible, and may make old ones easier to answer, surely the hypotheses should drive the field, and not the other way

around. In other words, we do not need data in search of a hypothesis. This does not mean, however, that genomics cannot revolutionize behavioral ecology. Here we outline some studies in which using a genomic approach can help to address some new questions.

GENOMICS IN BEHAVIORAL ECOLOGY: RECENT EXAMPLES

Convergence in sexual signal loss

The Pacific field cricket, *Teleogryllus oceanicus*, occurs throughout northern Australia and the Pacific islands, and has been introduced to Hawaii. In the latter, the acoustically orienting parasitoid fly *Ormia ochracea*, which uses the calling song of male crickets to locate its host, parasitizes *T. oceanicus* (Zuk et al. 1993). The ensuing conflict between natural selection, which is expected to favor lessening the song's conspicuousness, and sexual selection, which is expected to favor increasing it, appears to have driven a number of changes in the Hawaiian populations' behavior and call structure (Zuk et al. 1995; Rotenberry et al. 1996; Zuk et al. 1998).

Recently, a wing mutation that renders males unable to call has spread on 2 of the 3 islands where the cricket and fly co-occur, Kauai and Oahu (Zuk et al. 2006; Pascoal et al. 2014). The mutation protects the crickets from the parasitoid, but poses difficulties in mate attraction and acceptance, difficulties that may be ameliorated through the adoption of alternative reproductive behavior and relaxed female choosiness (Bailey et al. 2010). Crosses of lab populations revealed that the flatwing trait is inherited as a sex-linked single gene (Tinghitella 2008), but whether the 2 populations exhibited the similar phenotypes because of gene flow or convergence was unknown.

Close examination of flatwings from the 2 islands showed consistent morphological differences, and Pascoal et al. (2014) undertook a genomic analysis of the genetic divergence between them. Using restriction-site-associated DNA markers, Pascoal et al. (2014) determined that of the 7226 flatwing-associated SNP markers, only 22 (0.30%) of all associated SNPs were shared between islands. These patterns of allelic association and genomic architectures are consistent with 2 mutational events on the X chromosome, demonstrating convergent evolution in real time.

This study illustrates one of the uses of genomic analysis that sheds light on a question about behavioral evolution that could not have been answered easily with more traditional tools. Without a nucleotide's-eye-view of the differences between the populations, it would have been difficult to determine the relative roles of gene flow, phenotypic plasticity, and novel mutation.

THE SOCIOGENOMICS REVOLUTION: CONFINED TO HYMENOPTERA?

The social insects have been at the forefront of our thinking about behavior and adaptation at least since Darwin, who highlighted the challenges of altruism under natural selection using the honeybees and their kin as examples. Similarly, researchers over the last decade have eagerly applied new genomic techniques to questions such as the genetic control of division of labor, the ontogeny of worker tasks, and how the members of social insect colonies communicate with each other. Dolezal and Toth (2014) suggest that the sequencing of the honeybee genome and related advances have permitted scientists to "integrate the molecular underpinnings and ultimate explanations of social life." They further claim that the genome can—and potentially should—be the fulcrum for studies of behavior in bees, and perhaps other organisms, more generally.

For example, the ontogeny of a worker into a forager has been delineated with breathtaking detail, incorporating changes in gene expression, epigenetics, the environment, and inherited elements that result in a given phenotype (Dolezal and Toth 2014). A variety of genomic tools have shown that many of the steps are reversible, that genes are not a simple on/off switch for behavior, and that similar pathways can be used to produce different outcomes, such as those in bees versus wasps (Weiner and Toth 2012; Weiner et al. 2013; Dolezal and Toth 2014).

Using a different approach, Harpur et al. (2014) scanned genomes for evidence of selection, looking for clues to the pathway to eusociality. They discovered that novel genes were particularly important in social evolution, and that positive selection was more likely to be associated with worker-biased proteins than with those from queens, providing interesting new data to the longstanding debate about the relative roles of castes in driving sociality. Other work continues to link environmental influences to the operation of genes at a molecular level; worker fire ants in queenless colonies exhibit different gene expression profiles than those in colonies with queens, a difference that can be induced experimentally (Manfredini et al. 2014).

GENOMICS AND ALTERNATIVE MATING TACTICS

What other areas in behavioral ecology, besides the study of social insects, might profit from incorporating genomic techniques? One candidate is the evolution of alternative mating tactics, which has received such attention in the last few years (Snell-Rood et al. 2011; Pointer et al. 2013; Fraser et al. 2014; Schunter et al. 2014; Stuglik et al. 2014). Alternative mating tactics represent a spectrum of behaviors that may or may not have a strong genetic determinant, may be fixed at various stages throughout development, or may remain plastic throughout an individual's lifetime. Several research groups have used a variety of genomic techniques, including high-throughput gene expression technologies (microarrays and RNAseq), to examine transcriptional differences associated with alternative phenotypes in comparison to those arising from sexual dimorphism. Such methods have the potential to help determine how the structure and function of the genome itself influences the evolution of alternative mating tactics and how the phenotypes evolved in turn affect genome plasticity and function.

For example, bulb mites (*Rhizoglyphus robini*) exhibit 2 heritable male reproductive types—fighters and scramblers. Fighter males have a larger third pair of legs for fighting, whereas scramblers have legs similar to females and do not engage in fighting behaviors. In a study of the transcriptomes of both male reproductive types as well as females, genes showing male-biased expression were found to evolve faster than female-biased genes (Stuglik et al. 2014). Furthermore, 4 times as many genes were biased towards expression in the fighter morph than in the scrambler morph (Stuglik et al. 2014). The authors interpret these findings to mean that the degree of expression bias is related to the degree of sexual dimorphism. More interestingly, however, the authors found that morph-specific genes evolve differently than do genes involved in processes common to both morphs. They suggest that this may be due to interlocus conflict, which is driving the more rapid evolution of male-biased genes (Stuglik et al. 2014).

Black-faced bennies (*Tripterygion delaisi*) are fish with highly plastic male reproductive strategies. Territorial males change color, build a nest, and defend the nesting territory during the breeding season, whereas sneaker males maintain a female-like coloration and sneak fertilizations when females lay their eggs. If a territorial male is removed, a nearby sneaker can quickly change its color and behavior in the process of taking over the empty territory. Schunter et al. (2014) found that, during the reproductive period, more genes were differentially expressed between the brains of the 2 male morphs than between the sexes in general. Interestingly, none of the previously identified genes thought to be associated with social dominance in the context of alternative mating types were identified by this study, and most of the differentially expressed genes identified between the 2 male mating types were novel (i.e., unannotated).

We do not yet understand the importance of the genomic background in constraining and/or facilitating the evolution of alternative mating tactics. Until more studies are conducted, it will be difficult to say whether patterns of expression or responses of specific genes or networks are particular to the evolutionary history of the system in question, or if there are general patterns that can be identified across species with similar mating tactics. Of the studies conducted to date, only 1 study had a reference genome (Pointer et al. 2013). Understanding the role of genomic architecture in the evolution of alternative mating types will require this additional information, and we expect to see systems like the bulb mite and black-faced blennies developed further once genome sequences are available.

Genomics data may also help us to understand when similar pathways or genes are involved in producing alternative mating types. Do we see similar allelic variants or expression patterns in systems with continuous variation in male phenotypes, as we do with distinct morphs (Pointer et al. 2013), or is the underlying mechanism different? Is the tight relationship commonly observed between physical morph type (e.g., large body size) and behavior (e.g., aggression or territoriality) the result of modularity of gene expression? If so, does the manner in which gene expression is regulated influence whether alternative mating types evolve or can become established? One would certainly expect that without coordination, these morphological and behavioral traits would be far less fit (e.g., an aggressive male with small legs is unlikely to be successful). Are the molecular mechanisms underlying plastic social dominance generally distinct in different lineages, as was the case in black-faced blennies (Schunter et al. 2014), and will we see a more conserved list of genes if mating type is less plastic and fixed during development? To what extent does this influence the likelihood of the evolution of alternative mating types?

GENOMICS AND SEXUAL CONFLICT

Another area where we think genomics can contribute to behavioral ecology is sexual conflict. Interlocus and intralocus sexual conflict, which are hypothesized in some cases to be driving sexual dimorphism, rapid evolution of sperm traits, and biased sex ratios stem from the fact that males and females have divergent interests and selective pressures while sharing most if not all of their genome (Parker 1979; Arnqvist and Rowe 2005; Bonduriansky and Chenoweth 2009; Pennell and Morrow 2013). In cases of intralocus conflict, selection on a single locus is sexually antagonistic, such that 1 or both sexes cannot reach their phenotypic optimum at that trait due to selection on the other sex (Bonduriansky and Chenoweth 2009). In a recent review, Pennell and Morrow (2013) suggested that intralocus sexual conflict could be better understood through the use of molecular and genomic tools, which would "allow the location and function of sexually antagonistic genes to be identified." Identification of the genes underlying sexual conflict and their place within the genome would likely advance our

understanding of why some sexual conflicts can be resolved and some continue to escalate. Genomic context of the trait under sexually antagonistic selection may in fact be crucial to whether and how quickly a solution can be arrived at (Pennell and Morrow 2013). At the molecular level, sexual conflict has been postulated to be resolved by sexual dimorphisms in gene expression, hormonal regulation, alternative splicing of RNA, sex chromosomes, and genomic imprinting (Bonduriansky and Chenoweth 2009; Pennell and Morrow 2013).

Empirical studies identifying possible mechanistic pathways to sexual conflict resolution have primarily been demonstrated in *Drosophila*, and the genetic basis of sexual antagonism was recently described as a black box (Parsch and Ellegren 2013). For instance, although sex-biased gene expression is theoretically capable of resolving sexual conflicts, it can occur for other reasons as well (e.g., the gene is located on a sex chromosome). We do not know whether the ability to, for instance, identify sex-biased gene expression using high-throughput RNA sequencing and microarray analyses will allow us to generalize about intralocus sexual conflict resolution. Although Mank (2009) argued that "sex-biased expression can be used as a beacon of previously resolved sexual antagonism," she also acknowledged that time lags and pleiotropy mean that sex-biased expression will not always be correlated with sexual antagonism. Nonetheless, it remains the best and easiest proxy currently available.

NEW MODEL SYSTEMS

Model systems have been essential to progress in biology for centuries. They can facilitate research because the basic methodology for rearing, handling, and manipulation has already been developed by previous workers using the same system. New knowledge is thus easier to accumulate because researchers are not constantly reinventing the wheel. At the same time, however, model systems have their risks (Zuk et al. 2014). Because they are often chosen for practical, rather than conceptual, reasons, they may inadvertently canalize our thinking; we run the risk of over-generalizing about the behavior of all flies, or all insects, or all animals, based on studies of *Drosophila*, which were selected as a model system for somewhat arbitrary reasons (Kohler 1994).

For many years, our ability to examine the genetics of behavior was hampered by lack of basic knowledge of the genomes for more than a handful of species. Now, however, genomics and related approaches, such as quantitative trait locus analysis, allows us to take observations of behavior in previously obscure animals and use them to examine genetic differences in behavior, as was recently accomplished using oldfield mice (*Peromyscus polionotus*; Weber et al. 2013). The mice build complex burrows in the sand, and Weber et al. (2013) were able to demonstrate that the behavior is produced in modules, with tunnel length influenced by at least 3 independent genetic regions, whereas a single locus affects the presence of an escape tunnel. Oldfield mice provide a much better window into the operation of selection on behavior in nature than do the more widely used laboratory mice (*Mus musculus*), and genomics will make the use of such nonmodel systems more accessible than ever.

Interestingly, an editorial in *Nature* accompanying the report on the mouse research warned, "If every interesting animal becomes fair game, there is a risk that behavioral genetics will be fragmented" (Anonymous 2013). This statement suggests that we are striving for generality above all else, an arguable conclusion at best. Model systems are most useful when they allow us to construct general principles that transcend the details of an individual species'

natural history. *Drosophila* is thus ideal for inferring how traits are inherited. Similarly, although some animal psychologists objected (Beach 1950), the early 20th century focus by comparative psychologists on laboratory rats (*Rattus norvegicus*) and pigeons (*Columba livia*) was useful for developing a general theory of mind that could eventually be applied to all other animals, including humans.

But what happens when the ability to generalize is not always so useful, or at least not so much of an end in itself? Perhaps being "fragmented"—or at least acknowledging that 1 or a few species do not stand for all the rest—is actually a virtue. For example, sexual selection theory has relied heavily on insects, particularly a few classic species, including the handy *Drosophila*, as models. *Drosophila* was chosen, of course, because of their ease in husbandry and experimental manipulation for testing hypotheses in genetics, rather than behavioral ecology (Zuk et al. 2014). How might that adoption, and subsequent domination of the field by this and a few other taxa, has colored out thinking?

For example, *Drosophila* males transfer seminal fluid proteins that reduce the longevity and fitness of the female but deter her from mating with subsequent males, which is advantageous to the first male to mate. This striking example of sexually antagonistic evolution has prompted numerous studies on sexual conflict, with the underlying assumption that mating is harmful to females. But few researchers have considered the generality of the findings; how many insects possess such toxic ejaculates? And if they do not, what is the implication for sexual conflict and how widespread is sexual antagonism expected to be? In fact, *Drosophila* seminal fluid composition may in fact be very different from the norm, and females from many species do not necessarily experience harm from multiple mating (Zuk et al. 2014).

We are not advocating abandoning model systems to pursue an eclectic variety of study organisms. As genomes become easier and cheaper to sequence, however, more species may be taken up as models. This in turn could mean that we begin to question our assumptions about basic concepts in behavioral ecology, which can only be to the good. Owens (2006) complained that "behavioral ecology has failed to make use of the wealth of genetic information accumulated in the biomedical literature, or exploit the genomic resources already available for commercially important model species." We believe that this complaint has now been addressed, and with resounding success.

MECHANISMS, GENES, AND PLASTICITY

The role of mechanisms in the study of behavior has a long and somewhat anguished history, with some researchers claiming that Tinbergen's question about development, for example, had received far less attention than those of function and adaptation. Whether the mechanisms in question are hormonal, immunological, neurological, or genetic, similar questions about their value as ends of research in themselves recur. And discussions about the relative importance of, and the distinctions between, proximate and ultimate mechanisms in behavior have been going on for decades. Genomics provides us with another set of mechanisms, those occurring at the transcriptome and nucleotide level. Does this make a difference?

Many people who are mostly interested in the evolution of behavior, which presumably includes behavioral ecologists, tend to treat physiological mechanisms underlying that behavior as a convenient if not particularly interesting black box. This box is convenient in 2 directions: if one wants to say that some process cannot evolve, one can invoke a vague "physiological constraint" preventing it. In this

case, mechanisms serve to cover up ignorance of the reason that behaviors evolve one way and not another. Alternatively, if the goal is instead to gloss over the details of a process as being endlessly flexible under the pressures of selection, one can simply claim that any change in the mechanism is possible given enough time.

In reaction to this dismissal, a number of behavioral biologists have argued for a reemphasis on mechanisms; for example, the 2013 winter meeting of the Association for the Study of Animal Behaviour was titled *The Evolution of Behavioural Mechanisms* and addressed questions such as, "Why is behavior sometimes irrational? Why are cognitive systems biased? [and] How do animals deal with uncertainty?" (https://sites.google.com/site/winterasab2013/) The organizers noted that "functional studies of animal behavior traditionally ignore the psychological and physiological mechanisms involved." The black box, in other words, needs to be opened, and its contents examined.

But mechanisms, and a focus on them, can also be distracting. What if, in fact, we open the black box only to find a red herring inside, something that leads us down a path different from the one we intended? It is easy to become enamored of a new technique that uses sophisticated technology, without necessarily considering whether it gets us close to a goal of understanding function.

Genomics fits in well with a resurgence of interest in mechanisms because at heart the powerful new techniques are providing us with more detailed genetic mechanisms behind traits, including behaviors, than we may have previously had. But a genomics approach also runs the risk of encouraging a focus on "finding the gene"—a goal that, even if achieved, is of dubious value. In addition, those of us who study behavior know that the genotype–phenotype map mentioned as a goal by Travisano and Shaw (2013) is even less possible for behavioral traits than for other aspects of the phenotype. Popular misconceptions aside, single genes "for" any particular behavior do not exist, and hence sequencing an entire genome, or looking for associations between genes and behaviors, is not a productive avenue to pursue. Sequences in and of themselves do not inform without an understanding of the developmental circumstances in which the genes occur, which is part of why the nature–nurture controversy is so pointless—all behaviors are the result of input from both the genes and the environment. Perhaps the complexity of genomic mechanisms will help us put the controversy to rest.

THE TAKE-HOME

We hope that behavioral ecologists will be encouraged to embrace the genomic revolution, albeit with some cautions. The following summarizes our conclusions.

1. Having a sequenced genome, or chromosomal region, or a transcriptome, is not necessarily helpful.
2. At the same time, it is not a crime to use new technology, genomic or not, to obtain answers faster, more accurately, or at a heretofore unimagined scale.
3. Genomics has the potential to reinvigorate the study of mechanisms in behavior, though the same risk of becoming distracted by details remains.
4. New model systems, facilitated by the ease of obtaining genome sequences for a variety of organisms, could be extremely salutary for behavioral ecology (but see #1).

FUNDING

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