Please cite this article in press as: Jourjine and Hoekstra, Expanding evolutionary neuroscience: insights from comparing variation in behavior, Neuron (2021), https://doi.org/10.1016/j.neuron.2021.02.002

Neuron

CellPress

Perspective

Expanding evolutionary neuroscience: insights from comparing variation in behavior

Nicholas Jourjine[1],* and Hopi E. Hoekstra[1],*
[1]Department of Molecular and Cellular Biology, Department of Organismic and Evolutionary Biology, Center for Brain Science, Museum of Comparative Zoology, Harvard University and the Howard Hughes Medical Institute, 16 Divinity Avenue, Cambridge, MA 02138, USA
*Correspondence: nicholas_jourjine@fas.harvard.edu (N.J.), hoekstra@oeb.harvard.edu (H.E.H.)
https://doi.org/10.1016/j.neuron.2021.02.002

SUMMARY

Neuroscientists have long studied species with convenient biological features to discover how behavior emerges from conserved molecular, neural, and circuit level processes. With the advent of new tools, from viral vectors and gene editing to automated behavioral analyses, there has been a recent wave of interest in developing new, "nontraditional" model species. Here, we advocate for a complementary approach to model species development, that is, model clade development, as a way to integrate an evolutionary comparative approach with neurobiological and behavioral experiments. Capitalizing on natural behavioral variation in and investing in experimental tools for model clades will be a valuable strategy for the next generation of neuroscience discovery.

INTRODUCTION

Neuroscientists and evolutionary biologists have long shared an interest in animal behavior (Darwin, 1897). For much of the histories of their respective fields, however, they have pursued this interest using different research strategies. On the one hand, neuroscientists have focused on questions about the molecular and neural mechanisms of behavior using controlled experiments in single species (i.e., "model" species) chosen for convenient tools or biological features. By contrast, evolutionary biologists have focused on questions about the diversification of behavior by comparing multiple species that share a common ancestor (i.e., a clade) chosen because they vary in a behavior.

These distinct approaches are valuable on their own merits, but each also has its limitations. While manipulative experiments have led to fundamental discoveries about the neural and molecular mechanisms of behavior, neuroscientists have been limited in their ability to test if and how those mechanisms contribute to behavioral variation between species, in part due to the relatively infrequent application of comparative methods commonly used by evolutionary biologists. Conversely, evolutionary comparisons between species have provided insight into the ultimate causes of and constraints on behavioral diversity, but it is often challenging to test hypotheses about the cellular and molecular underpinnings of that diversity, in part due to a lack of the very tools for manipulative experiments common in modern neuroscience research. The complementarity of these limitations suggests that there is much to be gained by a research program that integrates controlled experimentation within species and quantitative comparisons between species (Figure 1).

Such an approach has been a long-standing goal of many neuroscientists, in particular neuroethologists, who have for decades championed the comparative approach to understanding both the proximate (mechanistic) and ultimate (evolutionary) causes of animal behavior. But achieving this goal requires taking two difficult steps. The first is to develop tools for experimentation in multiple species that differ in behavior. The second is to incorporate evolutionary comparative methods to generate and test hypotheses about the historical causes of behavioral differences between species. In just the last few years, neuroscientists have made hard-won progress toward the first of these steps, and barriers are falling as tools (from traditional approaches such as pharmacology and electrophysiological stimulation to more recent tools such as optogenetics, viral vectors, and CRISPR-Cas9 genome editing) are spread to new species. Even as the difficult task of turning single species into model species continues at an increasingly rapid pace, studies that explicitly integrate comparative and experimental approaches remain relatively rare.

Here, we discuss a research strategy at the interface of neuroscience and evolutionary biology to promote this integration. Specifically, we argue that as neuroscientists choose to develop and study nontraditional study systems, they should consider not only the biological features of single species that make experimentation especially convenient or exciting but also features of the evolutionary groups (or clades) to which those species belong that make phylogenetic comparisons especially convenient or exciting. We refer to study systems that balance these features as "model clades" (as a contrast to "model species") and suggest this strategy as an exciting way to capitalize on new approaches and tools to make fundamental insights into both the nervous system and behavior.

Neuroscientists now have unprecedented options when deciding how to study animal behavior and its underlying mechanisms. This is therefore an important time to think carefully about both the merits and limitations of the next generation of

Please cite this article in press as: Jourjine and Hoekstra, Expanding evolutionary neuroscience: insights from comparing variation in behavior, Neuron (2021), https://doi.org/10.1016/j.neuron.2021.02.002

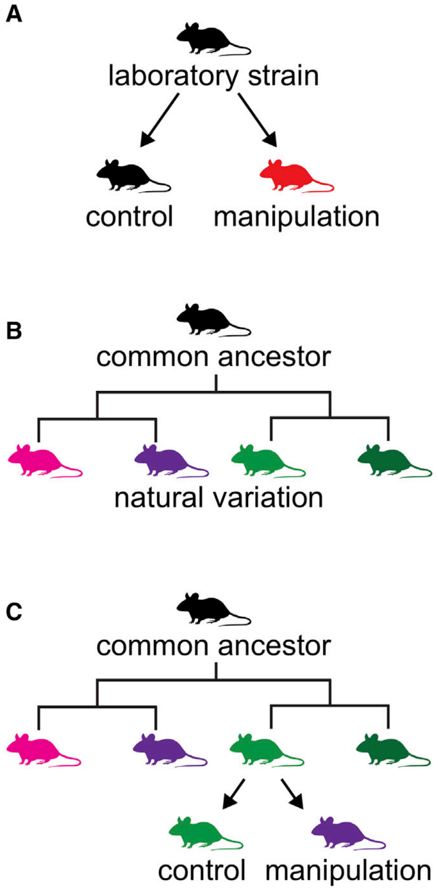

Figure 1. Integrating manipulative and comparative approaches for behavioral neuroscience

(A) Manipulative approaches common in neuroscience. Tools in laboratory strains are used to manipulate genes, molecules, neurons, or behavior itself to test for effects (red) relative to controls (black).

(B) Comparative approaches common in evolutionary biology. Natural variation in behavior is correlated with genetic or (more rarely) neural variation to understand the causes of behavioral differences.

(C) Combining manipulative and comparative approaches. Experimental manipulation can test relationships between molecular or neural variants and natural variation in behavior. For example, gene editing or optogenetics can target candidate genes or neurons in one species (green) to test if such manipulation can recapitulate behaviors observed in another species (purple).

study systems and how they are chosen. Using the evolutionary relationships and behavioral differences in carefully chosen clades will help inform these choices and promote a close integration of experimental and comparative approaches. Below, we summarize historical motivations that make the present particularly timely for developing not just model species but also model clades as study systems for neuroscience discovery, discuss the tradeoffs that neuroscientists with different interests and questions might face in choosing a clade to study, outline questions that model clades are well poised to help answer, and discuss some of the unique challenges of implementing a clade-based approach. Finally, we describe three recent model clades as exciting examples of feedback between neurobiological experiments and comparative methods to serve as inspiration.

A BRIEF HISTORY OF STUDY SYSTEMS IN NEUROSCIENCE: WHY THE TIME IS RIGHT TO CONSIDER CLADES

For much of the early history of neuroscience, researchers started their careers by first defining the questions that interested them and then seeking out species in which those questions could most easily be answered (Shepherd, 2010, 2016). This strategy is possible because for almost any biological question, there is an organism whose biology is conveniently or even uniquely suited to answering it, an observation famously made by the Danish physiologist August Krogh (Krogh, 1929) that has since been referred to as Krogh's principle, although it was also articulated 60 years earlier by the French physiologist Claude Bernard (Bernard, 1864). The discovery of the action potential in the squid axon (Hodgkin and Huxley, 1945), mechanisms of learning and memory in sea slugs (Carew et al., 1971), and properties of central pattern generators in crustaceans (Dickinson et al., 1990) are just a few oft-cited examples of Krogh's principle in action.

Despite these famous examples, the recent history of neuroscience has been characterized by studies on a tiny fraction of species diversity—the model species. While this focus has long been criticized as myopic (Beach, 1950; Katz, 2019), it has also been extremely successful, in part because focusing a large number of scientists on a small number of lab-friendly species has facilitated the development of a common set of tools. Among the most powerful of these are transgenic tools for observing or manipulating genetically defined cell types, thereby testing the role of specific neurons or circuits while leaving others unaltered—a crucial ability for neuroscientists interested in studying how patterns of animal behavior arise from neural circuits and the genes they express. Together with transgenics, additional tools such as electrophysiological manipulation (Desai et al., 2017), viral vectors (Fenno et al., 2020), and standardized psychophysical paradigms (Siemann et al., 2015), combined with descriptive resources like genome sequences, brain atlases, and connectomes, have come to comprise what might be referred to as the "model species toolkit."

While this toolkit has largely been restricted to a small number of model species, a recent wave of transferrable tools for neuroscience research has been allowing researchers to develop additional species in the image of these traditional models. As a result, neuroscientists are now (once again) increasingly facing the decision of which species to study, often using Krogh's principle to guide their choices (Laurent, 2020; Yartsev, 2017). Some of these species have behaviors that make them well suited to answer questions that are intractable in "traditional" model systems (e.g., understanding the neural basis of vocal turn-taking using vocal duets in singing mice; Banerjee et al., 2019; Okobi et al., 2019), while others have idiosyncratic, understudied behaviors that may harbor novel insights into nervous system function (e.g., vision in spiders, color changing in cuttlefish, and chemotactile touch in octopus; Menda et al., 2014; Reiter et al., 2018; van Giesen et al., 2020). Still others are well suited for answering questions enabled by new tools, such as the ability to record whole-nervous-system activity from a freely moving animal (e.g., the cnidarian Hydra; Dupre and Yuste, 2017).

Please cite this article in press as: Jourjine and Hoekstra, Expanding evolutionary neuroscience: insights from comparing variation in behavior, Neuron (2021), https://doi.org/10.1016/j.neuron.2021.02.002

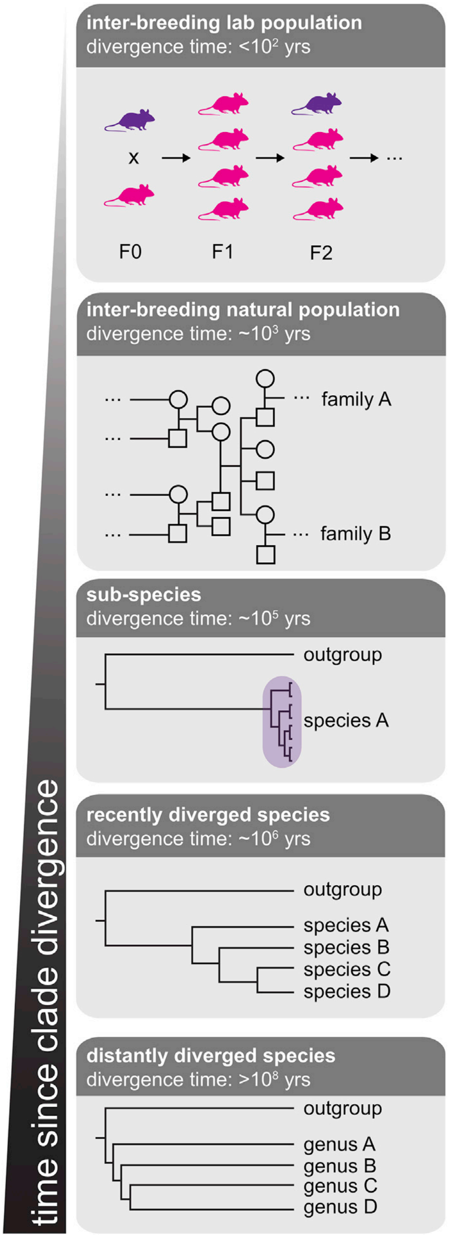

Figure 2. Clades are groups defined by a shared common ancestor
Different clades arranged by how recently they share a common ancestor. (Top) Lab-derived lineages and interbreeding natural populations share a recent common ancestor. These clades are often excellent genetic resources but also tend to exhibit limited behavioral variation due to their recent shared ancestry. F0, founder generation of a laboratory cross; F1, first cross generation; F2, second cross generation. (Bottom) Species and groups of genera can be isolated for tens of millions of years or more. These clades are more likely to have evolved large, easy-to-quantify differences in behavior but are also likely to have diverged in many other ways, which can make it difficult to identify homologous behaviors or link genetic and neural causes to behavioral variation. (Middle) "Subspecies" or recently diverged species are lineages sufficiently diverged to warrant separate designations but that may still be interfertile in the lab or in the wild and may have only diverged in a small number of behavioral or morphological traits. These clades can represent a balance for the needs of comparative methods with those of manipulative experiments.

However, there is another crucial reason for diversifying the species in behavioral neuroscience research: the ability to compare traits (which may be molecular, neural, or behavioral) that vary across species. Such comparisons are the foundation for inferring the evolution of neural and behavioral diversity, and as we discuss below, using this evolutionary approach can also lead to the discovery of fundamental principles in neuroscience (e.g., Katz, 2016a). Phylogenetic comparative methods—statistical approaches created to model and make inferences about evolutionary histories—have grown dramatically in both scope and rigor in recent years (e.g., Pennell and Harmon, 2013; Smith et al., 2020; Symonds and Blomberg, 2014). Despite the successes other fields have seen by embracing them (notably, evolutionary developmental biology, or "evo-devo"; Church and Extavour, 2020), these methods remain relatively rare in behavioral neuroscience.

One reason for the dearth of phylogenetic comparison in neuroscience research is that much of the field, including research in emerging nontraditional models, is focused on mechanisms and behaviors that are conserved. This is rightly motivated by the desire to understand features of the human nervous system (without directly performing experiments on humans). However, there is also much to be gained by studying neural mechanisms and behaviors that have diverged over evolutionary time, which can complement studies of evolutionary conservation to inform human biology as well as basic neuroscience principles. For example, comparisons between humans and non-human primates can identify differences in genetic or neural traits unique to the human lineage and responsible for human-specific behavior (e.g., cognitive ability). More generally, because evolution has had far longer than any laboratory experiment to "sample" cellular and molecular solutions for regulating behavior in different contexts, studying the diversity of solutions it permits can address the extent to which behavioral repertoires are promoted or constrained by the underlying features of their nervous systems, a fundamental question in neuroscience.

Studying behavioral differences between species thus has important contributions to offer for our understanding of both how behavior evolves and how molecular and cell mechanisms shape behavioral repertoires. But how best to study these differences? Here, we suggest an approach that remains relatively rare in neuroscience (see Gallant and O'Connell, 2020 for some notable exceptions), but is common in evolutionary biology (Donoghue and Edwards, 2019), ecology (Cavender-Bares, 2019), and, increasingly, developmental biology (Church and Extavour, 2020): study clades. Loosely defined, a clade is group in which all members are related by a common ancestor. Thus, a clade can comprise populations of individuals, subspecies, or (most commonly) species, from individuals that share grandparents to genera that have been reproductively isolated for millions of years (Figure 2). Treating clades as units of study as opposed to single species makes it possible to explicitly incorporate species differences and their evolutionary histories to test hypotheses about behavior and its underlying causes. This promotes feedback between neurobiological experiments within species and evolutionary comparisons across species, feedback that can provide key insight into animal behavior, its mechanistic basis, and its evolution.

Please cite this article in press as: Jourjine and Hoekstra, Expanding evolutionary neuroscience: insights from comparing variation in behavior, Neuron (2021), https://doi.org/10.1016/j.neuron.2021.02.002

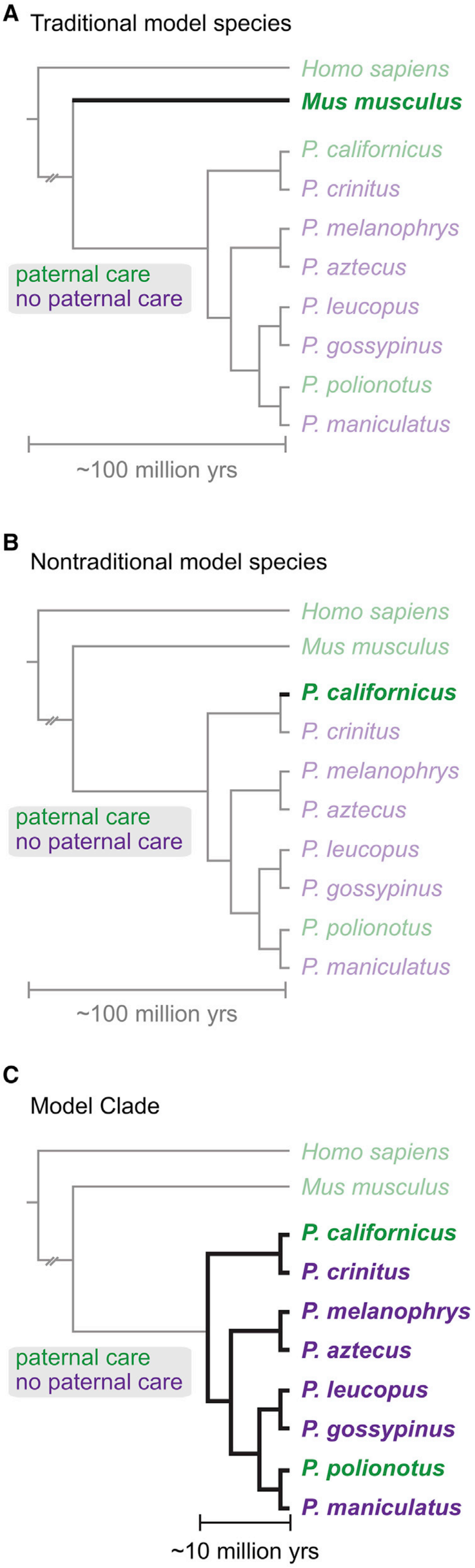

Figure 3. Model species versus model clades

Both traditional model species and nontraditional model species are often chosen because they exhibit a behavioral similarity with humans (e.g., paternal care, a behavior rare in mammals but present in humans).

(A) One traditional model species for studying this behavior is *Mus musculus*, which also exhibit paternal care.

(B) A nontraditional system for studying paternal care is *Peromyscus californicus*, a species of deer mouse.

(C) By contrast, a model clade is a group of related species in which behavioral differences, not just similarities, can be used to study a trait. The genus *Peromyscus* contains at least two species that have evolved paternal care independently (*P. californicus* and *P. polionotus*), which can be compared to closely related species in the clade that do not have paternal care (*P. crinitus* and *P. maniculatus*, respectively) to understanding the neural and molecular mechanisms that make these species behaviorally distinct.

HOW TO CHOOSE MODEL CLADES FOR NEUROSCIENCE

As with any study system, there is no single clade that is "perfect," and a clade-centric approach will not answer all questions in neuroscience. However, Krogh's principle applies just as effectively to groups as it does to individual species; if there is a question that will benefit from an integration of experimentation and evolutionary comparisons, there is probably a clade well suited to answering that question. While the term model has been rightly criticized as vague (Katz, 2016b; Marder, 2020b), we find it useful to think of such clades as model clades to draw a parallel with the widely used term model species; both describe a focal study system with features (e.g., experimental resources or convenient biology) that make it particularly useful for addressing a specific set of well-defined questions (Figure 3). Below, we outline features for guiding the choice of a model clade to study, focusing on two considerations: first, characteristics that will promote well-powered comparative methods; and second, characteristics that will make it feasible to perform neurobiological and behavioral experiments.

Features that promote powerful evolutionary comparisons

Two important requirements for phylogenetic comparisons are the number of well-established evolutionary relationships within the clade and the manner in which the trait (be it a behavior, circuit or molecule) of interest varies relative to those relationships. The more species that vary in behavior in a clade, the more power comparative methods will have to, for example, reconstruct ancestral states and test for correlations between different behaviors (but, as we note below, the more work it will be to develop tools for manipulative experiments). Even moving from one species to comparing two can help identify behavioral differences that can motivate a search for underlying mechanistic causes; moving to three species allows one to polarize behavioral differences (i.e., identify on which evolutionary branch that change occurred); and the addition of more species allows one to reconstruct traits (e.g., circuits or behaviors) that likely occurred in ancestors of present-day species or correlate behavioral variation with other traits and environmental variables. For example, comparing only seven species of deer mice using one of the most common phylogenetic comparative methods, phylogenetic generalized least-squares, revealed large differences in nesting behavior among closely related

Please cite this article in press as: Jourjine and Hoekstra, Expanding evolutionary neuroscience: insights from comparing variation in behavior, Neuron (2021), https://doi.org/10.1016/j.neuron.2021.02.002

species but no effect of body size or climate on the evolution of this behavior (Lewarch and Hoekstra, 2018). More branches mean more data on how a trait is distributed in a clade, which, in turn, means more power to make inferences about its evolution. At an extreme, Miles and colleagues recently used acoustic recordings of drumming displays from 134 woodpecker species to discover that drumming rhythm has significantly constrained the evolution of other behavioral features, specifically drumming speed and duration, as drumming display has evolved in this clade (Miles et al., 2020).

How behavioral, neural, and molecular traits of interest vary with respect to evolutionary relationships is a second feature that has an important impact on the power of evolutionary comparisons. Clades in which the same behavior has evolved independently (i.e., convergently) in multiple species are especially valuable, because they make it possible to test the extent to which similar or different mechanisms give rise to behaviors that have evolved repeatedly. For example, the ability to produce electric fields has evolved independently at least six times in distantly related vertebrates (separated by hundreds of millions of years in some instances), each time using an electric organ derived from skeletal muscle precursors and transcription factors regulating skeletal muscle size and excitability (Gallant and O'Connell, 2020). This evidence suggests that vertebrate behaviors involving electrical discharge have evolved multiple times using a common genetic toolkit, potentially because the physical requirements of this behavior constrain the biological mechanisms available to produce it (see Gallant and O'Connell, 2020 for more detail on the value of clades containing convergently evolved behavior). Thus, while the comparison of even two species can reveal novel insights, the more species for which data can be collected, the more powerful phylogenetic analyses will be, both because of increased sample sizes for reconstructing the evolutionary history of traits and because of the increased chance of finding multiple examples of convergent behavioral evolution.

Features that promote functional tests of neural and molecular mechanism

Not all species are equally convenient for performing controlled, manipulative experiments. However, those that are usually have biological features (like size, generation time, life cycle, or ecology) that make it possible to rear them in the lab in large numbers or to develop transgenic tools. Clades with these features often already contain either traditional or nontraditional model species. Thus, model species with established experimental (potentially transgenic) toolkits can be used to seed or anchor the development of clades containing them. For example, transgenic tools previously restricted to the classic model species *Drosophila melanogaster* have recently been developed in parallel in a clade of closely related species (Stern et al., 2017).

Not all biologically convenient systems need to include a "traditional" model species, however. For example, *Laupala* are a genus of forest dwelling Hawai'ian crickets whose mating songs have rapidly diversified over the last ~4 million years, resulting in a behaviorally diverse clade that still contains interfertile species that can be reared and studied in the lab (Mendelson and Shaw, 2005; Xu and Shaw, 2019). The existence of stable germline transgenics in a related cricket species, the Mediterranean field cricket (*Gryllus bimaculatus*) (Kulkarni and Extavour, 2019; Nakamura et al., 2010), and the recent annotation of the *Laupala kohalensis* genome (Ylla et al., 2020) are exciting steps toward developing crickets as a model clade to study neural and molecular causes of variation in behavior.

Tradeoffs inherent in the study of clades

Clades need not have all of the above features to provide an informative context in which to study animal behavior, and, as with any research strategy, tradeoffs will always exist. Two of the most common tradeoffs include the number of taxa in a clade and the time since those taxa have diverged from a common ancestor.

First, one must consider how many species is feasible to study to maximize the power of evolutionary inferences. Clades with fewer taxa will require fewer of the (often technically challenging) manipulative experimental approaches needed to deeply understand how variation in neural or molecular mechanism produce behavioral variation, but also typically contain too few branches for well powered comparative methods. Conversely, clades with many taxa provide statistical power for robust phylogenetic comparisons but also increase the work needed to comprehensively identify genetic and neural mechanisms underlying variation among species. For example, the clade of woodpeckers mentioned above (Miles et al., 2020) is an excellent system for performing phylogenetic comparisons because of the large number of species for which behavioral data can be collected, but it is not convenient as a system for discovering neural and molecular mechanisms of behavior because of the difficulty (or impossibility) of performing functional tests in >100 species of a large bird that is difficult to maintain in the lab. On the other hand, stable germline transgenics make precise manipulative experiments possible in a small number of *Drosophila* species, but this is likely too few species for making confident inferences from phylogenetic comparative methods alone.

A second consideration is the evolutionary time separating species within the clade of interest. For example, clades in which species have diverged recently are more likely to be convenient contexts to use genetic approaches made possible by closely related, interfertile species. However, the tradeoff is that recently diverged species are also less likely (though not always) to exhibit large, and therefore more easily measured, heritable differences in behavior. On the other hand, distantly related species have had more time to evolve large differences in behavior, but this comes with two challenges. First, distantly related species have often evolved numerous genetic and morphological differences that can both prevent successful genetic crosses and, importantly, confound comparison of the causes of behavioral differences. Perhaps even more challenging is that the more distantly related species are, the more difficult it becomes to determine if two behaviors are in fact the "same"; i.e., that one is comparing "apples to apples" (e.g., Lickliter and Bahrick, 2013). This difficulty is related to the broader challenge of defining homology for behavior, which we discuss below.

Please cite this article in press as: Jourjine and Hoekstra, Expanding evolutionary neuroscience: insights from comparing variation in behavior, Neuron (2021), https://doi.org/10.1016/j.neuron.2021.02.002

Box 1. Ten questions in evolutionary neuroscience that model clades can help answer

Here, we highlight 10 fundamental questions in evolutionary neuroscience that the field is increasingly well positioned to answer. Questions 1–5 are focused on the specific causes of behavioral differences between species that can start to be addressed by case studies, while questions 6–10 relate to more general issues about how and why behavioral evolution proceeds as it does. While these questions can be broadly addressed by a comparative approach, we think that an integration of experimental and comparative studies in model clades will be a particularly effective way to discover answers. Starred (*) questions are discussed in more detail in the main text.

1. Do evolutionary changes occur more often in neural systems for detecting (peripheral) or processing (central) sensory cues?
2. Are particular brain regions more likely to lead to behavioral evolution than others?
3. What is the contribution of new cell types to behavioral differences between species?*
4. Are some changes to neural circuitry more common than others during the course of behavioral evolution?*
5. Are some genes or gene families more likely to lead to behavioral evolution than others?
6. How many molecular and neural solutions are there for a given behavioral problem?
7. What are the respective roles of natural selection and genetic drift (e.g., historical accident) in determining which mechanisms are used by a species for generating a particular behavior?
8. To what extent does neural circuit architecture promote or constrain behavioral differences between species?*
9. To what extent does the evolution of morphology constrain or facilitate the evolution of behavior?
10. To what extent can we learn about neural innovations underlying human-specific abilities and pathologies by studying natural behavioral variation in other species?

Weighing tradeoffs for neuroscience discovery in model clades

Ultimately, what makes a clade a model clade will depend on the questions and interests of individual researchers, which in turn will determine how these tradeoffs are weighed. When weighing tradeoffs related to species number, researchers interested in testing hypotheses about the evolutionary history and ecological context of a behavior may focus on clades with many taxa, in which comparative methods are most powerful, such as woodpeckers or crickets, but those interested in the genetic or neural mechanism of a single behavioral variant might choose to delve deeper into mechanism in fewer species where manipulative tools for functional tests already exist, such as *Drosophila*. When weighing tradeoffs related to evolutionary distance, researchers interested in the genetic causes of behavioral variation may want to consider focusing on clades with closely related, interfertile species, while researchers interested primarily in understanding neural mechanisms of behavior may choose to focus on clades in which homologous neurons, circuits, and brain regions are identifiable across species even if those species diverged long ago. For example, Kocher and colleagues have recently used behavioral variation in closely related populations of a single species of sweat bees, *Lasioglossum albipes*, some populations of which form social colonies but others of which are solitary, to begin identifying genetic contributions to social behavior (Kocher et al., 2018). On the other end of the spectrum, Fischer and colleagues have focused on independently evolved parental behaviors in two lineages of poison dart frog separated by 140 million years of evolution, finding that homologous brain regions are involved in parental provisioning of chemical defenses to tadpoles in these distantly related lineages (Fischer et al., 2019b).

Tradeoffs between the ability to perform phylogenetic comparisons and neurobiological experiments are inevitable. However, clades in which some features allow for well-powered comparative analyses and some features allow for carefully controlled experimentation will set the stage for feedback between these two approaches, a central goal of evolutionary neuroscience that we think will prove important for addressing long-standing questions (Box 1).

QUESTIONS FOR MODEL CLADES IN NEUROSCIENCE

Feedback between comparative and experimental approaches will be critical to address questions of interest to behavioral neuroscientists, evolutionary biologists, and the next generation of scientists bridging the gaps between these two fields. Below, we present three key questions where we expect answers may be tractable. We also outline additional questions that may benefit from a clade-based approach in Box 1.

What is the contribution of new cell types to behavioral differences between species?

For example, when a difference in behavior evolves between species, is this difference caused by changes in the number of neurons, the relative proportion of cell types comprising those neurons, or novel cell types? With the advent of single-cell RNA sequencing and its accompanying computational toolkits, it is now possible to define cell types and quantitatively compare those cell types across, for example, brain regions within single species. These same tools also will allow for the comparison of cell types between species, opening the door to understanding how cell-type evolution contributes to natural variation in neural circuits underlying behaviors.

Are some changes to neural circuitry more common than others during the course of behavioral evolution?

For example, are evolved behavioral differences caused by differences in the size or wiring of specific projections, by duplication of specific circuit motifs, or by the creation of entirely new circuit architectures? Exciting progress toward answering this question has been made in *Drosophila*, where transgenic tools

Please cite this article in press as: Jourjine and Hoekstra, Expanding evolutionary neuroscience: insights from comparing variation in behavior, Neuron (2021), https://doi.org/10.1016/j.neuron.2021.02.002

have made it possible to quantitatively compare and manipulate single genetically and morphologically defined neurons (see Auer et al., 2020 for an exceptionally thorough example). But the development of tools for quantitative mapping and manipulation of neural circuits has also made questions about variation in neural circuit architecture more accessible in other clades. Low-cost sequencing-based connectomics (Huang et al., 2020b) and cell-type-specific viral vectors (Vormstein-Schneider et al., 2020) are both exciting examples of tools that can be transferred with increasing ease across vertebrate species to ask how changes to neural circuit architecture give rise to behavioral evolution and whether the answer differs between vertebrates and invertebrates.

To what extent does neural circuit architecture promote or constrain behavioral differences between species?

There are several striking examples of convergent evolution of neural mechanisms, from closely related populations of cave fish that have evolved parallel changes in the size of specific brain regions (Jaggard et al., 2020) to distantly related poison dart frogs that have coopted similar brain regions to control maternal provisioning behaviors (Fischer et al., 2019b) to convergently evolved acoustic behaviors across tetrapods (Chen and Wiens, 2020). At the same time, seminal work in crustaceans (Marder, 2011; Marder and Taylor, 2011), as well as more recent experiments from clades of flies (Auer et al., 2020), sea slugs (Sakurai and Katz, 2019), and frogs (Kelley et al., 2020), have shown that different solutions can give rise to the same neural circuit features, and different circuit features can, in turn, give rise to the same behavioral patterns, suggesting that at least for some behaviors in some clades, behavioral variation is not strongly constrained by changes to underlying circuits. An important next step is to understand why behavior sometimes appears constrained by underlying circuits and other times does not. This will require discovering general principles underlying the constraints (or lack thereof) imposed by neural circuits (as well as by genes, gene networks, and development) that shape the path behavioral evolution takes over long and short timescales.

CHALLENGES FOR MODEL CLADES IN NEUROSCIENCE

While model clades provide an exciting way to integrate comparative and experimental approaches to address key questions in neuroscience, this integration does not come without some unique challenges. Indeed, a clade-based approach has at least three hurdles: (1) defining a behavior of interest and making meaningful comparisons of that behavior among species, (2) using experimentation to test hypotheses about causes of variation between species, and (3) moving beyond case studies to general principles underlying behavioral variation. Below, we outline some of the challenges (and possible paths forward) for each of these hurdles.

The challenge of defining, measuring, and comparing behavior in clades

Deciding how behavior should best be defined, quantified, and understood remains a major open question and has been discussed at length in relation to the traditional model species paradigm (Gomez-Marin and Ghazanfar, 2019; Krakauer et al., 2017). Comparing behaviors across species in a clade presents two additional challenges: deciding whether behavioral variants are homologous (and how much it matters for the question of interest) and then deciding on the metrics through which those variants can be meaningfully compared.

Determining whether traits are homologous raises several pervasive and complex issues in evolutionary biology. While we will not rehash these issues here (they have been discussed at length elsewhere; for starting points, see Rendall and Di Fiore, 2007; Wake, 1999), when comparing behaviors across species, the importance of homology will often depend on the questions being asked. If the goal is to trace the historical changes that gave rise to a set of present-day behaviors in different species, then it is important that those behaviors are in fact descended with modification from a common ancestor (i.e., that they are indeed homologous). In this case, it may be useful to focus on clades with very recently diverged species, where establishing homology is less likely to be confounded by evolutionary distance. However, comparisons of distantly diverged species with nonhomologous behaviors can, of course, also be informative. For example, aquarium-raised flamboyant cuttlefish (*Metasepia pfefferi*) are capable of ‘‘walking’’ using tetrapodal gaits extremely similar to those of true tetrapods (Thomas and MacDonald, 2016), and another cuttlefish species (*Sepia smithi*) was recently observed in the wild imitating the morphology and walking style of a decapod crustacean (van Elden and Meeuwig, 2020). Studying nonhomologous behaviors like these walking gaits can inform how species converge on quantitatively similar behaviors using different neural and morphological toolkits.

A second challenge is choosing an appropriate metric by which behaviors can be compared across species. The emerging field of computational neuroethology (Datta et al., 2019) suggests one possible path forward (see Box 2 for more on specific tools): high-throughput quantification of behavioral repertoires followed by dimensionality reduction strategies (e.g., principal-component analysis [PCA]) to define modules within those repertoires and distances among them. This approach can also provide metrics (e.g., distance in PCA space) that may be useful for identifying behavioral modules across species (see Hernández et al., 2020 for an example) and quantifying differences between them. An important caveat is that interspecific behavioral differences are almost certain to have multiple sources, such as body size, differences in overall activity level, or propensity to perform a behavior of interest, that can confound comparisons (although see Machado et al., 2015 for a possible way around this caveat). Another challenge is that even closely related species are likely to differ in behavioral repertoires along many axes. Sophisticated computational approaches are therefore powerful, but not sufficient; careful human observation and a deep understanding of which behaviors are actually performed in nature (and, perhaps more importantly, which matter for survival) will remain essential for guiding which of the potentially many possible behavioral comparisons are most interesting and what potentially confounding species differences need to be controlled for before those comparisons can be made.

Please cite this article in press as: Jourjine and Hoekstra, Expanding evolutionary neuroscience: insights from comparing variation in behavior, Neuron (2021), https://doi.org/10.1016/j.neuron.2021.02.002

Box 2. Tools to study behavioral neuroscience in model clades

Tools to measure behavioral traits and access genetic and neural mechanisms underlying those traits have advanced quickly in the last few years. Below, we highlight some of these, focusing on tools for asking questions about genetic or neural contributions to behavior and for quantifying behavior itself both in the lab and in the wild.

TOOLS TO STUDY BEHAVIOR

Leaps in the field of machine learning and their application to the study of animal behavior have led to a recent wave of packages for high-resolution quantification of animal position and pose, including MoSeq (Wiltschko et al., 2015), DeeplabCut (Mathis et al., 2018), and DeepPoseKit (Graving et al., 2019), as well as vocal behavior, such as MUPET (Van Segbroeck et al., 2017), DeepSqueak (Coffey et al., 2019), Bat Detective (Mac Aodha et al., 2018), and others (Goffinet and Pearson, 2019; Sainburg et al., 2019). Cheap, efficient strategies to quantify behavior and perform behavioral manipulations in parallel for large numbers of animals are also being developed (Saunders and Wehr, 2019; Werkhoven et al., 2019), which enable the comparison of behavioral repertoires of (potentially many) species in a clade of interest. For example, Hernández and colleagues have recently used automated tracking of freely moving *Drosophila* to reconstruct ancestral behavioral repertoires during the evolution of a small clade (Hernández et al., 2020), an approach that will only become more powerful as more species are included. These tools have potential for comparative approaches, because they can often be applied as easily to traditional laboratory models as nontraditional models and even to animals behaving in natural environments with minimal or no human intervention (Hoffmann et al., 2019; Zanette et al., 2019; Mac Aodha et al., 2018).

TOOLS TO STUDY NEURONS AND CIRCUITS

New advances in connectomics, including both EM and more recent technologies (Kuan et al., 2020; Pende et al., 2020), are beginning to identify changes in neural wiring which may contribute to behavioral differences. While this may be challenging to do for many species, new genomic approaches for mapping connectomes (e.g., MAPseq; Huang et al., 2020b) may provide a more high-throughput strategy that can be applied to almost any species. Additional tools to study neurons and circuits include an increasingly diverse panel of channel rhodopsins to manipulate neural activity (Cho et al., 2019; Govorunova et al., 2015; Oppermann et al., 2019), genetically encoded calcium indicators and dense electrode arrays to monitor neural activity (Dana et al., 2019; Jun et al., 2017), and viral strategies to quantify neural anatomy and deliver experimental tools to genetically defined neuronal types (Callaway and Luo, 2015; Guenthner et al., 2013). For example, Scribner and colleagues have recently performed wide-field calcium imaging with virally delivered GCaMP in the brains of awake prairie voles and identified candidate populations involved in the formation of the male/female pair bonds that famously occur in this species, but not closely related montane voles (Scribner et al., 2020) (also see Horie et al., 2020 for an exciting use of cre lines to study the neural basis of pair-bonding behavior in prairie voles).

TOOLS TO STUDY NEURAL IDENTITY

Recent progress toward understanding neural contributions to behavioral evolution has been made possible by advances in sequencing (Gehring et al., 2020; Wenger et al., 2019), tools to combine spatial neuroanatomical information with single-cell gene expression (Moffitt et al., 2018), and tools to determine the molecular identities of neurons that are active during specific behaviors (Knight et al., 2012). Importantly, many of these tools do not require preexisting transgenic lines (e.g., Knight et al., 2012; Moffitt et al., 2018) and can be implemented cheaply in many parallel samples (e.g., Gehring et al., 2020). For example, Kebschull and colleagues have recently used single-cell comparative transcriptomics to investigate the evolution of cerebellar nuclei by comparing mice, chickens, and, remarkably, humans, and find evidence of an archetypal cerebellar nucleus that has diversified through duplication and subsequent divergence of specific cell types (Kebschull et al., 2020). Single-cell sequencing approaches like this are promising avenues to understand the evolution of cell types and their contribution to behavioral diversity between species.

TOOLS TO STUDY GENES AND GENOMES

Behavior evolves because it has a genetic basis, and a major goal of understanding behavioral evolution is to identify the genes responsible for inherited behavioral variation (Arguello and Benton, 2017). Moreover, these genes can be used as a handle to identify the neurons and circuits whose function they influence. Understanding how genetic variation acts in or on neural circuits to influence behavioral variation represents a ‘‘Holy Grail’’ of sorts. Comparative genomics, admixture mapping in natural interbreeding populations, and quantitative trait locus (QTL) mapping, for example, are approaches that describe the genetic architecture of trait variation and that are now being reinvigorated by increasingly cheap genome sequencing and genotype by sequencing

(Continued on next page)

Please cite this article in press as: Jourjine and Hoekstra, Expanding evolutionary neuroscience: insights from comparing variation in behavior, Neuron (2021), https://doi.org/10.1016/j.neuron.2021.02.002

Box 2. *Continued*

methods. These approaches, which have been used successfully to identify the genetic basis of morphological variation, are increasingly being applied to behavioral variation (Fitzpatrick et al., 2005; Niepoth and Bendesky, 2020). However, the challenge remains how to narrow down candidate genes and test their causal roles with functional tests (e.g., Stern, 2014). While these causal tests are without doubt extremely difficult, they are crucial for linking genetic evolution with the evolution of nervous systems and behavior.

The challenge of carrying out manipulative experiments in multiple species

Once behavioral variants are identified and compared, a crucial next step is to carry out experiments to discover underlying causes of the observed variation. As discussed above, some of the most powerful of these experiments are cell-type-specific manipulations using transgenic tools enabled by CRISPR-Cas9 genome editing. However, even when issues of husbandry and reproductive biology are overcome, transgenic approaches most likely need to be re-optimized for each species (even closely related ones), strains are expensive to generate (and, just as importantly, maintain), and interpreting results from neural manipulation experiments comes with serious caveats (Otchy et al., 2015).

Alternatively, experiments that manipulate behavior can be just as (and sometimes more) informative than cell-type-specific transgenic manipulations. As a classic example, simply extending or shortening the leg length of desert ants (*Cataglyphis*) was decisive in showing that these ants use a form of path integration as they navigate (Wittlinger et al., 2006). More recently, virtual reality has been increasingly useful as a tool to perform controlled behavioral experiments (Haberkern et al., 2019; Huang et al., 2020a). When combined with neural measurements, these behavioral experiments are powerful ways to link neural processes and behavioral patterns. The barriers to performing such experiments in multiple species are often relatively low, which can make them more feasible as a starting point for comparative studies than transgenic tools. While studying more than one species will necessarily increase the amount of work required to carry out any well-designed experiments, we think there is reason for hope as it becomes easier to quantify behavior and the transferability of tools across species is being prioritized (see Box 2 for more on these tools).

The challenge of moving beyond case studies to general principles

One step toward understanding the causes of behavioral variation is to accumulate case studies in specific model clades. However, an ultimate goal of the approach we describe here is to understand why some behaviors or neural mechanisms are used in some lineages, but not others, and this requires moving beyond case studies to identifying general principles. An important strategy for accomplishing this goal will be to complement the comparative and experimental approaches with theoretical modeling of behavior and its evolution. Feedback between theory (to motivate and define experimentation) and experimentation (to test theoretical predictions and hone models) has a long and successful history in neuroscience (Marder, 2020a), and this relationship should be crucial as comparative approaches in model clades become more common. Once again, it is useful to look to evo-devo as an example. This field began with a few nontraditional species following a model species ‘‘bottleneck’’ (Milinkovitch and Tzika, 2007) and is now (albeit after many years) at the point where general principles of how morphology evolves are beginning to emerge, often as a result of systematic experimentation, evolutionary comparisons, and theoretical modeling in clades chosen to answer well-defined questions (see Church et al., 2019a, 2019b; Hoke et al., 2019 for some recent success stories). Thus, evolutionary developmental biology provides an example of what a path forward for evolutionary neuroscience might look like as it moves from case studies to general principles shaping behavioral variation.

RECENT INSIGHTS FROM MODEL CLADES

To illustrate how a clade-based approach can be successful, we outline below examples of clades that have been used to understand animal behavior and how it evolves through changes in circuits, neurons, and genes (also see Table 1 for a more complete, but not exhaustive, list). For each clade, we provide a brief summary of the system, examples of the behavioral variation present and highlight a recent study that demonstrates how feedback between experimental and comparative approaches is beginning to address some of the questions and overcome some of the challenges discussed above (Figure 4).

Insights from snails and slugs (*Euthyneura*)

The system

Euthyneura is an ancient and diverse group of gastropods (snails and slugs) in which complex brains may have evolved independently more than once (Kocot et al., 2011). This clade is notable in the history of neuroscience because it contains *Aplysia californica*, an early powerhouse for studies to understand synaptic plasticity (Carew et al., 1971). It is also notable as one of the most diverse and well-studied clades in the field of neuroethology (Katz and Quinlan, 2019) and has contributed in particular to the study of circuits that cause rhythmic behaviors (i.e., central pattern generators [CPGs]) (Katz, 2016a). *Euthyneura* has several features that make a model clade an attractive context for comparative methods. First, homologous neurons can be identified and recorded electrophysiologically in multiple species. Second, those species exhibit a diverse array of fascinating behaviors, including examples of convergent behavioral evolution.

The behaviors

Many of the best-studied behaviors in *Euthyneura* involve feeding, which is accomplished in many ingenious ways in this group. Phylogenetic comparative methods support the hypothesis that the most recent common ancestor of euthyneurans

Please cite this article in press as: Jourjine and Hoekstra, Expanding evolutionary neuroscience: insights from comparing variation in behavior, Neuron (2021), https://doi.org/10.1016/j.neuron.2021.02.002

Table 1. Examples of model clades for behavioral neuroscience

Clade	Natural behavioral variation	References
Stickleback fish	Schooling	Greenwood et al., 2015, 2016; McKinnon and Rundle, 2002
Mexican cave fish	feeding, sleep, thermal preference	Aspiras et al., 2015; Jaggard et al., 2018; Riddle et al., 2018; Tabin et al., 2018; Yoshizawa et al., 2015
Electric fish	electric communication	Crampton, 2019; Picq et al., 2020
Cichlid fish	bower building	Baran and Streelman, 2020; York et al., 2018, 2019
Poison frogs	parental care	Fischer et al., 2019a, 2019b
Xenopus frogs	vocal behavior	Kelley et al., 2020
Domesticated dogs	learning, aggression	Hecht et al., 2019
Voles	pair bond formation	Horie et al., 2019, 2020; Scribner et al., 2020
Singing mice	vocal behavior	Banerjee et al., 2019; Campbell et al., 2010
Deer mice	burrowing, parental care, diet, nesting	Bendesky et al., 2017; Glendinning et al., 1988; Kingsley et al., 2017; Lewarch and Hoekstra, 2018; Weber et al., 2013
Mosquito	host preference	McBride et al., 2014, 2016
Drosophila	courtship song, feeding	Auer et al., 2020; Ding et al., 2019; Seeholzer et al., 2018
Hawai'ian cricket	courtship song	Xu and Shaw, 2019
Sweat bee	social colony formation	Kocher et al., 2018
Ants	social behavior	Chandra et al., 2018
Butterflies	toxin metabolism	Karageorgi et al., 2019; Taverner et al., 2019
Wolf spiders	mating display	Stratton and Uetz, 1986
Rove beetle	ant mimicry	Maruyama and Parker, 2017; Parker, 2016; Parker et al., 2018
Slugs and snails	rhythmic motor behaviors	Gunaratne et al., 2017; Katz and Quinlan, 2019; Sakurai and Katz, 2017

was herbivorous and that carnivory has evolved independently multiple times (Sakurai and Katz, 2015). In addition to feeding behaviors, euthyneurans exhibit a diversity of locomotory behaviors. Specific swimming patterns have also evolved multiple times independently (Sakurai and Katz, 2015), making it a promising group in which to ask about the extent to which evolution has repeatedly found the same neural solutions to a common behavioral problem.

Integrating experimental and comparative approaches

Melibe leonina and *Dendronotus iris* are two species within *Euthyneura* (specifically the subgroup *Cladobranchia)* that exhibit homologous left-right swimming patterns. Sakurai and colleagues have recently used a combination of pharmacology and an electrophysiological technique known as dynamic clamp to manipulate the circuits underlying left-right body flexion in *Melibe* and *Dendronotus* (Sakurai and Katz, 2017). They found that connectivity of homologous neurons differed, demonstrating that divergent wiring patterns are used to produce the same behavioral rhythm in each species. This observation suggests that properties of neural circuits may "drift" away from ancestral states over the course of evolution without producing a measurable effect on behavior, a process conceptually similar to genetic drift, in which mutations accumulate in genomes without affecting fitness. Is "neural drift" common or rare during behavioral evolution? The wealth of species and electrophysiological tools available in *Euthyneura* make it a promising clade in which to address this question.

Insights from fruit flies (*Drosophila*)

The system

Most neuroscientists likely associate fruit flies with one of the oldest and best-developed traditional model species, *D. melanogaster*. However, the genus to which this species belongs comprises more than 1,600 species distributed around the globe and has provided some of the best-documented cases of naturally occurring variation in behavior. In contrast to snails and slugs, this group is relatively recently diverged, and while electrophysiology is somewhat more difficult in *Drosophila* compared to *Euthyneura* due to their (typically) small neurons, it has the advantage of an abundance of transgenic tools for cell-type-specific manipulative experiments, several of which have recently been moved from *D. melanogaster* (where they were initially developed) to four species belonging to the *melanogaster* subgroup (Ding et al., 2016, 2019; Seeholzer et al., 2018; Stern et al., 2017).

The behaviors

Perhaps the best-studied example of behavioral variation in *Drosophila* involves the courtship songs produced by males (and sometimes females) as they vibrate their wings prior to copulation. While these songs are required for successful mating, species differ in the number of wings vibrated (Ding et al., 2019), the frequency of vibration (Ding et al., 2016), and whether or not females vibrate their wings in duets with males (LaRue et al., 2015). While most studies of natural behavioral variation in *Drosophila* have focused on mating song, there are many more examples of behavioral variation in fruit flies, such as food specialization, in which exciting progress has already been made (Auer et al., 2020), as well as habitat choice (Cooper et al., 2018) and circadian rhythm (Yang and Edery, 2018), among others (Dobzhansky and Spassky, 1969; Yamamoto, 1994) whose genetic and neural basis is waiting to be uncovered.

Integrating experimental and comparative approaches

Ding and colleagues have recently combined cell-type-specific neural manipulations with a comparative approach to understand

Please cite this article in press as: Jourjine and Hoekstra, Expanding evolutionary neuroscience: insights from comparing variation in behavior, Neuron (2021), https://doi.org/10.1016/j.neuron.2021.02.002

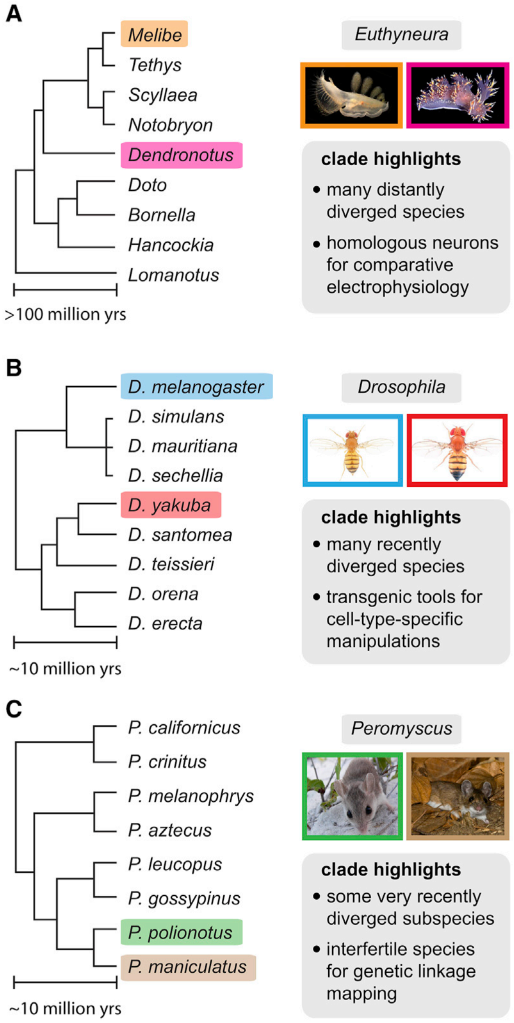

Figure 4. Three examples of model clades

(A) Sea slugs (belonging to *Euthyneura*), specifically those in the *Cladobranchia* group shown, have been used to understand the extent to which rhythmic behaviors are (or are not) constrained by the connectivity of underlying central pattern generators. Sakurai and Katz used electrophysiological and pharmacological tools to discover that different neural circuit architectures give rise to similar rhythmic swimming behaviors (Sakurai and Katz, 2017).

(B) *Drosophila* species in the melanogaster subgroup have been used to understand the neural basis of behavioral variation in courtship song. Ding and colleagues used transgenic tools to identify a single, genetically defined neuron involved in initiation of song in two species (Ding et al., 2019).

(C) Mice in the genus *Peromyscus* have been used to understand the genetic contributions to natural variation in parental behavior. Bendesky and colleagues used linkage mapping between interfertile sister species to identify and functionally validate the effect of the neuropeptide Avp on paternal care in these species (Bendesky et al., 2017).

the neural basis for song type evolution in in the *D. melanogaster* species subgroup (Ding et al., 2019). First, by comparing song types from nine closely related species, they show that *D. yakuba* and *D. santomea* have evolved a novel song type (the "clack" song) since the most recent common ancestor in the clade. Then, by moving transgenic lines that label specific neurons in the *melanogaster* song pathway to *yakuba*, they identify a single neuron, pIP10, that appears to be morphologically and functionally conserved between *yakuba* and *melanogaster*. In both species, this neuron descends from the brain to the ventral nerve cord, where singing pre-motor circuits are located, and in both species, high levels of pIP10 activation cause species-specific song. This effect of activation is also observed in headless males, arguing that the evolved neural differences that give rise to song variation lie downstream of pIP10 in the ventral nerve cord, close to the motor output for fly song (wing muscles).

A complementary study from Seeholzer and colleagues focusing on the evolution of a different aspect of courtship initiation, which is caused by gustatory detection by males of female cuticular hydrocarbons, identified a difference between *D. melanogaster* and another species, *D. simulans*, in the functional connectivity of a genetically defined circuit in the central brain. This difference contributes to the ability of males to distinguish between females from each of these species (Seeholzer et al., 2018). While in both studies, the specific evolving neurons remain to be identified, continued feedback between cell-type-specific manipulation and evolutionary inferences makes *Drosophila* a promising clade to determine which parts of the nervous system are likely (and which are unlikely) to give rise to behavioral evolution.

Insights from deer mice (*Peromyscus*)

The system

Deer mice are a group of exceptionally abundant and diverse North American rodents. Their natural history has been extensively documented over the course of the last century, creating a rich literature that has served as the foundation for many studies on morphological and behavioral variation among species in the wild (Bedford and Hoekstra, 2015). Despite this extensive variation, many species of deer mice remain interfertile in the laboratory, a feature that has facilitated recent forward-genetic studies (e.g., linkage mapping) to reveal the genetic architecture underlying natural variation in complex yet heritable mammalian behaviors. Thus, while transgenics and electrophysiology are still in their infancy in *Peromyscus*, two powerful strengths of this clade are the presence of closely related species with well-documented natural histories and highly divergent behaviors and the fact that several of these species can be crossed with each other to uncover the genetic underpinnings of neural circuitry and ultimately behavioral evolution.

The behaviors

Behavioral variation in *Peromyscus* spans social behaviors such as vocalization and parental care (Bendesky et al., 2017; Hart and King, 1966), environmental engineering such as burrowing and nest building (Lewarch and Hoekstra, 2018; Weber et al., 2013), and feeding behaviors such as the striking seasonal specialization on overwintering colonies of monarch butterflies by *P. melanotis* (Glendinning et al., 1988). We have recently focused our attention on a clade of *P. maniculatus* subspecies that have independently evolved behavioral and morphological adaptations to forested habitats in eastern and western North America (Kingsley et al., 2017). These subspecies have diverged extremely recently (~8,000–10,000 years ago), are interfertile, and exhibit variation in both morphology and behavior related to skilled climbing. Together with recently developed viral and

Please cite this article in press as: Jourjine and Hoekstra, Expanding evolutionary neuroscience: insights from comparing variation in behavior, Neuron (2021), https://doi.org/10.1016/j.neuron.2021.02.002

transgenic tools in *Peromyscus*, they provide an example of a model clade in which linkage mapping between very closely related species can uncouple the relative genetic contributions of neural anatomy and (non-neural) morphology to natural variation in behavior.

Integrating experimental and comparative approaches

We have also recently used linkage mapping to characterize the genetic architecture of parental care differences between two *Peromyscus* species, *P. maniculatus* and *P. polionotus*. *P. maniculatus* breed promiscuously and females primarily provide care for young, while *P. polionotus* form pair bonds in which both parents provide parental care (Bendesky et al., 2017). These complex differences in behavior are heritable and correlated with a small number of genomic loci. In particular, one parental behavior, nest building, is associated with inter-specific differences in expression of the pleiotropic gene arginine vasopressin (*avp*), which is expressed ~2-fold higher in *P. maniculatus*, where males rarely build nests for their litters, than in *P. polionotus*, where males often exhibit this behavior. Remarkably, application of exogenous AVP protein specifically suppresses nest building, but not other aspects of parental care, in *P. polionotus.* Thus, evolutionary modification in the expression of an ancient peptide hormone contributes to differences in social behavior between these species.

SUMMARY

The history of neuroscience has been profoundly shaped by its search for study systems. While 19th century neuroanatomists dissected as many species as they could get their hands on (the neuron doctrine was founded on observations Ramon y Cajal made of nervous systems from more than 50 species comprising 11 taxonomic orders; Garcia-Lopez et al., 2010), early 20th century physiologists and ethologists used Krogh's principle to search for species with just the right biology to answer specific questions that interested them. The molecular revolution of the mid-20th century then spurred on the era of the classic model species in which large communities shared knowledge and tool development for a relatively small number of species.

In the last decade, neuroscience has overseen a revolution of its own. Optogenetics has made cell-type-specific neural manipulations common practice, and CRISPR-Cas9, together with an increasingly diverse and versatile array of viral vectors and non-transgenic electrophysiological tools, is bringing a style of experimental neuroscience that was once restricted to a few model species to more and more nontraditional models. Thus, having emerged from the model species bottleneck (Brenowitz and Zakon, 2015), neuroscience is once again being shaped by a search for just the right study systems to answer long-standing and challenging questions. Here, we have argued in favor of groups of species related by a common ancestor (i.e., clades) as particularly powerful study systems in which to answer some of those questions.

While this approach comes with its own challenges, model clades bring with them the possibility of exciting discoveries that feedback between experimental and comparative approaches will foster. For example, by identifying clades in which the same behavior has independently evolved multiple times, we can gain insight into not only its evolutionary history but also its underlying mechanisms by finding the genes, neurons, or circuits that repeatedly evolve to produce the behavior (e.g., Gallant and O'Connell, 2020). By identifying the neural and molecular causes of behavioral differences within clades, we can uncover examples of behavioral adaptation (e.g., Campbell et al., 2019; Rowe and Rowe, 2008) and novel mechanisms that may underlie those adaptations (e.g., Okobi et al., 2019; Rowe et al., 2013). In other words, comparative studies of natural behavioral variation can be used to discover basic principles of neuroscience at the same time that experimental tools developed by neuroscientists can help discover causes of behavioral diversity in the natural world—a win-win situation.

ACKNOWLEDGMENTS

We would like to thank Vanessa Ruta, David Stern, Paul Katz, Michael Long, Sandeep Robert Datta, Michael Yartsev, one anonymous reviewer, and the members of the Hoekstra lab for their insightful comments on the manuscript. We also thank Paul Katz and Nicolas Gompel for kindly providing the organism photos in Figures 4A and 4B, respectively. Finally, we thank Renate Hellmiss, Harvard MCB graphics group, for help designing figures. Image credits: *Melibe leonina*, Charuni Gunaratne; *Dendronotus iris*, Bruce Wight and Ralph and Dale Marie Gonzales; *D. melanogaster* and *D. yakuba*, Nicolas Gompel; *P. polionotus*, Matt Falcy; *P. maniculatus*, Shawn Carey. Mouse silhouettes in Figures 1 and 2 were modified from the original by Anthony Caravaggi (http://phylopic.org; https://creativecommons.org/licenses/by-nc-sa/3.0/). N.J. is funded by the Jane Coffin Childs Memorial Fund; H.E.H. is an Investigator of the Howard Hughes Medical Institute.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

Arguello, J.R., and Benton, R. (2017). Open questions: Tackling Darwin's "instincts": the genetic basis of behavioral evolution. BMC Biol. *15*, 26.

Aspiras, A.C., Rohner, N., Martineau, B., Borowsky, R.L., and Tabin, C.J. (2015). Melanocortin 4 receptor mutations contribute to the adaptation of cavefish to nutrient-poor conditions. Proc. Natl. Acad. Sci. USA *112*, 9668–9673.

Auer, T.O., Khallaf, M.A., Silbering, A.F., Zappia, G., Ellis, K., Álvarez-Ocaña, R., Arguello, J.R., Hansson, B.S., Jefferis, G.S.X.E., Caron, S.J.C., et al. (2020). Olfactory receptor and circuit evolution promote host specialization. Nature *579*, 402–408.

Banerjee, A., Phelps, S.M., and Long, M.A. (2019). Singing mice. Curr. Biol. *29*, R190–R191.

Baran, N.M., and Streelman, J.T. (2020). Ecotype differences in aggression, neural activity and behaviorally relevant gene expression in cichlid fish. Genes Brain Behav. *19*, e12657.

Beach, F. (1950). The Snark Was a Boojum. Am. Psychol. *5*, 115–124.

Bedford, N.L., and Hoekstra, H.E. (2015). *Peromyscus* mice as a model for studying natural variation. eLife *4*, e06813.

Bendesky, A., Kwon, Y.M., Lassance, J.M., Lewarch, C.L., Yao, S., Peterson, B.K., He, M.X., Dulac, C., and Hoekstra, H.E. (2017). The genetic basis of parental care evolution in monogamous mice. Nature *544*, 434–439.

Bernard, C. (1864). Introduction à l'étude de la médecine expérimentale (Libraires de L'Académie Impériale de Médecine: J.B. Baillière et Fils).

Brenowitz, E.A., and Zakon, H.H. (2015). Emerging from the bottleneck: benefits of the comparative approach to modern neuroscience. Trends Neurosci. *38*, 273–278.

Please cite this article in press as: Jourjine and Hoekstra, Expanding evolutionary neuroscience: insights from comparing variation in behavior, Neuron (2021), https://doi.org/10.1016/j.neuron.2021.02.002

Callaway, E.M., and Luo, L. (2015). Monosynaptic circuit tracing with glycoprotein-deleted rabies viruses. J. Neurosci. *35*, 8979–8985.

Campbell, P., Pasch, B., Pino, J.L., Crino, O.L., Phillips, M., and Phelps, S.M. (2010). Geographic variation in the songs of neotropical singing mice: testing the relative importance of drift and local adaptation. Evolution *64*, 1955–1972.

Campbell, P., Arévalo, L., Martin, H., Chen, C., Sun, S., Rowe, A.H., Webster, M.S., Searle, J.B., and Pasch, B. (2019). Vocal divergence is concordant with genomic evidence for strong reproductive isolation in grasshopper mice (*Onychomys*). Ecol. Evol. *9*, 12886–12896.

Carew, T.J., Castellucci, V.F., and Kandel, E.R. (1971). An analysis of dishabituation and sensitization of the gill-withdrawal reflex in Aplysia. Int. J. Neurosci. *2*, 79–98.

Cavender-Bares, J. (2019). Diversification, adaptation, and community assembly of the American oaks (*Quercus*), a model clade for integrating ecology and evolution. New Phytol. *221*, 669–692.

Chandra, V., Fetter-Pruneda, I., Oxley, P.R., Ritger, A.L., McKenzie, S.K., Libbrecht, R., and Kronauer, D.J.C. (2018). Social regulation of insulin signaling and the evolution of eusociality in ants. Science *361*, 398–402.

Chen, Z., and Wiens, J.J. (2020). The origins of acoustic communication in vertebrates. Nat. Commun. *11*, 369.

Cho, Y.K., Park, D., Yang, A., Chen, F., Chuong, A.S., Klapoetke, N.C., and Boyden, E.S. (2019). Multidimensional screening yields channelrhodopsin variants having improved photocurrent and order-of-magnitude reductions in calcium and proton currents. J. Biol. Chem. *294*, 3806–3821.

Church, S.H., and Extavour, C.G. (2020). Null hypotheses for developmental evolution. Development *147*, dev178004.

Church, S.H., Donoughe, S., de Medeiros, B.A.S., and Extavour, C.G. (2019a). A dataset of egg size and shape from more than 6,700 insect species. Sci. Data *6*, 104.

Church, S.H., Donoughe, S., de Medeiros, B.A.S., and Extavour, C.G. (2019b). Insect egg size and shape evolve with ecology but not developmental rate. Nature *571*, 58–62.

Coffey, K.R., Marx, R.G., and Neumaier, J.F. (2019). DeepSqueak: a deep learning-based system for detection and analysis of ultrasonic vocalizations. Neuropsychopharmacology *44*, 859–868.

Cooper, B.S., Sedghifar, A., Nash, W.T., Comeault, A.A., and Matute, D.R. (2018). A maladaptive combination of traits contributes to the maintenance of a *Drosophila* hybrid zone. Curr. Biol. *28*, 2940–2947.

Crampton, W.G.R. (2019). Electroreception, electrogenesis and electric signal evolution. J. Fish Biol. *95*, 92–134.

Dana, H., Sun, Y., Mohar, B., Hulse, B.K., Kerlin, A.M., Hasseman, J.P., Tsegaye, G., Tsang, A., Wong, A., Patel, R., et al. (2019). High-performance calcium sensors for imaging activity in neuronal populations and microcompartments. Nat. Methods *16*, 649–657.

Darwin, C. (1897). The Expression of Emotions in Man and Animals (D. Appleton and Company).

Datta, S.R., Anderson, D.J., Branson, K., Perona, P., and Leifer, A. (2019). Computational neuroethology: A call to action. Neuron *104*, 11–24.

Desai, N.S., Gray, R., and Johnston, D. (2017). A dynamic clamp on every rig. eNeuro *4*, ENEURO.0250-0217.2017.

Dickinson, P.S., Mecsas, C., and Marder, E. (1990). Neuropeptide fusion of two motor-pattern generator circuits. Nature *344*, 155–158.

Ding, Y., Berrocal, A., Morita, T., Longden, K.D., and Stern, D.L. (2016). Natural courtship song variation caused by an intronic retroelement in an ion channel gene. Nature *536*, 329–332.

Ding, Y., Lillvis, J.L., Cande, J., Berman, G.J., Arthur, B.J., Long, X., Xu, M., Dickson, B.J., and Stern, D.L. (2019). Neural evolution of context-dependent fly song. Curr. Biol. *29*, 1089–1099.

Dobzhansky, T., and Spassky, B. (1969). Artificial and natural selection for two behavioral traits in *Drosophila pseudoobscura*. Proc. Natl. Acad. Sci. USA *62*, 75–80.

Donoghue, M.J., and Edwards, E.J. (2019). Model clades are vital for comparative biology, and ascertainment bias is not a problem in practice: a response to Beaulieu and O'Meara (2018). Am. J. Bot. *106*, 327–330.

Dupre, C., and Yuste, R. (2017). Non-overlapping neural networks in *Hydra vulgaris*. Curr. Biol. *27*, 1085–1097.

Fenno, L.E., Ramakrishnan, C., Kim, Y.S., Evans, K.E., Lo, M., Vesuna, S., Inoue, M., Cheung, K.Y.M., Yuen, E., Pichamoorthy, N., et al. (2020). Comprehensive dual- and triple-feature intersectional single-vector delivery of diverse functional payloads to cells of behaving mammals. Neuron *107*, 836–853.

Fischer, E.K., Roland, A.B., Moskowitz, N.A., Tapia, E.E., Summers, K., Coloma, L.A., and O'Connell, L.A. (2019a). The neural basis of tadpole transport in poison frogs. Proc. Biol. Sci. *286*, 20191084.

Fischer, E.K., Roland, A.B., Moskowitz, N.A., Vidoudez, C., Ranaivorazo, N., Tapia, E.E., Trauger, S.A., Vences, M., Coloma, L.A., and O'Connell, L.A. (2019b). Mechanisms of convergent egg provisioning in poison frogs. Curr. Biol. *29*, 4145–4151.

Fitzpatrick, M.J., Ben-Shahar, Y., Smid, H.M., Vet, L.E., Robinson, G.E., and Sokolowski, M.B. (2005). Candidate genes for behavioural ecology. Trends Ecol. Evol. *20*, 96–104.

Gallant, J.R., and O'Connell, L.A. (2020). Studying convergent evolution to relate genotype to behavioral phenotype. J. Exp. Biol. *223*, jeb213447.

Garcia-Lopez, P., Garcia-Marin, V., and Freire, M. (2010). The histological slides and drawings of cajal. Front. Neuroanat. *4*, 9.

Gehring, J., Hwee Park, J., Chen, S., Thomson, M., and Pachter, L. (2020). Highly multiplexed single-cell RNA-seq by DNA oligonucleotide tagging of cellular proteins. Nat. Biotechnol. *38*, 35–38.

Glendinning, J.I., Mejia, A.A., and Brower, L.P. (1988). Behavioral and ecological interactions of foraging mice (*Peromyscus melanotis*) with overwintering monarch butterflies (*Danaus plexippus*) in México. Oecologia *75*, 222–227.

Goffinet, J.M.R., and Pearson, J. (2019). Inferring low-dimensional latent descriptions of animal vocalizations. bioRxiv. https://doi.org/10.1101/811661.

Gomez-Marin, A., and Ghazanfar, A.A. (2019). The Life of Behavior. Neuron *104*, 25–36.

Govorunova, E.G., Sineshchekov, O.A., Janz, R., Liu, X., and Spudich, J.L. (2015). Natural light-gated anion channels: A family of microbial rhodopsins for advanced optogenetics. Science *349*, 647–650.

Graving, J.M., Chae, D., Naik, H., Li, L., Koger, B., Costelloe, B.R., and Couzin, I.D. (2019). DeepPoseKit, a software toolkit for fast and robust animal pose estimation using deep learning. eLife *8*, e47994.

Greenwood, A.K., Ardekani, R., McCann, S.R., Dubin, M.E., Sullivan, A., Bensussen, S., Tavaré, S., and Peichel, C.L. (2015). Genetic mapping of natural variation in schooling tendency in the threespine stickleback. G3 (Bethesda) *5*, 761–769.

Greenwood, A.K., Mills, M.G., Wark, A.R., Archambeault, S.L., and Peichel, C.L. (2016). Evolution of schooling behavior in threespine sticklebacks is shaped by the Eda gene. Genetics *203*, 677–681.

Guenthner, C.J., Miyamichi, K., Yang, H.H., Heller, H.C., and Luo, L. (2013). Permanent genetic access to transiently active neurons via TRAP: targeted recombination in active populations. Neuron *78*, 773–784.

Gunaratne, C.A., Sakurai, A., and Katz, P.S. (2017). Variations on a theme: species differences in synaptic connectivity do not predict central pattern generator activity. J. Neurophysiol. *118*, 1123–1132.

Haberkern, H., Basnak, M.A., Ahanonu, B., Schauder, D., Cohen, J.D., Bolstad, M., Bruns, C., and Jayaraman, V. (2019). Visually guided behavior and optogenetically induced learning in head-fixed flies exploring a virtual landscape. Curr. Biol. *29*, 1647–1659.

Hart, F.M., and King, J.A. (1966). Distress vocalizations of young in two subspecies of *Peromyscus maniculatus*. J. Mammal. *47*, 287–293.

Hecht, E.E., Smaers, J.B., Dunn, W.D., Kent, M., Preuss, T.M., and Gutman, D.A. (2019). Significant neuroanatomical variation among domestic dog breeds. J. Neurosci. *39*, 7748–7758.

Hernández, D.G., Rivera, C., Cande, J., Zhou, B., Stern, D.L., and Berman, G.J. (2020). A framework for studying behavioral evolution by reconstructing ancestral repertoires. bioRxiv. https://doi.org/10.1101/2020.1107.1117.209361.

Hodgkin, A.L., and Huxley, A.F. (1945). Resting and action potentials in single nerve fibres. J. Physiol. *104*, 176–195.

Hoffmann, S., Trost, L., Voigt, C., Leitner, S., Lemazina, A., Sagunsky, H., Abels, M., Kollmansperger, S., Maat, A.T., and Gahr, M. (2019). Duets recorded in the wild reveal that interindividually coordinated motor control enables cooperative behavior. Nat. Commun. *10*, 2577.

Hoke, K.L., Adkins-Regan, E., Bass, A.H., McCune, A.R., and Wolfner, M.F. (2019). Co-opting evo-devo concepts for new insights into mechanisms of behavioural diversity. J. Exp. Biol. *222*, jeb190058.

Horie, K., Inoue, K., Nishimori, K., and Young, L.J. (2020). Investigation of Oxtr-expressing neurons projecting to nucleus accumbens using oxtr-ires-Cre knock-in prairie voles (*Microtus ochrogaster*). Neuroscience *448*, 312–324.

Horie, K., Inoue, K., Suzuki, S., Adachi, S., Yada, S., Hirayama, T., Hidema, S., Young, L.J., and Nishimori, K. (2019). Oxytocin receptor knockout prairie voles generated by CRISPR/Cas9 editing show reduced preference for social novelty and exaggerated repetitive behaviors. Horm. Behav. *111*, 60–69.

Huang, K.H., Rupprecht, P., Frank, T., Kawakami, K., Bouwmeester, T., and Friedrich, R.W. (2020a). A virtual reality system to analyze neural activity and behavior in adult zebrafish. Nat. Methods *17*, 343–351.

Huang, L., Kebschull, J.M., Fürth, D., Musall, S., Kaufman, M.T., Churchland, A.K., and Zador, A.M. (2020b). BRICseq Bridges brain-wide interregional connectivity to neural activity and gene expression in single animals. Cell *182*, 177–188.

Jaggard, J.B., Stahl, B.A., Lloyd, E., Prober, D.A., Duboue, E.R., and Keene, A.C. (2018). Hypocretin underlies the evolution of sleep loss in the Mexican cavefish. eLife *7*, e32637.

Jaggard, J.B., Lloyd, E., Yuiska, A., Patch, A., Fily, Y., Kowalko, J.E., Appelbaum, L., Duboue, E.R., and Keene, A.C. (2020). Cavefish brain atlases reveal functional and anatomical convergence across independently evolved populations. Sci. Adv. *6*, eaba3126.

Jun, J.J., Steinmetz, N.A., Siegle, J.H., Denman, D.J., Bauza, M., Barbarits, B., Lee, A.K., Anastassiou, C.A., Andrei, A., Aydın, Ç., et al. (2017). Fully integrated silicon probes for high-density recording of neural activity. Nature *551*, 232–236.

Karageorgi, M., Groen, S.C., Sumbul, F., Pelaez, J.N., Verster, K.I., Aguilar, J.M., Hastings, A.P., Bernstein, S.L., Matsunaga, T., Astourian, M., et al. (2019). Genome editing retraces the evolution of toxin resistance in the monarch butterfly. Nature *574*, 409–412.

Katz, P.S. (2016a). Evolution of central pattern generators and rhythmic behaviours. Philos. Trans. R. Soc. Lond. B Biol. Sci. *371*, 20150057.

Katz, P.S. (2016b). ‘Model organisms’ in the light of evolution. Curr. Biol. *26*, R649–R650.

Katz, P.S. (2019). The conservative bias of life scientists. Curr. Biol. *29*, R666–R667.

Katz, P.S., and Quinlan, P.D. (2019). The importance of identified neurons in gastropod molluscs to neuroscience. Curr. Opin. Neurobiol. *56*, 1–7.

Kebschull, J.M., Richman, E.B., Ringach, N., Friedmann, D., Albarran, E., Kolluru, S.S., Jones, R.C., Allen, W.E., Wang, Y., Cho, S.W., et al. (2020). Cerebellar nuclei evolved by repeatedly duplicating a conserved cell-type set. Science *370*, eabd5059.

Kelley, D.B., Ballagh, I.H., Barkan, C.L., Bendesky, A., Elliott, T.M., Evans, B.J., Hall, I.C., Kwon, Y.M., Kwong-Brown, U., Leininger, E.C., et al. (2020). Generation, coordination, and evolution of neural circuits for vocal communication. J. Neurosci. *40*, 22–36.

Kingsley, E.P., Kozak, K.M., Pfeifer, S.P., Yang, D.S., and Hoekstra, H.E. (2017). The ultimate and proximate mechanisms driving the evolution of long tails in forest deer mice. Evolution *71*, 261–273.

Knight, Z.A., Tan, K., Birsoy, K., Schmidt, S., Garrison, J.L., Wysocki, R.W., Emiliano, A., Ekstrand, M.I., and Friedman, J.M. (2012). Molecular profiling of activated neurons by phosphorylated ribosome capture. Cell *151*, 1126–1137.

Kocher, S.D., Mallarino, R., Rubin, B.E.R., Yu, D.W., Hoekstra, H.E., and Pierce, N.E. (2018). The genetic basis of a social polymorphism in halictid bees. Nat. Commun. *9*, 4338.

Kocot, K.M., Cannon, J.T., Todt, C., Citarella, M.R., Kohn, A.B., Meyer, A., Santos, S.R., Schander, C., Moroz, L.L., Lieb, B., and Halanych, K.M. (2011). Phylogenomics reveals deep molluscan relationships. Nature *477*, 452–456.

Krakauer, J.W., Ghazanfar, A.A., Gomez-Marin, A., MacIver, M.A., and Poeppel, D. (2017). Neuroscience needs behavior: correcting a reductionist bias. Neuron *93*, 480–490.

Krogh, A. (1929). The Progress of Physiology. Science *70*, 200–204.

Kuan, A.T., Phelps, J.S., Thomas, L.A., Nguyen, T.M., Han, J., Chen, C.L., Azevedo, A.W., Tuthill, J.C., Funke, J., Cloetens, P., et al. (2020). Dense neuronal reconstruction through X-ray holographic nano-tomography. Nat. Neurosci. *23*, 1637–1643.

Kulkarni, A., and Extavour, C.G. (2019). The cricket *Gryllus bimaculatus*: techniques for quantitative and functional genetic analyses of cricket biology. In Evo-Devo: Non-model Species in Cell and Developmental Biology, W. Tworzydlo and S.M. Bilinski, eds. (Springer), pp. 183–216.

LaRue, K.M., Clemens, J., Berman, G.J., and Murthy, M. (2015). Acoustic duetting in *Drosophila virilis* relies on the integration of auditory and tactile signals. eLife *4*, e07277.

Laurent, G. (2020). On the value of model diversity in neuroscience. Nat. Rev. Neurosci. *21*, 395–396.

Lewarch, C.L., and Hoekstra, H.E. (2018). The evolution of nesting behavior in *Peromyscus* mice. Anim. Behav. *139*, 103–115.

Lickliter, R., and Bahrick, L.E. (2013). The concept of homology as a basis for evaluating developmental mechanisms: exploring selective attention across the life-span. Dev. Psychobiol. *55*, 76–83.

Mac Aodha, O., Gibb, R., Barlow, K.E., Browning, E., Firman, M., Freeman, R., Harder, B., Kinsey, L., Mead, G.R., Newson, S.E., et al. (2018). Bat detective-Deep learning tools for bat acoustic signal detection. PLoS Comput. Biol. *14*, e1005995.

Machado, A.S., Darmohray, D.M., Fayad, J., Marques, H.G., and Carey, M.R. (2015). A quantitative framework for whole-body coordination reveals specific deficits in freely walking ataxic mice. eLife *4*, e07892.

Marder, E. (2011). Variability, compensation, and modulation in neurons and circuits. Proc. Natl. Acad. Sci. USA *108* (*Suppl 3*), 15542–15548.

Marder, E. (2020a). Theoretical musings. eLife *9*, e60703.

Marder, E. (2020b). Words without meaning. eLife *9*, e54867.

Marder, E., and Taylor, A.L. (2011). Multiple models to capture the variability in biological neurons and networks. Nat. Neurosci. *14*, 133–138.

Maruyama, M., and Parker, J. (2017). Deep-time convergence in rove beetle symbionts of army ants. Curr. Biol. *27*, 920–926.

Mathis, A., Mamidanna, P., Cury, K.M., Abe, T., Murthy, V.N., Mathis, M.W., and Bethge, M. (2018). DeepLabCut: markerless pose estimation of user-defined body parts with deep learning. Nat. Neurosci. *21*, 1281–1289.

McBride, C.S. (2016). Genes and odors underlying the recent evolution of mosquito preference for humans. Curr. Biol. *26*, R41–R46.

McBride, C.S., Baier, F., Omondi, A.B., Spitzer, S.A., Lutomiah, J., Sang, R., Ignell, R., and Vosshall, L.B. (2014). Evolution of mosquito preference for humans linked to an odorant receptor. Nature *515*, 222–227.

McKinnon, J.S., and Rundle, H.D. (2002). Speciation in nature: the threespine stickleback model systems. Trends Ecol. Evol. *17*, 480–488.

Menda, G., Shamble, P.S., Nitzany, E.I., Golden, J.R., and Hoy, R.R. (2014). Visual perception in the brain of a jumping spider. Curr. Biol. *24*, 2580–2585.

Mendelson, T., and Shaw, K. (2005). Rapid speciation in an arthropod. Nature *433*, 375–376.

Miles, M.C., Schuppe, E.R., and Fuxjager, M.J. (2020). Selection for rhythm as a trigger for recursive evolution in the elaborate display system of woodpeckers. Am. Nat. *195*, 772–787.

Milinkovitch, M.C., and Tzika, A. (2007). Escaping the mouse trap: the selection of new Evo-Devo model species. J. Exp. Zoolog. B Mol. Dev. Evol. *308*, 337–346.

Moffitt, J.R., Bambah-Mukku, D., Eichhorn, S.W., Vaughn, E., Shekhar, K., Perez, J.D., Rubinstein, N.D., Hao, J., Regev, A., Dulac, C., and Zhuang, X. (2018). Molecular, spatial, and functional single-cell profiling of the hypothalamic preoptic region. Science *362*, eaau5324.

Nakamura, T., Yoshizaki, M., Ogawa, S., Okamoto, H., Shinmyo, Y., Bando, T., Ohuchi, H., Noji, S., and Mito, T. (2010). Imaging of transgenic cricket embryos reveals cell movements consistent with a syncytial patterning mechanism. Curr. Biol. *20*, 1641–1647.

Niepoth, N., and Bendesky, A. (2020). How natural genetic variation shapes behavior. Annu. Rev. Genomics Hum. Genet. *21*, 437–463.

Okobi, D.E., Jr., Banerjee, A., Matheson, A.M.M., Phelps, S.M., and Long, M.A. (2019). Motor cortical control of vocal interaction in neotropical singing mice. Science *363*, 983–988.

Oppermann, J., Fischer, P., Silapetere, A., Liepe, B., Rodriguez-Rozada, S., Flores-Uribe, J., Peter, E., Keidel, A., Vierock, J., Kaufmann, J., et al. (2019). MerMAIDs: a family of metagenomically discovered marine anion-conducting and intensely desensitizing channelrhodopsins. Nat. Commun. *10*, 3315.

Otchy, T.M., Wolff, S.B., Rhee, J.Y., Pehlevan, C., Kawai, R., Kempf, A., Gobes, S.M., and Ölveczky, B.P. (2015). Acute off-target effects of neural circuit manipulations. Nature *528*, 358–363.

Parker, J. (2016). Myrmecophily in beetles (*Coleoptera*): evolutionary patterns and biological mechanisms. Myrmecol. News *22*, 65–108.

Parker, J., Taro Eldredge, K., Thomas, I.M., Coleman, R., and Davis, S.R. (2018). Hox-logic of body plan innovations for social symbiosis in rove beetles. bioRxiv. https://doi.org/10.1101/198945.

Pende, M., Vadiwala, K., Schmidbaur, H., Stockinger, A.W., Murawala, P., Saghafi, S., Dekens, M.P.S., Becker, K., Revilla-I-Domingo, R., Papadopoulos, S.C., et al. (2020). A versatile depigmentation, clearing, and labeling method for exploring nervous system diversity. Sci. Adv. *6*, eaba0365.

Pennell, M.W., and Harmon, L.J. (2013). An integrative view of phylogenetic comparative methods: connections to population genetics, community ecology, and paleobiology. Ann. N Y Acad. Sci. *1289*, 90–105.

Picq, S., Sperling, J., Cheng, C.J., Carlson, B.A., and Gallant, J.R. (2020). Genetic drift does not sufficiently explain patterns of electric signal variation among populations of the mormyrid electric fish *Paramormyrops kingsleyae*. Evolution *74*, 911–935.

Reiter, S., Hülsdunk, P., Woo, T., Lauterbach, M.A., Eberle, J.S., Akay, L.A., Longo, A., Meier-Credo, J., Kretschmer, F., Langer, J.D., et al. (2018). Elucidating the control and development of skin patterning in cuttlefish. Nature *562*, 361–366.

Rendall, D., and Di Fiore, A. (2007). Homoplasy, homology, and the perceived special status of behavior in evolution. J. Hum. Evol. *52*, 504–521.

Riddle, M.R., Aspiras, A.C., Gaudenz, K., Peuß, R., Sung, J.Y., Martineau, B., Peavey, M., Box, A.C., Tabin, J.A., McGaugh, S., et al. (2018). Insulin resistance in cavefish as an adaptation to a nutrient-limited environment. Nature *555*, 647–651.

Rowe, A.H., and Rowe, M.P. (2008). Physiological resistance of grasshopper mice (*Onychomys spp.*) to Arizona bark scorpion (*Centruroides exilicauda*) venom. Toxicon *52*, 597–605.

Rowe, A.H., Xiao, Y., Rowe, M.P., Cummins, T.R., and Zakon, H.H. (2013). Voltage-gated sodium channel in grasshopper mice defends against bark scorpion toxin. Science *342*, 441–446.

Sainburg, T., Thielk, M., and Gentner, T.Q. (2019). Latent space visualization, characterization, and generation of diverse vocal communication signals. bioRxiv. https://doi.org/10.1101/870311.

Sakurai, A., and Katz, P.S. (2015). Phylogenetic and individual variation in gastropod central pattern generators. J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol. *201*, 829–839.

Sakurai, A., and Katz, P.S. (2017). Artificial synaptic rewiring demonstrates that distinct neural circuit configurations underlie homologous behaviors. Curr. Biol. *27*, 1721–1734.

Sakurai, A., and Katz, P.S. (2019). Command or obey? Homologous neurons differ in hierarchical position for the generation of homologous behaviors. J. Neurosci. *39*, 6460–6471.

Saunders, J.L., and Wehr, M. (2019). Autopilot: automating behavioral experiments with lots of Raspberry Pis. bioRxiv. https://doi.org/10.1101/807693.

Scribner, J.L., Vance, E.A., Protter, D.S.W., Sheeran, W.M., Saslow, E., Cameron, R.T., Klein, E.M., Jimenez, J.C., Kheirbek, M.A., and Donaldson, Z.R. (2020). A neuronal signature for monogamous reunion. Proc. Natl. Acad. Sci. USA *117*, 11076–11084.

Seeholzer, L.F., Seppo, M., Stern, D.L., and Ruta, V. (2018). Evolution of a central neural circuit underlies Drosophila mate preferences. Nature *559*, 564–569.

Shepherd, G. (2010). Creating Modern Neuroscience: The Revolutionary 1960s (Oxford University Press).

Shepherd, G. (2016). Foundations of the Neuron Doctrine (Oxford University Press).

Siemann, J.K., Muller, C.L., Bamberger, G., Allison, J.D., Veenstra-VanderWeele, J., and Wallace, M.T. (2015). A novel behavioral paradigm to assess multisensory processing in mice. Front. Behav. Neurosci. *8*, 456.

Smith, S.D., Pennell, M.W., Dunn, C.W., and Edwards, S.V. (2020). Phylogenetics is the new genetics (for most of biodiversity). Trends Ecol. Evol. *35*, 415–425.

Stern, D.L. (2014). Identification of loci that cause phenotypic variation in diverse species with the reciprocal hemizygosity test. Trends Genet. *30*, 547–554.

Stern, D.L., Crocker, J., Ding, Y., Frankel, N., Kappes, G., Kim, E., Kuzmickas, R., Lemire, A., Mast, J.D., and Picard, S. (2017). Genetic and transgenic reagents for *Drosophila simulans*, *D. mauritiana*, *D. yakuba*, *D. santomea*, and *D. virilis*. G3 (Bethesda) *7*, 1339–1347.

Stratton, G.E., and Uetz, G.W. (1986). The inheritance of courtship behavior and its role as a reproductive isolating mechanism in two species of *Schizocosa* Wolf Spiders (*Araneae*; *Lycosidae*). Evolution *40*, 129–141.

Symonds, M.R.E., and Blomberg, S.P. (2014). A primer on phylogenetic generalised least squares. In Modern Phylogenetic Comparative Methods and Their Application in Evolutionary Biology, L.Z. Garamszegi, ed. (Springer), pp. 105–130.

Tabin, J.A., Aspiras, A., Martineua, B., Riddle, M., Kowalko, J., Borowsky, R., Rohner, N., and Tabin, C.J. (2018). Temperature preference of cave and surface populations of *Astyanax mexicanus*. Developmental Biology *441*, 338–344.

Taverner, A.M., Yang, L., Barile, Z.J., Lin, B., Peng, J., Pinharanda, A.P., Rao, A.S., Roland, B.P., Talsma, A.D., Wei, D., et al. (2019). Adaptive substitutions underlying cardiac glycoside insensitivity in insects exhibit epistasis *in vivo*. eLife *8*, e48224.

Thomas, A., and MacDonald, C. (2016). Investigating body patterning in aquarium-raised flamboyant cuttlefish (*Metasepia pfefferi*). PeerJ *4*, e2035.

van Elden, S., and Meeuwig, J.J. (2020). Wild observation of putative dynamic decapod mimicry by a cuttlefish (*Sepia* cf. *smithi*). Mar. Biodivers. *50*, 93.

van Giesen, L., Kilian, P.B., Allard, C.A.H., and Bellono, N.W. (2020). Molecular basis of chemotactile sensation in Octopus. Cell *183*, 594–604.

Van Segbroeck, M., Knoll, A.T., Levitt, P., and Narayanan, S. (2017). MUPET-Mouse Ultrasonic Profile ExTraction: a signal processing tool for rapid and unsupervised analysis of ultrasonic vocalizations. Neuron *94*, 465–485.

Please cite this article in press as: Jourjine and Hoekstra, Expanding evolutionary neuroscience: insights from comparing variation in behavior, Neuron (2021), https://doi.org/10.1016/j.neuron.2021.02.002

Vormstein-Schneider, D., Lin, J.D., Pelkey, K.A., Chittajallu, R., Guo, B., Arias-Garcia, M.A., Allaway, K., Sakopoulos, S., Schneider, G., Stevenson, O., et al. (2020). Viral manipulation of functionally distinct interneurons in mice, non-human primates and humans. Nat. Neurosci. *23*, 1629–1636.

Wake, D.B. (1999). Homoplasy, homology and the problem of 'sameness' in biology. Novartis Found. Symp. *222*, 24–33, discussion 33–46.

Weber, J.N., Peterson, B.K., and Hoekstra, H.E. (2013). Discrete genetic modules are responsible for complex burrow evolution in *Peromyscus* mice. Nature *493*, 402–405.

Wenger, A.M., Peluso, P., Rowell, W.J., Chang, P.-C., Hall, R.J., Concepcion, G.T., Ebler, J., Fungtammasan, A., Kolesnikov, A., Olson, N.D., et al. (2019). Accurate circular consensus long-read sequencing improves variant detection and assembly of a human genome. Nat. Biotechnol. *37*, 1155–1162.

Werkhoven, Z., Rohrsen, C., Qin, C., Brembs, B., and de Bivort, B. (2019). MARGO (Massively Automated Real-time GUI for Object-tracking), a platform for high-throughput ethology. PLoS ONE *14*, e0224243.

Wiltschko, A.B., Johnson, M.J., Iurilli, G., Peterson, R.E., Katon, J.M., Pashkovski, S.L., Abraira, V.E., Adams, R.P., and Datta, S.R. (2015). Mapping sub-second structure in mouse behavior. Neuron *88*, 1121–1135.

Wittlinger, M., Wehner, R., and Wolf, H. (2006). The ant odometer: stepping on stilts and stumps. Science *312*, 1965–1967.

Xu, M., and Shaw, K.L. (2019). The genetics of mating song evolution underlying rapid speciation: linking quantitative variation to candidate genes for behavioral isolation. Genetics *211*, 1089–1104.

Yamamoto, A.H. (1994). Temperature preference of *Drosophila immigrans* and *D. virilis*: intra- and inter-population genetic variation. Jpn. J. Genet. *69*, 67–76.

Yang, Y., and Edery, I. (2018). Parallel clinal variation in the mid-day siesta of *Drosophila melanogaster* implicates continent-specific targets of natural selection. PLoS Genet. *14*, e1007612.

Yartsev, M.M. (2017). The emperor's new wardrobe: Rebalancing diversity of animal models in neuroscience research. Science *358*, 466–469.

Ylla, G., Nakamura, T., Itoh, T., Kajitani, R., Toyoda, A., Tomonari, S., Bando, T., Ishimaru, Y., Watanabe, T., Fuketa, M., et al. (2020). Insights into the genomic evolution of insects from cricket genomes. bioRxiv. https://doi.org/10.1101/2020.07.07.191841.

York, R.A., Patil, C., Abdilleh, K., Johnson, Z.V., Conte, M.A., Genner, M.J., McGrath, P.T., Fraser, H.B., Fernald, R.D., and Streelman, J.T. (2018). Behavior-dependent *cis* regulation reveals genes and pathways associated with bower building in cichlid fishes. Proc. Natl. Acad. Sci. USA *115*, E11081–E11090.

York, R.A., Byrne, A., Abdilleh, K., Patil, C., Streelman, T., Finger, T.E., and Fernald, R.D. (2019). Behavioral evolution contributes to hindbrain diversification among Lake Malawi cichlid fish. Sci. Rep. *9*, 19994.

Yoshizawa, M., Robinson, B.G., Duboué, E.R., Masek, P., Jaggard, J.B., O'Quin, K.E., Borowsky, R.L., Jeffery, W.R., and Keene, A.C. (2015). Distinct genetic architecture underlies the emergence of sleep loss and prey-seeking behavior in the Mexican cavefish. BMC Biol. *13*, 15.

Zanette, L.Y., Hobbs, E.C., Witterick, L.E., MacDougall-Shackleton, S.A., and Clinchy, M. (2019). Predator-induced fear causes PTSD-like changes in the brains and behaviour of wild animals. Sci. Rep. *9*, 11474.