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What genetic model organisms offer the study of behavior and neural circuits

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Abstract

The past decade has witnessed the development of powerful, genetically encoded tools for manipulating and monitoring neuronal function in freely moving animals. These tools are most readily deployed in genetic model organisms and efforts to map the circuits that govern behavior have increasingly focused on worms, flies, zebrafish, and mice. The traditional virtues of these animals for genetic studies in terms of small size, short generation times, and ease of animal husbandry in a laboratory setting have facilitated rapid progress, and the neural basis of an increasing number of behaviors is being established at cellular resolution in each of these animals. The depth and breadth of this analysis should soon offer a significantly more comprehensive understanding of how the circuitry underlying behavior is organized in particular animals and promises to help answer long-standing questions that have waited for such a brain-wide perspective on nervous system function. The comprehensive understanding achieved in genetic model animals is thus likely to make them into paradigmatic examples that will serve as touchstones for comparisons to understand how behavior is organized in other animals, including ourselves.

Keywords

Animal psychology; behavioral neurobiology; circuit-mapping; ethology; neuroethology; optogenetics

Classical behavioral genetics

Genetic model organisms, as their name implies, have been the workhorse for genetic studies of all stripes for many decades. The chromosomal organization of genes, the nature of the genetic material and of mutations, and the function of specific genes in biochemical, physiological, and developmental processes have all been productively investigated in a variety of organisms amenable to easy genetic manipulation. The power of mutational analysis also has been systematically used to identify the genetic determinants of behavior. This work began slowly, with the earliest evidence that individual genes might influence behavioral expression coming from the work of Margaret Bastock in Niko Tinbergen's

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laboratory in the mid1950s (Bastock, 1956; Cobb, 2007). Bastock carefully analyzed the stereotyped pattern of male courtship in *Drosophila* and discovered specific deficits linked to mutations in the body color gene, *yellow*. Perhaps because the connection between behavior and a gene associated principally with body color was not obvious, this work attracted little immediate attention and it was over a decade before Seymour Benzer began to more systematically use *Drosophila* mutational screens as a tool to identify behaviorally relevant genes in the fruit fly (Benzer, 1971). This work – as readers of this journal in particular will know – proved immediately productive and the investigation of genes associated with specific behavioral phenotypes was subsequently pursued in other model animals (Brenner, 1974; Brockhoff et al., 1995; Granato et al., 1996).

While the rich yield of these genetic studies is obvious, it was clear from the beginning that the study of single gene mutations would not lead to a full account of behavior. This is because genes supply the developmental building blocks and operational components of functional neuronal circuits, but it is the circuits and not genes *per se* that represent the proximate causes of behavior. Benzer's recognition of this fact led to his clever use of mosaic analysis to isolate the sites of action within the brain of specific mutations (Hotta & Benzer, 1972). An alternative approach was pursued by Martin Heisenberg and his school who sought to make and characterize *Drosophila* mutants with specific neuroanatomical deficits that could be correlated with behavioral phenotypes (Heisenberg, 1997). The latter work contributed to, among other things, the recognition that the brain region known as the mushroom body in insects is the neural structure critical for associative learning (DeBelle & Heisenberg, 1994; Heisenberg, Borst, Wagner, & Byers, 1985).

A different and more direct approach to understanding the neural circuitry underlying behavior that likewise relies on the use of genetic model animals has emerged over the past two decades. The new approach, however, relies not on the use of mutations, but instead on a host of new technologies that permit the direct manipulation and monitoring of brain cell function in living animals.

From genes to circuits

The rapid rise of thermogenetic tools in flies (Bernstein, Garrity, & Boyden, 2012; Hamada et al., 2008; Kitamoto, 2002; Peabody et al., 2009) and optogenetic tools in mice (Bernstein et al., 2012; Deisseroth, 2015), with their use of temperature- and light-activated proteins to acutely turn targeted brain cells on and off in awake, behaving animals, epitomizes the revolution that has occurred in the study of behavioral circuitry. These, and other equally potent tools for neuronal manipulation which rely on drugs (Sternson & Roth, 2014) for their activation, have largely supplanted the need for stimulating microelectrodes. Likewise, fluorescent tools for observing neuronal activity in living animals have made inroads into experimental territory previously occupied only by recording electrodes (Broussard, Liang, & Tian, 2014; Tian, Hires, & Looger, 2012). The beauty of the new tools is that they are proteins that can be genetically encoded as transgenes which can then be expressed selectively in targeted sets of neurons.

The tools for targeting transgene expression are themselves genetic and rely, in one way or another, on co-opting the transcriptional enhancers that normally govern the expression of an animal's native genes so that they now also regulate the expression of the foreign transgene (Huang & Zeng, 2013; Meinertzhagen & Lee, 2012). By using the enhancer of a native gene that normally expresses in a particular type of brain cell, one can target that class of cell for a particular type of manipulation or for activity monitoring. An increasingly sophisticated array of methods for genetically targeting neurons based on the activity of not one, but on two or more enhancers of the genes that they express is opening the door to progressively refined localization of neuronal function of the type necessary to identify and characterize the brain cells that participate in generating behavior (Diao et al., 2015; Luan & White, 2007).

Because the new tools for circuit-mapping are genetic in nature, their development and use has benefited from the already well-developed genetic toolkits available in nematodes, flies, fish, and mice (Fang-Yen, Alkema, & Samuel, 2015; Huang & Zeng, 2013; Oswald, Lin, & Waddell, 2015; Portugues, Severi, Wyart, & Ahrens, 2013). Indeed, these animals have served both as technology incubators and principal end users for the new techniques and the last decade has seen explosive growth in the investigation of neural circuits in these organisms. Given the rapid expansion of such studies, it is worth examining the promise of these new efforts to see what they offer the study of behavior.

I will focus here not on the tools themselves, but instead on their application to circuit-mapping studies in genetic model animals. These animals join an already crowded arena of molluscs, crustacea, insects, fish, amphibians, reptiles, birds, and mammals used to study behavioral circuitry, and it is worth asking what can be expected of studies in the small group of genetic model organisms now being intensively investigated: only incremental progress along already well-established lines of investigation? Or something uniquely different? I will argue that the traditional virtues of genetic model animals, when combined with the power of the new genetic tools for neuronal manipulation and monitoring, will offer a more comprehensive understanding of how behavior is organized by nervous systems than we might otherwise hope for.

Fast, good, cheap: pick all three

Historically, the animals used to investigate the neural substrates of behavior had to satisfy one critical criterion: their nervous systems and neurons had to be amenable to the electrophysiological methods, focal pharmacological manipulations, and lesions that were until recently the standard tools for neural circuit-mapping. Molluscs, such as *Aplysia*, with their large and accessible neurons, or rats, cats, monkeys, and other animals with brains large enough to accommodate stereotactically-guided instruments for recording or manipulation, clearly met this criterion. This criterion, however, is not among the principal requirements of genetic model organisms, which must have small size, short generation times, and the capacity to be easily reared in the laboratory (Figure 1). The latter requirements, however, when coupled with genetic tools that facilitate neurophysiology in a small brain, provide a combination that not only potently expedites the neurobiological

investigation of behavior, but allows it to proceed in the context of other circuits operating in the same nervous system, rather than one circuit at a time.

Small is beautiful

The brain of a behaving animal is engaged at many levels: sensory and hormonal inputs are being integrated and evaluated for their importance, possible responses to these stimuli are being weighed against each other, and any selected response must be generated by activation of appropriate elements of the motor circuitry and coordinated with on-going activities. The number of neurons involved in this process is large and the neurons themselves are widely distributed within the nervous system. Identifying and characterizing these brain cells, by monitoring their activity and manipulating them to determine how they contribute to the production of behavior is a daunting task. To accomplish this task, it is useful to reduce its complexity by working with fewer neurons, a strategy that has previously been productive in elucidating the function of the “small circuits” that govern motor rhythms in invertebrates, such as the crustacean stomatogastric ganglion (Marder & Bucher, 2007). The same strategy can be applied to whole brains, by working with nervous systems that are relatively small.

Fortunately, “small” is a defining feature of genetic model animals, at least when compared to other animals in their respective classes. The nervous system of *Caenorhabditis elegans* has a mere 302 neurons (White, Southgate, Brenner, & Thomson, 1986), the activity of which can be imaged simultaneously in the intact animal using genetically encoded Ca^{++} indicators (Kato et al., 2015; Schrodell, Prevedal, Aumayr, Zimmer, & Vaziri, 2013). Although the central nervous systems of larval flies and zebrafish contain several orders of magnitude more neurons than that of nematodes, they are still compact enough to also permit whole brain imaging (Keller & Ahrens, 2015; Lemon et al., 2015). Indeed, whole brain imaging was pioneered in larval zebrafish with recordings at cellular resolution in an estimated 80% of the approximately 100 000 neurons in the brain (Ahrens, Orger, Robson, Li, & Keller, 2013). In this case, as in other cases so far, whole brain activity patterns have been recorded in restrained animals or in excised nervous systems, but in all cases many neurons were found to participate in endogenously generated rhythms, and correlated activity was observed across large brain regions suggestive of functional connectivity. The results thus demonstrate the power of this technology for exploring global brain rhythms and their role in governing behavior in a range of genetic model animals.

In addition to permitting brain-wide recordings of activity at single neuron resolution, the new genetically targetable tools are also permitting the mapping and functional characterization of dispersed neurons in circuits across multiple synapses and brain regions. Patterns of connectivity in an increasing number of such circuits have been mapped in *Drosophila* (Hampel, Francoville, Simpson, & Seeds, 2015; Helfrich-Forster, 2014; Ruta et al., 2010), with the circuit governing male courtship behavior among those most extensively characterized (Pavlou & Goodwin, 2013; Yamamoto & Koganezawa, 2013). The way in which this circuit has been elucidated is noteworthy in that it also showcases the significant synergies that can facilitate circuit-mapping in a genetic model animal. Genetic analysis of the sex determination pathway in *Drosophila* led to the identification of a sex-specific isoform of the transcription factor *fruitless*, which was subsequently found to express in

essential neurons of the male courtship circuit spanning the entire neuraxis from sensory input to motor output (Demir & Dickson, 2005; Manoli et al., 2005). In addition, independent investigation of olfactory, and gustatory processing in *Drosophila* together with characterization of fly pheromones, facilitated the recent identification and characterization of sensory circuits that converge onto the *fruitless*-positive command neurons that trigger initiation of male courtship (Clowney, Iguchi, Bussell, Scheer, & Ruta, 2015). The mapping of the male courtship circuit has thus benefited broadly from information derived from previous developmental and genetic studies of other biological processes. The wealth of background information already available in genetic model animals is increasingly being supplemented by large-scale anatomical maps of synaptic connectivity. A full connectome is already available for *C. elegans*, the small size of which permitted reconstruction of the animal's entire central nervous system by conventional electron microscopy (White et al., 1986). Emerging methods in electron microscopy are now being applied to map patterns of synaptic connectivity in the nervous systems of both fruit flies (Ohshima et al., 2015; Takemura et al., 2013) and mice (Kasthuri et al. 2015). Here again, the relatively small size of the nervous systems, and the availability of existing data to validate the maps, makes these genetic model animals attractive subjects of study, and the information derived will further support the broader quest to understand how nervous systems are organized to govern behavior.

In addition to permitting the brain-wide analysis of patterns of synaptic connectivity, the small nervous systems of genetic model animals are also well suited for understanding the action of signaling molecules that act non-synaptically, such as hormones and neuromodulators. These factors are extremely important in regulating behavioral state, but often act broadly to coordinate activity at multiple nodes across the neural connectome. By using genetic tools to target neurons that express the receptors of hormones and neuromodulators, researchers can identify and characterize the downstream partners of these factors (Diao et al., 2015, 2016). How neuromodulators alter activity in synaptically defined circuits to alter behavior has been most extensively explored in the nematode *C. elegans*, which, as noted above, is currently the only animal for which a nervous system wiring diagram is known (Bargmann, 2012). This research underscores the point that a connectome, while a necessary step in understanding brain function, is not sufficient to understand its operation. At the same time, it also emphasizes that circuit interactions can be quite dispersed and understanding them benefits from the nervous system-wide perspective achievable in genetic model organisms.

Laboratory-ready

The fact that genetic model animals consist largely of strains that have been bred for decades under laboratory conditions is, at first blush, not an obvious advantage for behavioral studies. The controlled inbreeding and inadvertent selection that occur in laboratory-housed animals over generations can lead to behavior and genetic backgrounds that may vary substantially from those found in their natural counterparts in the wild. For studies in the ethological tradition, where the goal is to examine and understand behavior under natural conditions, this is a potential impediment. However, the types of heritable, "species specific"

behaviors of interest to ethologists, which promote survival and reproductive success are in many cases well preserved even in laboratory strains and can thus be readily studied.

Further countering the potential disadvantages of domestication are precisely the advantages of being able to control environmental conditions and genetic background of the individual animals or populations being studied. Indeed, the ability to selectively breed animals of different genotypes is essential to the use of the new genetic technologies. Importantly, controlled breeding has also permitted the logic of the classical genetic screen to be applied to circuit-mapping to allow investigators to identify in an unbiased fashion those neurons that participate in generating a particular behavior of interest (White & Peabody, 2009). This strategy has proved tremendously productive in *Drosophila*, where it is central to many circuit-mapping efforts, especially those carried out on a large-scale using collections of transgenic strains made for this purpose (Pfeiffer et al., 2008). The strategy has similarly been exploited in zebrafish (Scott et al., 2007). It should also be noted that while these screening approaches favor simple behavioral assays that meet the “high throughput” ideal traditional in genetic research (Anderson & Perona, 2014), the diminutive size of most model organisms does not preclude laboratory experiments under “semi-natural” conditions to investigate more complex behavioral phenomena, a strategy sometimes used in mice (Weissbrod et al., 2013).

It is also worth noting that while mice are unpractical candidates for the kinds of neuronal screening methods mentioned above, the investigation of ethologically relevant “survival circuits” has benefited from the new genetic tools for circuit-mapping in this genetic model organism. It has done so by building on the considerable base of observations previously made using neurophysiological, pharmacological, and anatomical methods (Sternson, 2013). These methods had crudely identified substrates for such motivated behaviors as eating, drinking, mating, and defensive aggression within the mammalian hypothalamus. Because this small structure consists of nuclei with densely intermingled populations of neurons expressing different neuromodulatory peptides, it was, however, difficult to resolve which cell types were critical for any given behavior using classical methods. Genetic techniques that allow cells to be targeted for manipulation based on their expression of specific peptides or other molecules has now made it possible to unambiguously assign functions to selected populations of hypothalamic neurons. As a result, significant advances have been made in understanding the circuits mediating such things as feeding, sleep–wake rhythms, reproduction, and aggressive attack (Arrigoni & Saper, 2014; Betley, 2013; Carter Soden, Zweifel, & Palmiter, 2013; Krashes et al., 2014; Lin et al., 2011; Scott, Prigge, Yizhar, & Kimichi, 2015).

The study of learning and memory – a domain once ruled by the quintessential laboratory-bred animal, the rat – has also been revolutionized by recent research in transgenic mice that has only become possible using the new genetic technologies (Tonegawa, Pignatelli, Roy, & Ryan, 2015). The power of the new approaches is readily apparent in experiments that have allowed circuits involved in forming an associative memory to be selectively marked and optogenetically reactivated to promote recall (Liu et al., 2012). In addition, false memories have been implanted (Liu, Ramirez, & Tonegawa, 2014; Ramirez et al., 2013), lost memories reawakened (Ryan, Roy, Pignatelli, Arons, & Tonegawa, 2015), and traumatic

memories erased (Han et al., 2009). Studies in the fly are yielding similarly spectacular advances in the realm of learning and memory. Recent research implicates dopaminergic neurons in the process of active forgetting in the fly (Berry, Cervantes-Sandoval, Chakraborty, & Davis, 2015; Shuai et al., 2015), and suggests that such neurons are downregulated in sleep, thus linking this important biological process with the preservation of old memories, as well as the consolidation of new ones. In addition, memories have been induced by direct thermogenetic stimulation of sparse populations of Kenyon cells, definitively demonstrating that these Mushroom Body neurons are substrates of memory engrams (Vasmer, Pooryasin, Riemensperger, & Fiala, 2014). Understanding how the Mushroom Body supports associative learning in *Drosophila* has also been significantly advanced by the recent mapping of this structure's functional circuit organization in exquisite detail (Aso, Hattori, et al., 2014; Aso, Sitaraman, et al., 2014).

As these examples make clear, behaviors traditionally within the provinces of both psychology and neuroethology are yielding to analysis using the new genetically based circuit-mapping tools. Application of these tools requires the carefully controlled production of specific genotypes that can only be achieved in laboratory-maintained stocks. The use of such stocks thus facilitates the mapping of circuits, but leaves researchers considerable latitude in studying the neural substrates of a wide range of behaviors.

Live fast; die old

The short generation times of genetic model animals are perhaps their most prized trait. Advances in genetics have critically relied on the experimenter's ability to quickly produce and characterize heritable mutations and to observe and manipulate the effects of these mutations across multiple generations of animals. The capacity to facilitate rapid progress in research is obviously also an advantage to circuit analysis in genetic model animals, but an additional advantage relates to the ease of conducting developmental and longitudinal studies, which may require observations of an animal over its entire lifetime.

Behavioral phenotypes vary considerably over the lifespan of most animals and may change as a function of genetically programmed events (e.g. sexual maturation), environmental cues (e.g. changing seasons), and learning. Understanding the circuit mechanisms that underlie such changes is a critical aspect of behavioral science, and changes in behavioral circuitry across the lifespan can be readily studied in short-lived nematodes, flies, zebrafish, and mice. An added bonus of research with these animals is that the molecular genetic determinants of development, including those that govern nervous system development, have long been studied in these organisms, with the consequence that a bonanza of information is already available to facilitate behavioral investigation.

The way in which knowledge of neurodevelopment can be leveraged to enhance circuit-mapping in genetic model organisms is on display in a recently published study of the circuitry underlying prepulse inhibition (PPI) of the startle response, which is altered in human schizophrenic patients (Bergeron, Carrier, Li, Ahn, & Burgess, 2015). This study, which began as a neuronal screen carried out in zebrafish, led to the identification of not only critical neuronal determinants of PPI, but also a transcription factor likely involved in

their specification, *Gsx1*. This gene had previously been shown to be important for interneuron maturation in mice and the authors went on to show that *Gsx1* was likewise necessary for normal PPI in that species. Similarly, knowledge from developmental genetics, neuronal screens, and activity manipulations in *Drosophila* recently identified a population of *Eve/Evx1*-expressing interneurons that are conserved from flies to mice and are potentially ancient mediators of locomotor control (Heckscher et al., 2015).

The ability to manipulate environmental and genetic variables during development and to observe the effects of these manipulations on an animal's later behavior and its underlying neural circuitry is also essential for a variety of other kinds of study. This is most obviously true for behaviors that exhibit developmental critical periods, or which themselves play a key role in facilitating development. Included in the latter category are behaviors that mediate molting – or shedding of the exoskeleton of each previous developmental stage – in insects, and the new genetic tools are proving essential in characterizing the neural substrates of these behaviors in *Drosophila* (Diao et al., 2016; Luan, Diao, Peabody, & White, 2012). Examining the influence of early life experience on circuit development has also been productively addressed using the new techniques in genetic model animals. In one such study, the hypoxia response circuitry in nematodes was found to be alternatively wired in animals exposed to prolonged low oxygen conditions during growth (Chang & Bargmann, 2008).

The future: aiming for even distribution

With the introduction of new, genetically encoded tools for analyzing neuronal function, circuit-mapping studies in genetic model animals have become a major research focus. As I have tried to demonstrate here, the traditional strengths of these animals for genetic studies also translate into distinct advantages in the scale and speed of circuit-mapping studies and promise that a fine-grained understanding of the circuits underlying many different behaviors will be achievable in the relatively near future. Elucidating the structure, function, and activity of individual circuits in a small number of intensively investigated animals will necessarily lead to a concomitant understanding of how these circuits intersect, interact, and change over time. Indeed, it will be interesting to see to what extent the metaphor of static and discrete “circuits,” adopted from electronics, survives as the nervous systems of genetic model animals are examined from an increasingly holistic standpoint.

Such a standpoint is essential to resolving many of the outstanding questions in behavioral science, which relate to how nervous systems set priorities, adjudicate decisions, and orchestrate coherent responses to somatic and environmental changes. All of these are highly integrative tasks, which must engage diverse circuits and draw on information about environment, physiological state, and past experience represented in many parts of the brain. A more global understanding of how behavior is governed will likewise be required to answer long-standing questions about how actions are not only selected, but composed from fundamental behavioral units (motor primitives) common to many behavior patterns, and how general vs. specific motivational mechanisms are encoded to direct the execution of different behaviors toward desired goals.

The fact that we are moving toward a comprehensive understanding of how four quite different nervous systems govern behavior is a fortuitous advantage. In any discipline, general principles emerge by comparing multiple examples of the phenomenon in question. The examples of how nematodes, flies, zebrafish, and mice generate behavior from the activity of their neural circuitry will provide the material for just such a comparison: The similarities will allow general principles to be extracted and, at the same time, the differences will allow us to understand where and how other factors (e.g. selection pressures) can act to produce variation. Both similarities and differences can arise through multiple mechanisms, and comparison of the results obtained from the four genetic model organisms should cast light on questions related to how behavioral circuits evolve. The finding that the neuromodulatory control of reproductive behavior by oxytocin-like peptides may be ancient and shared by nematodes and humans (Garrison et al., 2012) is perhaps an indication of what types of insights may be in store. In any case, research in genetic model organisms can be expected to provide insights into not only the neural mechanics, but also the development and evolution of behavioral circuits.

In this sense, behavioral research in genetic model animals is not so much moving us towards new goals as it is moving us closer to old ones. In 1963, Niko Tinbergen articulated an influential framework for the study of behavior in an attempt to define the “Aims and Scope of Ethology” (Tinbergen, 2005). He called for the investigation of four principal questions that addressed not only the physiological basis of each specific behavioral pattern, but also its development, evolution, and adaptive function. All but the last can be productively framed as questions about neural circuitry, and circuit-level studies in model organisms are poised to provide answers to Tinbergen’s questions with a mechanistic depth that was scarcely attainable previously. To the extent that these answers are being achieved not for single behaviors studied in isolation, but for many at once in the same organism, means that the understanding gained will also have unprecedented scope.

Will the scope provided by studying only four species be sufficient to understand animal behavior in all its diversity? One of the inspiring aspects of behavioral studies pursued in the ethological tradition was the incredible richness of the subject matter. Neuroethologists, following Tinbergen, turned their electrodes to the neural circuitry of organisms spanning a wide range of animal phyla. This work remains a source of both biological and intellectual diversity and fully exploiting the studies done in genetic model organisms will require comparing their results to those obtained in diverse other animals. Work in other animals should, however, also profit from techniques now common in research on genetic model organisms, such as whole genome sequencing, Crispr/Casmediated genomic editing, viral-mediated transgene delivery, optogenetics, and RNAi-mediated gene silencing. These techniques which are already making it possible to extend the benefits of working with genetic model animals to traditionally “non-genetic” models, such as rats (Schonig et al., 2012), monkeys (Han, 2012), zebra finches (Velho & Lois, 2014), and silk moths (Kiya, Morishita, Uchino, Iwami, & Sezutsu, 2014). This dissemination of genetic approaches is likely to represent an important way forward, and one can hope that as it leads to a better understanding of the organization of behavior in diverse animals we will better understand it in the animal most important to us: ourselves.

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Figure 1.

The four major genetic model animals used in behavioral research. The genetic model organisms discussed in depth in the text—*Mus musculus* (house mouse), *Danio rerio* (zebrafish), *Drosophila melanogaster* (fruit fly), and *Caenorhabditis elegans* (nematode worm)—are compared to *Homo sapiens* (human), the animal whose behavior we would most benefit from understanding. The comparison relates on the one hand to each animal's virtue as a genetic model (represented on the graph's ordinate axis by the shortness of its generation time, in days), and on the other to its evolutionary distance from humans (represented on the graph's abscissa as the time elapsed since the two species last shared a common ancestor, measured in millions of years). Several other indices are listed for each species to the right of its icon. Top to bottom, these also relate either to attributes that suit the species to genetic studies, as described in the text (smallness of size, indicated in grams; and shortness of lifespan, indicated in days), or speak to the species' relationship to humans (total number of protein-encoding genes, with the percentage having human homologs indicated in parentheses). Finally, the estimated number of neurons in the animal's nervous system is indicated as a measure of its complexity. All four animals discussed here are mature genetic model systems in that all have sequenced genomes, and for each there exist many in-bred wildtype strains and mutant strains with heritable deficits. In addition, for each

species mutagenesis screens have identified genes that participate in biological processes of interest, including behavior.

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