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Commentary

Social behavior and comparative genomics: new genes or new gene regulation?

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Molecular analyses of social behavior are distinguished by the use of an unusually broad array of animal models. This is advantageous for a number of reasons, including the opportunity for comparative genomic analyses that address fundamental issues in the molecular biology of social behavior. One issue relates to the kinds of changes in genome structure and function that occur to give rise to social behavior. This paper considers one aspect of this issue, whether social evolution involves new genes, new gene regulation, or both. This is accomplished by briefly reviewing findings from studies of the fish *Haplochromis burtoni*, the vole *Microtus ochrogaster*, and the honey bee *Apis mellifera*, with a more detailed and prospective consideration of the honey bee.

Keywords: brain, gene regulation, GnRH, *Haplochromis burtoni*, honey bee, PKG, social behavior, sociogenomics, vasopressin, vole

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A major goal in the brain and behavioral sciences is to identify genes that influence social behavior and understand how their protein products influence the structure and function of the nervous system. Other forms of behavior studied at the molecular level, such as learning (Dubnau & Tully 2001) and circadian rhythms (Panda *et al*. 2002), have focused mostly on the traditional model organisms used for genetic analysis, the fruit fly *Drosophila melanogaster* and the mouse *Mus musculus* (a notable exception in learning studies is the use of *Aplysia californica* (Kandel 2001)). Molecular analyses of social behavior, in contrast, involve a veritable menagerie. Genes related to social behavior have been studied in flies (Greenspan & Ferveur 2000), mice (Pfaff 1999), and the nematode *Caenorhabditis elegans* (de Bono & Bargmann 1998), but also in several organisms used as models for behavioral analysis: voles (reviewed by Insel & Young 2000), birds (reviewed by Clayton 2000b), fish (Hofmann & Fernald 2000), crayfish (Spitzer *et al*. 2001), ants (Krieger & Ross 2002), and bees (reviewed by Robinson 2002b).

A number of factors, including the following three, contribute to the diversity of models used to study genes and social behavior. First, employing *Drosophila* and *C. elegans* in this context has, until now, been limited to studies of elemental social behaviors; mating behavior (Greenspan & Ferveur 2000) and aggregation during feeding (de Bono & Bargmann 1998). Mating is the only activity in *Drosophila* known to involve structured interactions with conspecifics (but see Chen *et al*. 2002), however, mating does not distinguish social animals from most others, and is not featured prominently in the authoritative *Sociobiology* (Wilson 1975).

A second factor contributing to the diversity of models used to study genes and social behavior is that while powerful studies of social behavior can be performed in the laboratory (Pfaff 1999), there is keen interest in understanding the molecular machinery of social behavior in natural contexts (Jarvis *et al*. 1997). Studies conducted under ecologically relevant conditions make it easier to interpret molecular data within a broad integrative framework that includes both mechanistic and evolutionary perspectives (Wilson 1975; Robinson 1999; Robinson *et al*. 1999; Miklos & Maleszka 2000).

A third factor contributing to the diversity of models used to study genes and social behavior is the diversity of social behavior itself. There are many types of social behaviors, exhibited by species that differ dramatically in their level of sociality. There are solitary species that interact with conspecifics only when mating, and species that live in highly structured societies with complex social lives in which nearly all activities are influenced by interactions with conspecifics. Sociality, a term used to describe such all-encompassing social life, is highly derived and has evolved independently in many lineages of animals (Wilson 1975), providing diverse social systems for molecular analysis. Studying the most extreme manifestations of social behavior, exhibited by animals living in complex societies, provides experimental access to a process that is undoubtedly involved in all forms of social behavior, social regulation of gene expression. Studying diverse animal societies also allows for analysis, in molecular

terms, of the roles of convergence and conservation in social evolution.

It is reasonable to assume that the evolution of social behavior acted, in part, on conserved mechanisms that control responses to stimuli in the environment. Social cues, like other environmental cues, convey information critical for animal survival and reproduction. The basics of how individuals respond to their environment are similar in vertebrates and invertebrates and involve sensory structures, signal transduction cascades, and various forms of neural plasticity (Harris-Warrick 2000; Kandel *et al*. 2000). Genes involved in orchestrating the perception and processing of sensory information and the responses that are then triggered are thus likely to figure prominently in social evolution. However, our understanding of social evolution is limited by the fact that the identification of genes involved in social behavior is still in the early phases and no general principles have yet emerged.

Compelling as they are as exemplars of social behavior, the animals mentioned above, which are used as model behavioral systems, will never rival fruit flies and mice as engines of gene discovery (Sokolowski 2001; Bucan & Abel 2002), at least in terms of elucidating basic molecular functions. However, using diverse animal species, in addition to being advantageous for the reasons mentioned above, also provides the opportunity for comparative genomic analyses that address fundamental issues in the molecular biology of social behavior. One issue relates to the kinds of changes in genome structure and function that occur to give rise to social behavior. This paper deals with one aspect of this issue: is the evolution of social behavior associated with the evolution of genes with new functions, genes that are regulated in new ways, or both?

Harris-Warrick, (2000) discusses a larger set of evolutionary changes in the structure and localization of molecules in the nervous system that can influence behavior: (1) gene duplication and diversification that results in protein coding sequence; (2) changes in gene regulation that influence protein abundance; (3) changes in gene regulation that influence spatial patterns of gene expression; (4) changes in gene regulation that influence the complement of neurotransmitters released from single neurons; (5) changes in patterns of alternative splicing; and (6) changes in neuromodulation that can be caused by a variety of transcriptional and post-transcriptional processes. All of these processes likely will be experimentally linked to social behavior in the future (e.g. Kucharski & Maleszka 2002), but here we consider a version of the first one, gene diversification that results in changes in protein coding or regulatory sequence ('new genes') and a version of the second and third ones, changes in gene regulation that influence both spatial and temporal patterns of gene expression ('new gene regulation'). We focus on these processes because there is already evidence that they are involved in social behavior.

Evolution via gene duplication and diversification that results in changes in protein coding sequence has been studied in a number of contexts (Garczarek *et al.* 2001; Chen *et al.* 1997; Robertson 2001; Yokoyama 1995), including behavior (Scheller *et al*. 1983). Recently it was reported that there are differences between humans and chimpanzees in the sequence of FOXP2 (Enard *et al*. 2002b), a gene implicated in human speech, while another study found evidence for a gene (Neu5Gc) lacking in the human genome but present in other animals, including chimpanzees (Chou *et al*. 2002). These results suggest that evolution affecting protein coding sequences can have profound effects on social evolution.

Britten & Davidson (1971) and King & Wilson (1975) first suggested in general notion that changes in gene regulation, rather than gene sequences, can cause evolutionary novelty. Evidence supporting this idea has recently been presented by Enard *et al*. (2002a), who reported results from cDNA microarrays made from human sequences that suggest that there are large differences between humans and chimpanzees in patterns of gene expression in the brain. Given that human and chimpanzee genomes are around 99% identical (see Enard *et al*. 2002a), these findings highlight the possibility that similar genomes can produce different nervous systems with different bahavioral repertoires. With an estimated 6 million years of divergence between humans and chimpanzees (Hacia 2001), these results also suggest that evolutionary effects on brain transcriptomes can be relatively rapid.

We address the issue of whether social evolution involves new genes, new gene regulation, or both, by briefly reviewing findings from the fish *Haplochromis burtoni*, the vole *Microtus ochrogaster*, and the honey bee *Apis mellifera*, with a more detailed and prospective consideration of the honey bee. The same three systems were reviewed earlier (Robinson 1999), but from a more ecological perspective.

GnRH genes and dominance behavior in fish

Dominance in the African teleost (*Astatotilapia*) *Haplochromis burtoni* provides an example of the evolution of a social behavior that is associated with gene diversification and modification of sequence. *H. burtoni* has two forms of males. Dominant males, aggressively territorial, brightly colored and, with high levels of circulating testosterone, enjoy high levels of reproductive success. Subordinate males, lacking all these attributes, do not (reviewed by Hofmann & Fernald 2000).

In the brains of dominant males, neurons that release gonadotropin-releasing-hormone (GnRH) are larger than in subordinate males. GnRH is a neuropeptide that plays a pivotal role in coordinating physiological and behavioral aspects of reproduction in fish and other vertebrates. The difference in GnRH neuron size is most marked in the preoptic area (POA) of the hypothalamus, a brain region known to mediate reproductive behavior (neurosecretory cells in the POA release GnRH in the pituitary). Larger GnRH neurons also reflect increased expression of the GnRH gene (White *et al*. 2002).

The GnRH system is sensitive to changes in social context in *H. burtoni*. Subordinates that become dominant show an increase in neuron size and GnRH expression, whereas dominants that fall to subordinate status show a decrease. Dominance hierarchies in *H. burtoni* society are fluid, with a great

deal of turnover of territories; non-territorial males that move up in social rank and acquire a territory rapidly acquire a suite of characteristics including an increase in GnRH. These results demonstrate that GnRH signaling is sensitive to changes in the social environment and, in turn, influences behavioral and morphological traits that have profound effects on *H. burtoni* reproductive success.

Social regulation of GnRH signaling appears to be due to just one of the three genes in the *H. burtoni* genome that encode GnRH peptides (reviewed by Fernald & White 1999). GnRH is a highly conserved peptide, present in all vertebrates and some invertebrates. All species studied to date have two or three closely related forms, due to a combination of whole genome and GnRH gene duplication events (see White *et al*. 1995). There are three forms in *H. burtoni*, and they differ due to one or two amino acid substitutions at positions 5, 7, and 8. Only [ser^{8}]GnRH (GnRH1; White *et al*. in press) is present in the POA, and therefore is likely responsible for orchestrating the socially regulated reproductive state of males. The other two GnRH genes are expressed elsewhere in the brain and apparently are not sensitive to changes in dominance status. Changes in both coding and regulatory sequence are thus implicated in the *H. burtoni* social system, with regulatory changes also associated with changes in neuron size. The phylogeny of GnRH genes is complex and not completely resolved, but it appears that [ser^{8}]GnRH represents a member of the GnRH family that is specialized for social regulation (White *et al*. in press).

The vasopressin V_{1a} receptor gene and mating behavior in voles

Monogamy in voles provides an example of the evolution of a social behavior associated with changes in gene regulation that influence spatial patterns of gene expression (reviewed by Insel & Young 2000). Although the phylogeny of voles is not conclusive in this regard (Modi 1996), monogamy is a derived trait in most lineages of animals, especially mammals (Alcock 2001). The prarie vole, *Microtus ochrogaster*, is highly monogamous, with both long-term pair bonding and high levels of biparental care. Closely related species, such as the montane vole (*M. montanus*), are not monogamous.

Neuroendocrine studies have demonstrated that vole male affiliative behaviors associated with monogamy are strongly affected by vasopressin. Vasopressin (and the evolutionarily related vasotocin) are neuropeptide hormones that are widely associated with male reproductive behavior in vertebrates (e.g. Goodson & Bass 2000). For example, prarie vole males show a preference for their partner after mating, and treatment that enhances vasopressin signaling increases the expression of partner preference, even in the absence of mating. In contrast, vasopressin treatment does not similarly affect montane voles. This is consistent with findings showing that the species differences in behavior are not associated with differences in vasopressin brain titers, but instead are related to the distribution of vasopressin V_{1a} receptors in the male vole brain. Relative to non-monogamous montane voles, monogamous prarie voles have a higher density of V_{1a} receptors in the ventral pallidal area, which is part of the mesolimbic 'reward' system. Similar differences in spatial distribution of V_{1a} receptors are also seen in several other species of vole and mouse and they also are correlated with monogamy and non-monogamy.

Two studies have shown that the expression of vole monogamy depends on where the V_{1a} receptor is expressed in the brain. First, an ingenious experiment was performed that exploited established transgenic technology for another rodent species, the mouse. Transgenic mice that received a construct containing the promoter and coding sequence for the prarie vole V_{1a} receptor gene showed both prarie vole-like distribution of V_{1a} receptors in the brain and an increase in a form of affiliative behavior associated with monogamy (Young *et al*. 1999). The change in mouse behavior was striking because mice are not monogamous. Second, an adeno-associated viral vector was used to transiently augment the expression of the V_{1a} receptor in the ventral pallidal region of the vole itself, a technical breakthrough that augurs well for this field. Male prarie voles treated in this way showed pair-bond formation in the absence of mating (Pitkow *et al*. 2001). The key is a 428-bp insert in the promoter region of the prarie vole V_{1a} receptor gene that is absent in the montane vole; the coding sequences for this gene are 99% similar for the two species. This insert also is present in another monogamous species of vole, *M. pinetorum,* and absent in another non-monogamous species, *M. pennsylvanicus*.

It is not known whether the presence of V_{1a} receptors in the reward circuit of male prarie voles facilitated the evolution of monogamy in this lineage or whether receptor distribution evolved in concert with other aspects of neural organization that are associated with monogamy. Molecular genetic analyses of behavior conducted within a phylogenetic framework are required to address these issues, but it appears that variation in vole social behavior is associated with variation in gene regulation.

The *foraging* gene and division of labor in honey bees

Division of labor among workers in honey bee (*Apis mellifera*) colonies provides an example of the evolution of a social behavior that is associated with changes in gene regulation that influence temporal patterns of gene expression. The social Hymenoptera – ants, bees and wasps – and other social insects are characterized by 'eusociality'. This means they live obligately in colonies with overlapping generations, cooperative brood care, and a reproductive division of labor in which the queen reproduces directly, while the workers perform tasks related to colony growth and development and engage in little, if any, reproduction themselves (Wilson 1971).

Worker honey bees thus do not grow up and reproduce, but they do grow up (Robinson 1992). Worker bees begin their adult life by performing tasks in the nest such as brood care ('nursing') and nest maintenance. They then make a transition to foraging at about 2–3 weeks of age, which in-

volves changes in brain chemistry, brain structure, endocrine activity, and gene expression (reviewed by Robinson 2002b). Division of labor in honey bee colonies also is sensitive to changes in the environment, especially the social environment. For example, a shortage of foragers will cause some bees to develop prematurely into precocious foragers when they are as young as five days of age.

Ben-Shahar *et al*. (2002) studied the expression of the *foraging* gene (*for, Amfor*) in the brains of nurse bees and foragers to determine whether this gene is regulated in association with honey bee division of labor. *for*, which encodes a cGMP-dependent protein kinase (PKG), was known previously to affect naturally occurring variation in behavior in *Drosophila* (Osborne *et al*. 1997). Two *for* alleles are present commonly: for^R (rover) flies have higher levels of *for* mRNA and PKG activity and collect food over a larger area than do for^s (sitter) flies. Patchy food and high population densities provide a selective advantage for rovers while more uniformly distributed food and low population densities favor sitters (Sokolowski *et al*. 1997). These results suggest that behavioral evolution in flies has involved selection for alternative *for* alleles under different ecological conditions.

for was studied in honey bees because there are intriguing differences and similarities in the foraging behavior of *Drosophila* and honey bees. Foraging in flies is driven by personal hunger (Dethier 1976) but in honey bees foraging is socially regulated (Seeley 1995); forager bees collect food in response to the needs of their colony. Yet, nurse bees resemble sitter flies because they obtain food only in the more restricted confines of the beehive, while forager bees display rover-like behavior by ranging widely throughout the environment. This loose fly/bee analogy suggested that *for* may also be involved in developmentally regulated behavioral variation. Ben-Shahar *et al*. (2002) reported evidence to support this hypothesis; foraging in honey bees is associated with an increase in *for* transcript in the brain, with foragers having higher levels than nurses. *for* expression also was high in the brains of precocious foragers, which are obtained from experimental colonies that lack a typical forager force. This demonstrates that *for* up-regulation is associated primarily with foraging behavior rather than with advanced chronological age.

Ben-Shahar *et al*. (2002) employed a pharmacological approach to test the hypothesis that increased PKG activation causes an increase in the likelihood of precocious foraging. Bees were treated with 8-Br-cGMP, which elevates PKG activity in the brain (see Ben-Shahar *et al*. 2002). This treatment also caused foraging behavior in a dose-dependent fashion. These results suggest that *for* is part of a pathway that influences the transition from hive work to foraging.

PKG also plays a role in the control of feeding arousal in other species of invertebrates and vertebrates (Della-Fera *et al*. 1981; Morley *et al*. 1995; Moroz *et al*. 2000), suggesting that evolutionary changes in food-related behaviors, including complex social foraging, are based in part on changes in the regulation of *for* and other related genes (Ben-Shahar *et al*. 2002). The evidence from flies and bees indicates that *for* orthologs exert different types of influence on behavior, over an evolutionary time scale in flies and an ontogenetic time scale in bees.

Temporal aspects of *for* gene regulation appear to be evolutionary labile and so may have figured in the evolution of division of labor in social insects, particularly with respect to aspects of foraging behavior. Eusociality has evolved independently about a dozen times (Wilson 1971), so this idea can be explored by studying the expression of *for* in other social insect species as well as in solitary bees. It also might be fruitful to compare the promoter regions of *for* in *Drosophila* and honey bees for insights into the molecular mechanisms of social gene regulation.

Post-genomic prospects with honey bees

The study of Ben-Shahar *et al*. (2002) suggests that the issue of whether social evolution involves new genes, new gene regulation, or both, can be profitably addressed by comparative analyses of *Drosophila* and honey bees (see Miklos 1993). The possibilities for fly/bee comparative genomic analyses have just increased enormously with the recent announcement that the NIH National Human Genome Research Institute has selected the honey bee *Apis mellifera* to be among the next group of organisms for genome sequencing (Pennisi 2002).

Sequencing the honey bee genome will facilitate the identification of genes involved in social behavior via the candidate gene approach (e.g. Kucharski *et al*. 2000; Toma *et al*. 2000; Shapira *et al*. 2001; Ben-Shahar *et al*. 2002). Sequencing the honey bee genome will also aid positional cloning efforts that build on studies that have identified quantitative trait loci for several types of honey bee social behavior (reviewed by Page *et al*. 2002). It also will make possible comprehensive comparative analyses of the genomes of the honey bee and *Drosophila*, as well as those of other insects that will soon be completed, *Drosophila pseudoobscura* (Dalton 2000) and *Anopheles gambiae* (Adam 2001).

Results from all genome sequencing projects to date reveal a remarkable degree of gene conservation across all life forms, so it is reasonable to predict that *Drosophila* and the honey bee will be found to share the vast majority of genes. Consistent with this prediction is the following recent finding: in an analysis of 20 000 ESTs from the honey bee brain, over 95% of the assembled sequences had likely orthologs in *Drosophila* (Whitfield *et al*. 2002). Therefore, the striking differences between flies and bees in neural and behavioral complexity (including sociality) are likely due, in large measure, to differences in gene regulation. Yet as insects, flies and bees also have retained many similar characteristics, despite approximately 300 million years of divergence (Burmester 2001). Comparative microarray analyses (Enard *et al*. 2002) can be used to compare gene expression profiles; emerging high-throughput techniques that also provide information on spatial patterns of expression in the brain (Kallioniemi 2001; Brown *et al*. 2002) will be especially useful. Microarrays fabricated with over 7000 EST cDNA clones that represent putatively different transcripts from the bee brain (from a 20 000 brain EST (expressed se-

quence tag) project) are already available (Whitfield *et al*. 2002). Soon after completion of genome sequencing and annotation, microarrays that encompass the entire honey bee genome should be available.

Comparative genomics also can be used to identify conserved regulatory sequences, and then regulatory networks (Loots *et al*. 2000). This will involve new techniques to identify regulatory sequences from the honey bee genome using the *Drosophila* sequence (Berman *et al*. 2002) and new enhancer prediction algorithms to identify novel candidate *cis*-regulatory sequences in the honey bee genome (Ishihara *et al*. 2000; Papatsenko *et al*. 2002).

Are all differences between *Drosophila melanogaster* and *Apis mellifera* in genome structure and function related to the evolution from solitary to social lifestyles? Of course not! There are limitations on what can be inferred because *both* are highly derived species separated by over 300 million years of evolution. This means that genomic resources must be developed for other species to most effectively use honey bees to help develop the field of sociogenomics (Robinson 2002a, 1999). Sociogenomics seeks a comprehensive understanding in molecular terms of social life: how it evolved, how it is governed, and how it influences all aspects of genome structure, gene expression, and organismal development, physiology, and behavior.

The Hymenoptera are distinguished by having species that span the entire range of sociality, from solitary to highly eusocial. This diversity provides special advantages for identifying genes and gene pathways involved in social behavior. Two approaches can be taken, particularly with the hymenopteran ants and bees. First, genomic resources can be developed for other species of social insects that, like honey bees, are well studied and show the most advanced forms of eusociality, such as fire ants (Krieger & Ross 2002) and leaf-cutting ants (Hölldobler & Wilson 1990). Second, genomic resources can be developed for strategically selected species of bees that differ in level of sociality. For example, within the Apini tribe alone are species that are solitary, primitively eusocial, and highly eusocial: the euglossines, bumble bees, and honey bees and stingless bees, respectively (Lockhart & Cameron 2001). It is important to develop genomic resources for the study of behavior; cloning and sequencing on a piecemeal basis are too inefficient for rapid discovery, especially because the regulation of social behavior no doubt involves many genes and pathways.

Genomics makes it possible to use the Hymenoptera and other non-traditional model species to identify genes that influence social behavior. While it is not now realistic to expect to obtain whole genome sequences for many of these species, new techniques promise to dramatically reduce sequencing costs (http://www.usgenomics.com/technology/index.shtml), and some envision the day when whole genome sequencing of animals will become widespread. However, much can be done with genomic resources that can already be developed relatively economically, such as expressed sequence tags, microarrays, and BAC libraries. These can be used in various ways (e.g. (White *et al*. 1999; Band *et al*. 2000; Summers *et al*. 2001) to discover genes and genomic regions that are of particular relevance to social behavior. Once genes have been implicated, functional tests of genes and regulatory sequences can be conducted with transgenic constructs introduced to *Drosophila*. Furthermore, recent reports suggest that genomic manipulations with honey bees will be possible. There is preliminary success making transgenic bees by inseminating a queen with semen mixed with a DNA construct (Robinson *et al*. 2000) and gene expression in a specific region of the brain has been manipulated with antisense (Fiala *et al*. 1999). Experiments manipulating gene expression in bees with RNAi are in progress in several laboratories (S. Omholt and B.H. Smith, pers. comm).

The richness of the Hymenoptera will thus allow for comparisons among bee species that identify genomic differences associated with the evolution from solitary to social lifestyles as well as comparisons between honey bees and ants that identify which genomic differences are consistently associated with the highest levels of sociality. We believe that the potential of these approaches will likely result in a prominent position for social insects in sociogenomic analyses.

Conclusions

The purpose of this article was to consider some of the kinds of changes in genome structure and function that might occur to give rise to social behavior. We focused on the question of whether the evolution of social behavior involves the evolution of genes with new functions, genes that are regulated in new ways, or both. Evidence for both processes was presented, and no doubt both are involved, but it should be noted that in each specific case definitive conclusions are difficult to make, for several reasons. Sometimes it is difficult to distinguish orthologs from paralogs. Another complication is that it is also difficult to distinguish a new gene with a new function from an old gene with new regulation; for example, the proteins encoded by the three GnRH genes in *H. burtoni* are biochemically very similar (Robison *et al*. 2001), and the most striking difference about them is their spatial distribution. All three examples covered here illustrate that plasticity in social behavior can arise due to either temporal or spatial variation in gene expression, regardless of whether this variation is due to activity at a single or multiple loci.

Another complication is that genes that influence behavior are notoriously pleiotropic (Baker *et al*. 2001; Greenspan 2001; Sokolowski 2001; Berenbaum 2002), making it difficult to ascribe the presence of a change in gene sequence or gene regulation to a specific selective factor. We expect also other changes in the structure and localization of molecules in the nervous system to play important roles in the evolution and regulation of social behavior, such as alternative splicing and changes that affect a host of post-translational events (Harris-Warrick 2000). In addition, evolutionary changes in brain structure that have profound effects on social behavior (Arnold 1997) also will ultimately be understood at the molecular level (Krubitzer & Huffman 2000).

We applaud the fact that a broad array of animal models

are being used for molecular analyses of social behavior, and encourage even more diversity, especially species that can be studied in naturalistic contexts. In this way, studies on the molecular biology of social behavior can contribute to our understanding of how the genome responds to environmental change in general (Clayton 2000a; Berenbaum 2002). For insights into social behavior, special opportunities exist to creatively couple model genetic systems with model behavioral systems, such as mice with voles, and *Drosophila* with honey bees.

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