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COMMENTARY

# Co-opting evo-devo concepts for new insights into mechanisms of behavioural diversity

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## ABSTRACT

We propose that insights from the field of evolutionary developmental biology (or 'evo-devo') provide a framework for an integrated understanding of the origins of behavioural diversity and its underlying mechanisms. Towards that goal, in this Commentary, we frame key questions in behavioural evolution in terms of molecular, cellular and network-level properties with a focus on the nervous system. In this way, we highlight how mechanistic properties central to evo-devo analyses – such as weak linkage, versatility, exploratory mechanisms, criticality, degeneracy, redundancy and modularity – affect neural circuit function and hence the range of behavioural variation that can be filtered by selection. We outline why comparative studies of molecular and neural systems throughout ontogeny will provide novel insights into diversity in neural circuits and behaviour.

**KEY WORDS: Neural circuits, Neuroethology, Robustness, Plasticity, Modularity, Canalization**

## Introduction

Fifty-six years ago, Tinbergen's four questions challenged the field of animal behaviour to fully integrate four distinct categories of analysis: ontogeny (development), causation (physiological mechanisms), survival value (adaptive significance) and evolution (phylogeny) (Tinbergen, 1963). Recent reviews have celebrated the effects of this four-pronged approach to the study of animal behaviour while advocating even greater integration (e.g. Bateson and Laland, 2013; Nesse, 2013; Kapheim, 2018). Even so, many studies of behaviour understandably focus on only one of these questions, guided by disciplinary-specific methods of inquiry and perspectives on what is 'important'. Here, we aim to integrate Tinbergen's four questions into an evolutionary developmental ('evo-devo') framework for explaining patterns of variation in behavioural phenotypes and the underlying mechanisms. This framework encompasses (1) genetic mechanisms that direct development and (2) the modifications to developmental processes by the animal's experiences throughout ontogeny, including learning. Together, these developmental mechanisms influence behavioural outcomes via effects at molecular, cellular, and tissue or organ system levels of organization. Moreover, because some behavioural outcomes of these developmental processes are more likely to arise than others, these processes can bias the options available for selection, and thus the behavioural diversity observed within and among populations, and across species.

Evo-devo research includes both macroevolutionary comparisons of developmental genetics and population-level analyses. Macroevolutionary comparisons can identify modifications of ancestral developmental programs that give rise to evolutionary innovations, whereas population-level analyses can reveal developmental influences on the structure of morphological variation and covariation among phenotypes (Box 1; reviewed in Sanger and Rajakumar, 2019). Relevant to both macroevolutionary comparisons and populational analysis is understanding how phenotypic variation is generated. Syntheses by Marc Kirschner and John Gerhart (Kirschner and Gerhart, 1998; Gerhart and Kirschner, 2007) provide a framework to understand how developmental processes create phenotypic variation in an evolutionary context by exploiting molecular- and cellular-level properties such as weak linkage, versatile proteins and exploratory mechanisms, together with network-level properties such as criticality, degeneracy, redundancy and modularity (see Glossary). Guided by this framework, we describe how developmental processes operating over an individual's entire lifetime have important outcomes for behavioural variation by: (1) stabilizing behavioural phenotypes in the face of genetic and environmental variation, (2) mediating forms of developmental plasticity (see Glossary), including learning, that generate predictable behavioural outcomes in different environments and (3) biasing the set of behavioural phenotypes in the population, and thereby shaping the variation on which selection acts (Fig. 1).

The nervous system, which plays an essential role in generating behaviour, was little addressed by previous syntheses (Kirschner and Gerhart, 1998; West-Eberhard, 2003; Gerhart and Kirschner, 2007). Here, we focus on examples from a range of neural mechanisms that determine behavioural outcomes. We aim to look at neural mechanisms of behaviour through the lens of developmental biologists who share a common interest in the pattern and process of evolution with scientists who focus on neural and, more broadly, physiological mechanisms of behaviour. We propose that evo-devo concepts, often applied to explain the evolution of morphologies, can be repurposed to establish causal mechanisms underlying the evolution of neural systems and behavioural diversity.

## Developmental processes shape behavioural variation

The phenotypic consequences of mutations can be limited by developmental processes that buffer phenotypes from both stochasticity (noise) and environmental variation. In this section, we first discuss how developmental processes are canalized (see Glossary), producing stable phenotypic output despite noise, while simultaneously accommodating developmental plasticity to mediate

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## Glossary

**Canalization**
Process by which the available outcomes of a developmental pathway are limited, by being buffered against environmental and other variation.

**Criticality**
The state in which developmental processes are close to tipping points between trajectories that lead to different phenotypic outcomes.

**Cryptic genetic variation**
Genetic variants that create mechanistic differences in a population that have no discernible phenotypic effect in typical environments.

**Degeneracy**
The presence of different mechanisms to accomplish the same outcome, potentially capable of substituting for one another.

**Developmental plasticity**
The capacity of a genotype to produce quantitatively or qualitatively different phenotypes depending on the environment. Developmental plasticity encompasses responses to abiotic or biotic environmental conditions, including learning throughout life.

**Developmental pleiotropy**
A genetic variant that affects more than one trait.

**Evolvability**
'An organism's capacity to generate heritable phenotypic variation' (Kirschner and Gerhart, 1998).

**Exploratory mechanism**
The initiation of more elements than will finally persist, with the most functional elements persisting while the remainder disappear. For example, neural development includes overproduction of neurons followed by survival only of neurons that made productive synapses.

**Modularity**
The parsing of a process into separate independent units, each of which can develop or be regulated independent of what is happening with the other units.

**Neofunctionalization**
Process by which a paralogue of a gene takes on a function different from that of the ancestral gene.

**Paralogue**
Related copies of a gene in a single organism that arose as the product of gene duplication.

**Redundancy**
The presence of very similar or closely related elements that carry out similar functions and can thus substitute for one another.

**Subfunctionalization**
Process by which paralogues derived from an ancestral gene retain different subsets of the functions of that gene.

**Versatility**
Situation in which a molecule or process has some 'give' in its requirements or substrates. This flexibility can more easily allow for other substrates to come under its control.

**Weak linkage**
Situation in which two processes are coupled, but not in a substrate/product or direct biochemical capacity. Instead, one process switches the other to a particular state without direct transmission of molecular entities.

learning and to produce divergent phenotypes in different environments. We then discuss three properties that may contribute to this developmental buffering and plasticity (Table 1; after Kirschner and Gerhart, 1998), illustrated by examples with neural and behavioural consequences. One property is versatility (see Glossary), where a molecule or process has some 'give' in its requirements or substrates. A second property is weak linkage (see Glossary), the relative independence of pathway components that function in a switch-like rather than a lock-and-key fashion. A third property involves exploratory mechanisms (see Glossary), in which overproduced elements (e.g. neurons or synapses) are pruned to the most functional ones via activity-dependent processes.

## Trade-offs between canalization and developmental plasticity

The robustness of developmental processes underlies the canalization that produces consistent phenotypes despite environmental variation and noise at other levels of biological organization (e.g. cellular, genetic, biochemical) (Fig. 2A). Gene regulatory and biochemical mechanisms within neural networks buffer behavioural phenotypes from change, even in variable environments (Marder et al., 2014; Siegal and Leu, 2014; Drion et al., 2015). For example, circadian clock protein networks modulate organism-wide behavioural rhythms across multiple time scales, including fruit fly courtship songs (Kyriacou and Hall, 1986) and seasonal migration in birds (e.g. Bazzi et al., 2015; Saino et al., 2015). In ectotherms, the circadian network's regulatory structure allows coordinated compensatory regulation that preserves clock periodicity across temperatures, despite the temperature sensitivity of individual biochemical components (Zhou et al., 2015). Selection for this canalized rhythmicity despite varied environments might also buffer phenotypes from the effects of genetic variation, as the same properties that confer robustness of gene regulatory and biochemical networks in the face of environmental variability also confer mutational robustness (reviewed in Siegal and Leu, 2014). For example, the coupling among suprachiasmatic neurons both prevents resetting of behavioural rhythms by temperature in mice (Buhr et al., 2010) and preserves behavioural rhythmicity in mice with mutations in circadian clock proteins (Liu et al., 2007). Such mutational robustness means that varied genotypes produce similar phenotypes that are selectively equivalent under typical environmental conditions, enabling the accumulation of cryptic genetic variation (see Glossary; Paaby and Rockman, 2014).

Although some traits are canalized and develop in a consistent manner despite environmental variation, as described above, other traits develop differently in response to environmental variation. Developmental plasticity, in which genotypes predictably produce quantitatively or qualitatively different phenotypes depending on the environment (Fig. 2B), can be a source of phenotypic variation. Developmental environments, both internal and external, can predictably shift the likelihood of following alternative developmental trajectories, as can (for behaviours) learning throughout life. One example of developmental plasticity is temperature-dependent sex determination in some ectotherms (reviewed in Bachtrog et al., 2014). Masculinization or feminization of behaviour is mediated by temperature activating a dichotomous developmental switch that affects both neural and endocrine traits. Temperature-dependent sex determination invokes portions of the same gene regulatory networks that promote gonadal development in organisms with genotypic sex determination (Shoemaker and Crews, 2009; Matson and Zarkower, 2012). These developmental programs are even more flexible in some fish species, in which social opportunity can initiate adult sex changes and accompanying changes in circulating steroid hormone profiles and patterns of steroid-dependent neuropeptide expression in the brain (Grober et al., 1991; Perry and Grober, 2003; Marsh-Hunkin et al., 2013). The striking evolutionary lability of sex-determining mechanisms demonstrates that many gene regulatory network configurations can be translated through a threshold into two or more phenotypic outputs depending on genetic, hormonal or environmental factors (Matson and Zarkower, 2012). Sex determination illustrates a general principle: environmental sensitivity of developmental switches is a source of phenotypic variation, as genetic variants or environments affecting the function

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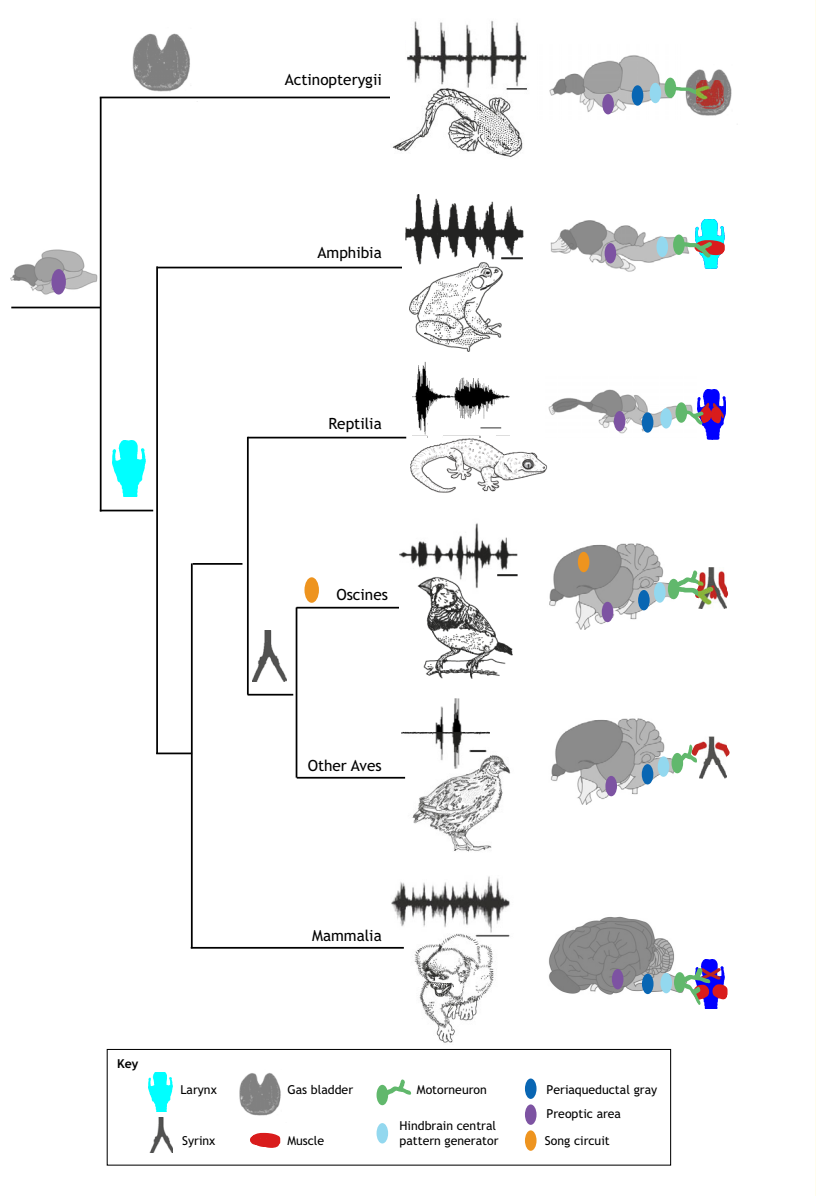
**Box 1. Origins of evolutionary innovations and diversity in neural circuits and behaviours**

A central tenet of evo-devo is that every feature of an organism has an antecedent. Even evolutionary innovations are built upon traits that originally served other functions (bricolage; Jacob, 1977; Duboule and Wilkins, 1998). We frequently see such phenomena at the behavioural level, as when predators localize prey by eavesdropping on mating or territorial displays (Zuk and Kolluru, 1998), and when females select mates using incidental features of males that indicate quality (Borgia, 2006). Such re-purposing is also ubiquitous at mechanistic levels, as in the use of nonapeptides for driving social behaviours (Godwin and Thompson, 2012; Johnson and Young, 2017), and in the neural circuit modifications underlying new cognitive functions (Anderson, 2007, 2010). The first major challenge in an evo-devo investigation is to identify core mechanistic elements sculpting behaviour – e.g. biochemical pathways that establish cell type and connectivity between neurons during development, as well as subsequent activity-dependent processes that adjust the structure and physiology of neural circuits as a part of learning. Comparative approaches can then reveal how the divergence of underlying mechanisms contributes to neural circuit and behavioural diversity. The main text of this paper highlights molecular, cellular and network properties that shape behavioural phenotypes, as these properties structure the phenotypic variation generated by developmental processes.

The figure shows evolutionary modifications to mechanisms mediating vocalization in vertebrates. This phylogeny of living bony vertebrates depicts ancestral states for vocal characters (modified from Bass et al., 2008; see also Bass, 2014). Behavioural outputs are indicated by oscillograms of representative vocalizations from (top to bottom): plainfin midshipman fish (*Porichthys notatus*), bullfrog (*Lithobates catesbeianus*), tokay gecko (*Gekko gecko*), zebra finch (*Taeniopygia guttata*), Japanese quail (*Coturnix japonica*) (prepared from recording number XC266707 by Albert Lastukhin, www.xeno-canto.org) and squirrel monkey (*Saimiri sciureus*) (scale bars 500, 1000, 20, 250, 400 and 200 ms top to bottom; see Bass et al., 2008 for more details). To the right are schematic sagittal views of the brains showing approximate positions of major components of the vocal network, with symbols identified in the key, and inferred time at which the structure arose represented by the placement of the symbols above branches on the tree. Evo-devo research will help determine how ancestral networks are modified to give these novel innervation and vocalization patterns, and identify molecular and cellular features that impact neural circuit function and behaviour.



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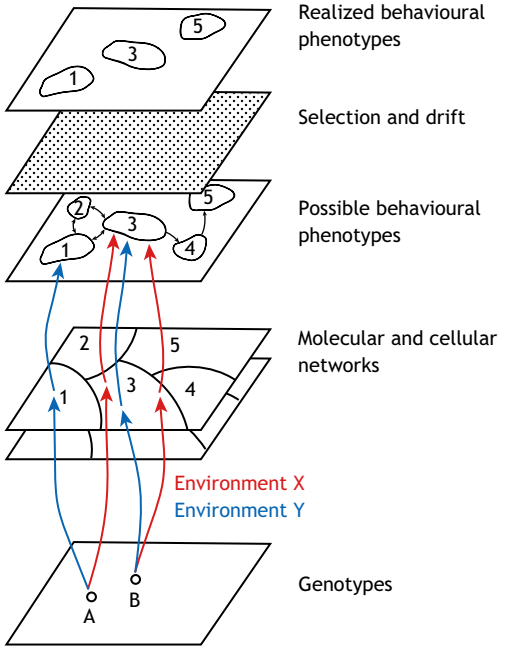


**Fig. 1. Evolution of behavioural phenotypes is shaped by developmental mechanisms.** Which suites of behavioural phenotypes are likely or even possible in a population depends, in part, on how genetic variants alter developmental processes. Mutations can impact behaviour via effects on diverse molecular and cellular networks throughout development. Different cellular or molecular mechanisms are depicted as layers in the diagram, with only two of the many cellular or molecular network states during development shown. Vertical arrows in the diagram mark developmental pathways for given genotypes (A or B); the abiotic environment, biotic environment and learning affect these pathways to mediate developmental plasticity in behaviour. Differently coloured vertical arrows, which represent any environmental differences, reflect the alternative developmental trajectories in environments X and Y that emerge from accumulating effects of experience on cellular and molecular networks. Genotype A adopts different probable states (indicated by numbers at each level of phenotype) in the two environments owing to developmental plasticity, potentially leading to two distinct behavioural phenotypes. By contrast, environmental influences on development of genotype B are buffered, leading to the same probable states during development and a single behavioural phenotype. The robustness of developmental processes means that many alleles might have no phenotypic consequences if pathways converge onto the same behavioural outcome (as in environment X, where both genotypes exhibit behavioural phenotype 3). Genetic variants with consequences can produce alternative behavioural phenotypes in a particular environment owing to the properties of exploratory mechanisms, versatility and weak linkage that characterize developmental processes. Arrows between the possible behavioural phenotypes depict likely transitions between behavioural states that can be brought about by mutations affecting developmental processes. In this way, developmental mechanisms can bias the behavioural variants present in a population. These biases thus shape the evolutionary consequences of natural selection and genetic drift (the dotted filter shown at the top of the figure) and hence the range of realized behavioural phenotypes present in a population. Figure adapted from Oster and Alberch (1982).

of these switches can produce diverse combinations of phenotypes (West-Eberhard, 2003).

Robust developmental processes affect the phenotypic consequences of mutations and hence evolvability (see Glossary), i.e. ‘the capacity to generate heritable, selectable phenotypic variation’ (Kirschner and Gerhart, 1998). Even small developmental genetic changes can shift behavioural outcomes and thus expose new (or different) behavioural variants to selection (Fig. 2C; Dingemanse et al., 2010). Neural/behavioural examples include allelic variation in monoamine oxidase A in rhesus monkeys modulating the effect of early-life social environment on later aggressiveness (Newman et al., 2005). Regulation of the cellular and molecular processes underlying neural plasticity tunes the environmental sensitivity and robustness of neural circuit output (Knudsen, 2004), yet the extent to which greater environmental sensitivity might destabilize neural or molecular networks and lead to unpredictable behaviours is not clear. Simulated gene regulatory networks with greater plasticity are more sensitive to the effects of mutations, harbour greater genetic variance, and have different evolutionary trajectories than non-plastic gene networks (Draghi and Whitlock, 2012). The activity-dependent homeostatic mechanisms that tune synaptic plasticity to preserve neural network stability and support ongoing plasticity (Tetzlaff et al., 2011; Gao et al., 2017; Zenke and Gerstner, 2017) have not been considered in the context of genetic variation. Thus we lack information about how variation in neural circuit plasticity might impact behavioural reliability, performance and evolvability. The ability to identify the genetic underpinnings of behavioural variation, such as the natural allelic variation in the *Drosophila Ten-a* gene, which predicts variation in the laterality of turns within inbred fly strains (Ayroles et al., 2015), offers opportunities to show how genetic variation shapes robustness and plasticity in the development of neural circuits, resulting in diverse behavioural phenotypes.

In summary, current evidence suggests that developmental processes confer both robustness and plasticity in molecular and cellular networks, including those of the nervous system, that contribute to behavioural variation. We next discuss how versatility, weak linkage and exploratory mechanisms may contribute to robustness and plasticity and, in turn, varied phenotypic outcomes in behaviours (Fig. 3A; Kirschner and Gerhart, 1998; Gerhart and Kirschner, 2007).

**Table 1. Mechanistic properties central to evo-devo that contribute to behavioural robustness and variation**

| Property | Example |
|---|---|
| Versatility | Transcription factors bind to a range of binding sites in promoters and enhancers, which confers robustness to mutations (Ding et al., 2016). |
| Weak linkage | Common sets of kinases mediate biochemical responses to diverse ligand–receptor pairings (Elion, 1998). |
| Exploratory mechanisms | Neural development incorporates activity-dependent processes that determine numbers of cell types and synaptic connections. |
| Criticality | Neural circuits are poised between distinct dynamical states such that context can shift neurons between alternative states (Hesse and Gross, 2014). |
| Redundancy | The set of similar GnRH neurons work together to regulate reproductive state, yet reproduction is possible with many fewer GnRH neurons (Herbison et al., 2008). |
| Degeneracy | Multiple sensory cues contribute to orientation in pigeons (Gagliardo et al., 2016; Wiltschko and Wiltschko, 2017). |
| Modularity | Behaviourally relevant vasopressin receptor levels are independently regulated in different brain regions (Okhovat et al., 2015). |

## Versatility

Genomes determine the available constituents for biochemical interactions, but they do not specify developmental processes with precision. This ‘give’ or versatility could play an important role in determining the range of behavioural variation. Examples of versatility include transcription factors that tolerate some variation

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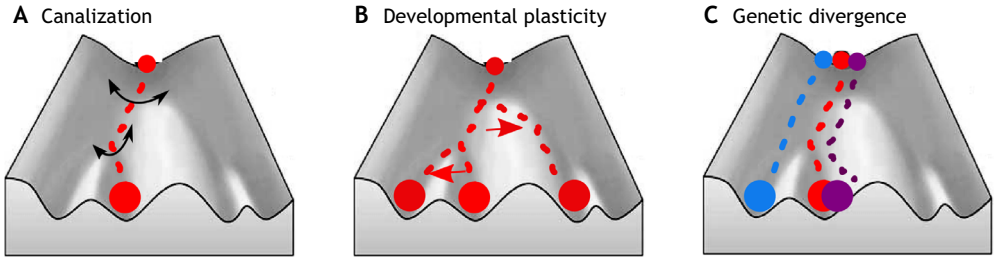


**Fig. 2. Waddington's landscape (adapted from Waddington, 1957) illustrates how phenotypic plasticity and genetic divergence shift robust developmental processes.** Time moves from top to bottom of each figure panel, and deep valleys indicate possible developmental trajectories. Circles represent phenotypes of individuals, with colour indicating genotype. Positions of the large circles after development portray adult phenotypes. (A) Canalization: selection for robustness has shaped developmental processes to produce similar phenotypes despite developmental noise. Typical noise levels encountered by the species at two time points are illustrated by curved arrows. Noise is buffered such that organisms stay on the same trajectory despite small deviations. (B) Developmental plasticity: predictable changes in developmental outcomes as a function of rearing environment and learning require overcoming the buffering systems inherent in development. Exposure to distinct environments or experiences at different times in ontogeny shifts developmental processes reliably onto either the right or left trajectories. These shifts require moving the organism outside the buffering capacity indicated in A (across the ridge in the figure). (C) Genetic divergence: trajectories represent three genotypes developing in a common environment. The effects of genetic variants may be buffered by developmental processes. For example, the mutations distinguishing the red and purple genotypes may reliably shift aspects of development without altering the adult phenotype. In contrast, some genetic variants, such as the blue genotype, reliably alter the phenotype by shifting developmental trajectories.

in the DNA sequences to which they bind (Ding et al., 2016), as well as some protein–protein interactions and enzyme–substrate binding (Kirschner and Gerhart, 1998). This biochemical versatility simultaneously produces robustness and flexibility. Some mutations will not disrupt existing transcription factor binding sites or protein–protein interactions. Other mutations will generate new transcription factor binding sites, protein–protein interactions or receptor binding affinities (Fig. 3C).

From a neural perspective, such molecular versatility underlies the evolution of sweet taste receptors in hummingbirds: sequence divergence in the ancestral umami receptor increases its binding affinity for carbohydrates and decreases amino acid binding, thereby enabling nectar feeding behaviour despite the loss of the vertebrate sweet receptor in ancestral bird lineages (Baldwin et al., 2014). That multiple receptor constituents can evolve to bind sugars illustrates that a diversity of genetic changes can alter expression patterns or biochemical network function to produce varied behavioural outputs, thereby facilitating evolutionary change (Kirschner and Gerhart, 1998; Gerhart and Kirschner, 2007; Maleszka et al., 2014).

## Weak linkage

Weak linkage, the quasi-independence of pathway members, contributes to the robustness of cell signalling. Weak linkage is typically associated with switch-like cascades, such as the kinase cascade triggered by a ligand binding to its receptor (Elion, 1998). Weak linkage also characterizes the integration by post-synaptic neurons of incoming presynaptic input signals in establishing patterns of action potentials (Gerhart and Kirschner, 2007); the immediate neurophysiological consequences of an action potential do not depend on the details of which inputs were active. We posit that weak linkage may also promote the evolution of neural circuits that mediate novel behaviours. For example, circuits in fruit flies link specific olfactory neurons to reproductive circuits that direct males to court females (Demir and Dickson, 2005). When olfactory neurons in drosophilids evolved to express the novel receptor IR84a, which responds to fruit volatiles, weak linkage in intracellular signaling pathways would have enabled these neurons to incorporate IR84a into their sensory machinery (Grosjean et al., 2011). Because these olfactory neurons innervate neurons that integrate environmental and social cues and trigger mating (Grosjean et al., 2011), pre-existing neurophysiological integration of weakly linked inputs likely facilitated the novel behaviour of flies mating on fruit once IR84a was expressed. Thus, similar to versatility, weak linkage of signalling cascades buffers cellular and biochemical networks from mutation or environmental variability, but also could facilitate behavioural innovations by allowing single mutations to have broad and coherent effects on biochemical systems and cellular physiology (Fig. 3B).

## Exploratory mechanisms

The lack of precision with which the genome specifies events mediating neural development can catalyze behavioural innovation by facilitating integrated shifts in neural circuit function due to exploratory mechanisms. These mechanisms are ones in which structural elements (e.g. neurons or synapses) are initially overproduced and are subsequently pruned to a functional subset. For example, neural development in most animals involves vast overproduction of neurons that are later pruned based on experience: only neurons with appropriate anatomical connections and activity patterns will survive and mature. Similarly, the surviving neurons make exuberant synaptic connections during development, and the resulting synapses are later pruned to a subset based on activity-dependent stabilization. These are examples of exploratory mechanisms in which early developmental processes promote overproduction of neurons and synaptic connections, and activity-dependent processes stabilize the most functional subset (Kirschner and Gerhart, 1998; Gerhart and Kirschner, 2007). Similar activity-dependent processes allow the diverse changes in neural circuits following experience that underlie learning (Titley et al., 2017). When zebra finches learn to sing stereotyped songs, they strengthen some synapses and eliminate others (Garst-Orozco et al., 2014).

Exploratory mechanisms such as these allow sampling of a broad set of neurophysiological patterns to develop and maintain functional neural circuits. This sampling buffers behavioural phenotypes from the effects of genetic variation and stochasticity. The ability of many neural network configurations to give rise to similar behavioural phenotypes could permit the accumulation of cryptic genetic variation. For example, the exploratory mechanisms that characterize development of human colour processing pathways can partially or fully compensate for opsin photopigment mutations (Neitz et al., 2002). These same exploratory mechanisms also enable

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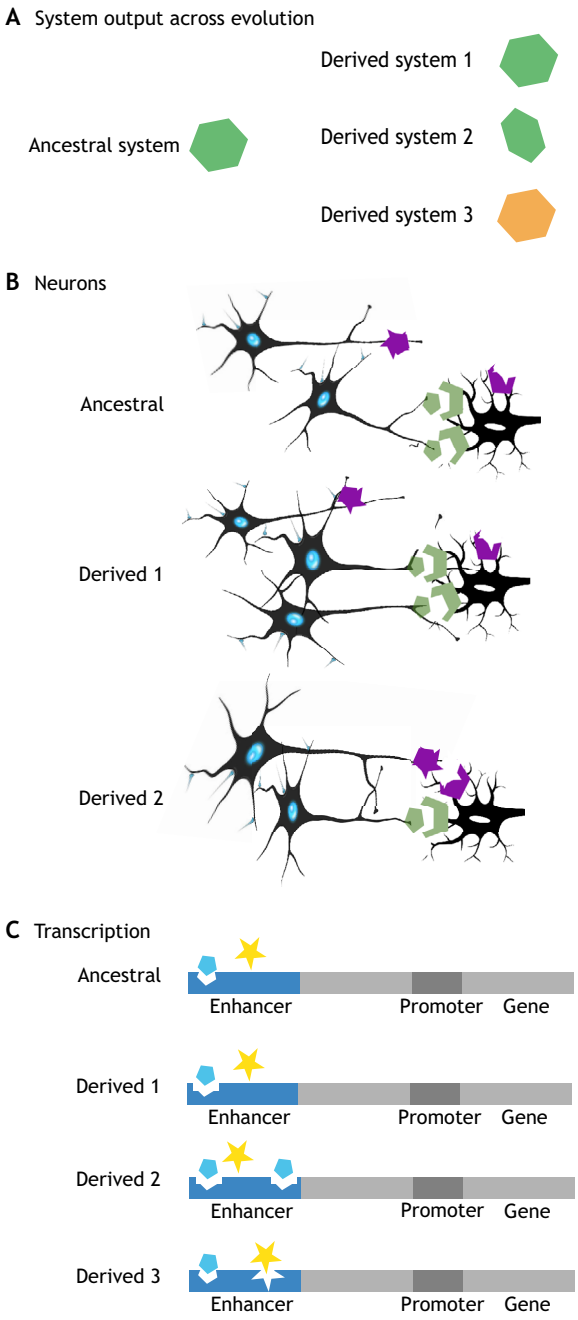


**Fig. 3. Analogies between transcriptional and cellular processes that confer robustness and plasticity in network output during evolution.** (A) Systems such as transcriptional and neural networks can produce similar network output from numerous system configurations. We depict here examples of systems-level evolution and the consequences for outputs (depicted by shape) in a generic sense, as such changes occur at all hierarchical levels of biological organization. Many components may shift during evolution without changing system output, as in derived system 1. Some component shifts might cause quantitative changes in the output, as in derived system 2. Adding or removing a new input to the system may cause qualitative changes in system output, as depicted for system 3. (B) Evolved changes in neural populations can produce similar network output via distinct mechanisms. The ancestral neural circuit involves a presynaptic neuron releasing the green neurotransmitter. When the presynaptic neurons increase in number in derived lineage 1, network output is largely similar because developmental processes reduce the number of synapses from each presynaptic neuron. A novel input from another neural population in derived lineage 2 (purple neurotransmitter) influences network output because the postsynaptic neuron has a receptor that can bind the new neurotransmitter. (C) Evolved changes in regulatory networks can produce similar network output via distinct mechanisms. The ancestral condition has an enhancer element that binds the blue transcription factor. In the evolutionary history of derived lineage 1, the enhancer element mutated, but the new enhancer still binds the same transcription factor moderately well. In derived lineage 2, a novel enhancer site arises by mutation and increases transcription by binding the blue transcription factor. Derived lineage 3 also evolves higher transcription levels, but this time the mutation causes novel regulation by the yellow transcription factor.

behavioural novelties, as even small genetic changes and the resulting activity-dependent responses can induce flexible shifts throughout neural circuits to give rise to novel circuit functions (Kirschner and Gerhart, 1998; Gerhart and Kirschner, 2007; Anderson, 2010). For example, genetic manipulation of squirrel monkeys to add novel opsin photopigments permits novel colour discrimination abilities, even in adulthood (Mancuso et al., 2009). Thus, the same developmental mechanisms that buffer neural circuit development from developmental noise or fluctuating environments also allow for coordinated shifts in neural circuits and resulting behaviours.

## Biochemical, genetic and cellular mechanisms shape behavioural variation

In summary, the effects of mutations and environmental context on phenotype are buffered by robust developmental processes. In many cases, these same developmental mechanisms can generate predictable phenotypic variation in response to environmental conditions and experiences. These robust developmental processes allow cryptic genetic variation to accumulate; this variation may subsequently be revealed in novel environments and exposed to natural selection (Paaby and Rockman, 2014). Understanding how developmental processes bias the phenotypic effects of mutations can help researchers distinguish key mechanistic contributions to the behavioural diversity within lineages. As described above, versatility, weak linkage and exploratory mechanisms can contribute to robustness and plasticity in neural output and consequent behavioural diversity.

## Network properties and the evolvability of behaviours within lineages

Which neural circuit configurations are represented in a population depends both on stochastic fixation of nearly neutral mutations as well as on selection for efficiency, performance and environment-dependent plasticity (Lynch, 2007). Although alternative neural or genetic networks might be selectively equivalent in some environments, they could have different consequences for the evolvability of behaviours within lineages.

In this section, we describe four basic properties of cellular and molecular networks that could impact evolvability: criticality, redundancy, degeneracy and modularity (Table 1). The network-level properties we discuss emerge from properties described above, such as versatility, weak linkage and exploratory mechanisms. We first discuss criticality (see Glossary), a property characterizing how close networks are to thresholds that distinguish alternative states (i.e. tipping points), and therefore whether small changes can cause dramatic shifts in network state and resulting behavioural phenotypes (reviewed in Hesse and Gross, 2014). We then highlight the built-in backups owing to redundancy and degeneracy (see Glossary) among network elements, in which identical (in the case of redundancy) or structurally distinct (in the case of degeneracy) molecules or neurons can substitute for one another in maintaining biochemical or neural network output (Tononi et al., 1999; Edelman

and Gally, 2001). Redundancy and degeneracy buffer network output from changes in one element and may also affect evolvability (Hebets et al., 2016). We describe network modularity (see Glossary), in which a small number of neurons or proteins are linked to each other, but decoupled from other elements (reviewed in Schlosser and Wagner, 2004). Modularity in neural or biochemical networks promotes coordinated evolution of behaviours that are influenced by the same modules, but allows independent evolution of behaviours regulated by distinct modules (Schlosser and Wagner, 2004). Criticality, redundancy, degeneracy and modularity of networks that contribute to behavioural variation will shape patterns of evolution within a lineage. Understanding these properties can open new avenues for research into physiological mechanisms underlying behavioural diversity.

## Tipping points

Consideration of tipping points (1) offers a conceptual way to view alternative developmental pathways, and (2) enables mathematical modelling to quantify how close dynamical systems are to criticality and to link behavioural variation to the effects of developmental plasticity and mutation. Some transcriptional networks may operate near criticality (Villegas et al., 2016), although we have little empirical data to determine how commonly cells are in this state. Similarly, neural systems often exist at tipping points between ordered and disordered neurophysiological dynamics (reviewed in Hesse and Gross, 2014). Most models of neurophysiological dynamics in a criticality framework come from large-scale networks in mammalian cortex, where near-critical dynamics may improve discrimination between stimuli (Clawson et al., 2017), but physiology of other neurons and circuits that function in distinct states can also be characterized in this framework. The proposed functional consequences of criticality in mammalian cortex (e.g. dynamic range of amplitudes over which stimuli can be processed, information transmission, information capacity; Shew and Plenz, 2013) prompts future research to identify which neural circuits function close to criticality and to relate those dynamics to developmental processes and behavioural outcomes.

Selection may push robust developmental systems toward tipping points between developmental pathways that lead to different behavioural phenotypes depending on the environment encountered (Hidalgo et al., 2014; Villegas et al., 2016). Hormones or neuromodulators are candidates for tuning criticality within cells or circuits that, in turn, adjust the sensory–motor coupling governing behaviours. For example, sex steroids can change the abundances of calcium-activated, large-conductance potassium (BK) channels (Rohmann et al., 2013) and drive adult plasticity in peripheral auditory encoding of the upper harmonics of advertisement calls in a vocalizing fish. Experimentally manipulating BK channels mimics natural increases in hearing sensitivity during the breeding season (Sisneros et al., 2004). Analyzing neurophysiological dynamics in auditory neurons in a criticality framework could reveal a mechanism by which neural circuits modulate criticality and help characterize more precisely the functional consequences of hormone-mediated plasticity in molecular traits. Similarly, the molecular interactions among signal transduction pathway components determine physiological features such as the alternation between two firing states in hunger-related neurons in the mouse hypothalamus (Yang et al., 2011). Computational models implicate specific calcium channel subtypes in supporting positive feedback necessary for alternative firing patterns (Franci et al., 2018; Drion et al., 2018); hence, specific biochemical changes could tune criticality and adjust neural firing state based on internal or external cues. Understanding how neural circuits adjust criticality more broadly will offer novel insights into the ways in which genetic changes and experience contribute to individual variation in neural system function and behaviour.

Criticality thus offers an important lens through which to understand sources of behavioural variation among animals; moreover, criticality of cellular or molecular networks could have important consequences for evolutionary trajectories. Computational modelling suggests that transcriptional networks near criticality have greater evolvability because small changes in abundance of one protein can transition the cell to a different functional output (Aldana et al., 2007; Torres-Sosa et al., 2012). Complementary analyses assessing the effects of criticality on the evolvability of neurons or neural circuits and their resulting behaviours are lacking, as are comparative studies on criticality in neural systems. Mounting evidence that the dynamics within mammalian cortex may operate not quite at criticality raises the possibility that critical dynamics may be too unstable or too difficult to achieve (Hesse and Gross, 2014), and thus neural circuit dynamics may constrain neural plasticity and behavioural evolution.

## Redundancy and degeneracy

One common property of networks is the existence of built-in backups that confer robustness to network output. Redundancy, the existence of multiple identical or very similar elements for a function, allows the system to tolerate failure of one element (Edelman and Gally, 2001). For example, a mutation that disrupts the migration of gonadotropin-releasing hormone neurons in mice shows that only one-third of the neurons are required for normal oestrous cycles and ovulation in females; the remainder are redundant (Herbison et al., 2008). Degeneracy refers to different structures or molecules that carry out similar functions and can act as backups (Tononi et al., 1999; Edelman and Gally, 2001). That multiple sensory systems can provide navigational cues to homing pigeons suggests degeneracy within orientation circuits (Gagliardo et al., 2016; Wiltschko and Wiltschko, 2017). Redundancy and degeneracy offer the possibility that individual network elements can adopt novel functions while preserving network output. For example, after gene duplication, one copy can mutate and adopt new functions (neofunctionalization; see Glossary), while the other copy maintains existing functions. For example, relaxed stabilizing selection on one paralogue (see Glossary) after sodium channel gene duplication enabled extensive sequence evolution that supports divergent patterns of electric signalling behaviour in fishes (Zakon et al., 2006; Arnegard et al., 2010). Alternatively, the two paralogues may retain different subsets of functions of the ancestral version, decoupling those sets of functions (subfunctionalization; see Glossary). Meta-analyses suggest that neofunctionalization is the more common outcome of gene duplication (He and Zhang, 2005; MacCarthy and Bergman, 2007), and hence the redundant genes produced by gene duplication may fuel novel phenotypes.

Divergence in molecular and neural systems, even if phenotype is preserved, could alter levels of degeneracy and redundancy in developmental processes and, hence, evolvability. To predict evolvability, we need a deeper understanding of the extent to which greater system complexity (e.g. larger number of neurons, more feedback regulatory mechanisms) implies greater system degeneracy, redundancy or robustness. Even small neural circuits can have sufficient developmental plasticity to buffer the effects of genetic variants that alter redundancy. For example, escape responses in fish require that only one of the two paired Mauthner neurons fire an action potential that leads to muscle contractions on one side of the body. Mutants having supernumerary Mauthner cells

exhibit effective escape responses (Liu et al., 2003), likely owing to exploratory mechanisms during development of the escape circuitry. The vast range in complexity of neural circuits across species offers the opportunity to investigate the extent to which redundancy or degeneracy, in this case at the level of the configuration of neural circuits, influences behavioural diversity.

## Modularity

In modular neural or molecular systems, a change in one node (e.g. mRNA, protein or neuron) often affects processes within its module without affecting other modules (Schlosser and Wagner, 2004). Here, we discuss modularity as it is used in the evo-devo literature, recognizing that the term is used in other fields in different ways (Melo et al., 2016). Although the same genes function in multiple brain regions, this need not imply rampant pleiotropic consequences of genetic variants (i.e. developmental pleiotropy; see Glossary; Paaby and Rockman, 2013), because transcriptional, biochemical and neural networks all exhibit modularity. For example, vasopressin receptor mutations in prairie voles are implicated in distinct mating strategies owing to epigenetic influences on receptor abundance within a single brain region independent of other brain regions (Okhovat et al., 2015). Recent estimates find little evidence that pleiotropy constrains evolution (Wagner and Zhang, 2011; Pavličev and Cheverud, 2015), consistent with modularity as a predominant feature of molecular and cellular systems, but the extent to which pleiotropy leads to genetic correlations in behavioural traits is unknown (Dochtermann and Dingemanse, 2013).

The modular structure of gene, protein and neural networks reflects regulatory and developmental processes that unite elements within a module. The abundance of the elements that comprise gene or protein network modules are sometimes coordinated by shared regulatory elements, such as common miRNA binding sites (Tsang et al., 2007; Ebert and Sharp, 2012). Exploratory mechanisms in neural development, as described above, can coordinate neural elements within structural modules, in which subsets of brain regions or neurons are strongly interconnected anatomically and less connected to brain regions in other modules (Sporns and Betzel, 2016). Functional modules, in which the activity dynamics of brain regions are more strongly coordinated within a module than are dynamics between regions in different modules (as found in human neuroimaging studies), may be coordinated by factors such as attention (Sporns and Betzel, 2016). Shared reliance on circulating hormones is another way biochemical and physiological systems may be integrated (reviewed in McGlothlin and Ketterson, 2008). For example, elevated androgen receptor levels in specific motor neurons and the muscles they innervate in the wings of male manakins facilitate their demanding courtship display without altering androgen signalling in other tissues (Schlinger et al., 2013). Modularity in some cases reflects phenotypic integration, in which coordinated regulation within a module might maintain an effective balance among module elements in cases in which that balance affects performance (Pigliucci and Preston, 2004).

The regulatory and developmental processes that unify protein or neural modules also have important implications for evolvability (Schlosser and Wagner, 2004). Mechanisms that coordinate elements within the module may evolve, decoupling unrelated elements or coupling new module elements (e.g. West-Eberhard, 2003; Schlosser and Wagner, 2004; Adkins-Regan, 2008). For example, population differences in expression of aromatase (which converts testosterone to oestrogens) and in androgen receptor distribution may alter the dependence of aggressive behaviours on circulating testosterone (Bergeon Burns et al., 2013). Because the modular structure of biochemical and neural systems can evolve, correlations among behaviours are not fixed (Dingemanse et al., 2010; Dochtermann and Dingemanse, 2013). Modularity can also facilitate evolutionary innovation. One example is the emergence of a 'supersoldier' phenotype in ants in which a novel late developmental sensitivity to juvenile hormone activates an ancestral plasticity module (Rajakumar et al., 2012). Entire cassettes of genes can be deployed jointly in novel developmental roles (Tabin et al., 1999); thus, altered timing of responsiveness to juvenile hormone could initiate a developmental cascade that coordinates the many phenotypic levels that comprise the supersoldier phenotype. The core regulatory structure that coordinates elements within modules, whether a common transcriptional regulatory network or a set of neural development processes, can promote recruitment of an entire functional module by adding one upstream input (Schlosser and Wagner, 2004).

## Developmental processes shape evolutionary trajectories of behaviour

In summary, although complexity may have evolved under selection for robust development in varied environmental conditions, details of the criticality, degeneracy, redundancy and modularity in complex biochemical and neural networks shape the likelihood that particular trait constellations will evolve in a lineage. As mechanistic studies provide a more sophisticated understanding of how variation is generated and accumulated, we will be able to understand the joint influences of variation in developmental processes and the action of natural selection on neural circuit function and behavioural outcomes.

## Conclusions

Applying the concepts of evo-devo to behaviours offers a fundamentally integrated approach to understanding patterns of behavioural diversity and their underlying mechanisms, which reflect both the consequences of selection and the developmental processes that determine the effects of mutations on variation. Lineage-specific biases of mutational effects shape the variation in neural phenotypes that arose within lineages and, hence, the resulting patterns of pleiotropy, modularity and evolvability of behaviours. Developmental plasticity and robust developmental processes together allow for the integrated effects of individual mutations on mechanisms that contribute to behavioural outcomes, as one change can have cascading consequences on all levels of biological organisation. As was urged for morphological studies (Sanger and Rajakumar, 2019), we encourage researchers to characterize the molecular, cellular and developmental mechanisms that translate genetic variation into behavioural variation at the population level. Understanding these developmental cascades may reveal which mechanistic changes are key innovations in a lineage and how ancestral developmental processes structure behavioural variation.

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#### Author contributions

All authors jointly conceived of the content. K.L.H. prepared the figures and drafted the text. All authors critically revised the text and figures, and approved the final version for publication.

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