

Systems biology as a framework to understand the physiological and endocrine bases of behavior and its evolution—From concepts to a case study in birds

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ARTICLE INFO

Keywords:

Systems biology
Animal behavior
Organismal physiology
Adaptive evolution
Manakin birds
Androgenic hormones
Robustness

ABSTRACT

Organismal behavior, with its tremendous complexity and diversity, is generated by numerous physiological systems acting in coordination. Understanding how these systems evolve to support differences in behavior within and among species is a longstanding goal in biology that has captured the imagination of researchers who work on a multitude of taxa, including humans. Of particular importance are the physiological determinants of behavioral evolution, which are sometimes overlooked because we lack a robust conceptual framework to study mechanisms underlying adaptation and diversification of behavior. Here, we discuss a framework for such an analysis that applies a “systems view” to our understanding of behavioral control. This approach involves linking separate models that consider behavior and physiology as their own networks into a singular vertically integrated behavioral control system. In doing so, hormones commonly stand out as the links, or edges, among nodes within this system. To ground our discussion, we focus on studies of manakins (Pipridae), a family of Neotropical birds. These species have numerous physiological and endocrine specializations that support their elaborate reproductive displays. As a result, manakins provide a useful example to help imagine and visualize the way

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systems concepts can inform our appreciation of behavioral evolution. In particular, manakins help clarify how connectedness among physiological systems—which is maintained through endocrine signaling—potentiate and/or constrain the evolution of complex behavior to yield behavioral differences across taxa. Ultimately, we hope this review will continue to stimulate thought, discussion, and the emergence of research focused on integrated phenotypes in behavioral ecology and endocrinology.

1. Introduction

Understanding the physiological mechanisms of behavior can shed a powerful light on the way ecological conditions and evolutionary forces shape animal life. Organismal biologists have long recognized this point and therefore have worked to incorporate ideas and principles from anatomy and physiology into behavioral ecology and evolutionary biology (Bell, 2008; Flatt and Heyland, 2011; Jablonka and Lamb, 2014; Pigliucci and Müller, 2010). This way of thinking has transformed the field of ethology into an integrative discipline that celebrates a combination of conceptual and technical advances in areas such as neuropsychology, genomics, and physiology (Blumstein et al., 2010; Fischer et al., 2021; Hofmann et al., 2014; Rubenstein et al., 2014). With the surge in technical, computational, and conceptual advances arising from genomics, transcriptomics, and proteomics, this integrative approach to studying the control of behavior is also rapidly growing. Central to this growth are studies of hormones and their ability to mediate behavioral output by acting throughout the brain and body, where they influence tissue functions to shape how an individual interacts with its physical and social environment.

Here we explore how general concepts and principles of systems biology can be used to help study the complex physiological underpinnings of an organism's behavioral repertoire. In doing so, we attempt to emphasize the importance of systems biology to fully understanding processes of behavioral evolution. To conceptualize this approach, we focus on a body of research in a family of birds called manakins (Pipridae). Biologists have worked with these birds to begin to understand how components of the physiological scaffold underlie elaborate sexual behavior and its diversification. Finally, we turn our attention to androgenic control systems and end our manuscript with a summary that encourages researchers to adopt systems perspectives when designing behavioral studies and interpreting their results. This approach promises to enhance our understanding of how behavioral traits in animals are evolutionarily constructed and regulated. Our primary thesis, which builds on previous work (Hofmann et al., 2014; Martin and Cohen, 2014; Martin et al., 2011), is that seemingly disparate networks that comprise an animal's physiological phenotype and behavioral phenotype are hierarchically integrated into a larger behavioral control system (Fig. 1).

Broadly speaking, a systems perspective is more than just an integrative view of behavior, one which articulates the general importance of multiple physiological systems to any given behavioral trait. Rather, a systems perspective on behavior and physiology appreciates the fact that *connectedness* among physiological processes confers its own set of behavioral effects that are functionally important and potentially adaptive. Studying behavior and its mechanisms through a systems level therefore means that one studies the nature of these connections and the way that they influence how animals behave, and how such behavior diversifies.

2. Principles of systems biology

Systems thinking provides an approach for researchers to explore the nature of interactions among different components of a biological process (Aderem, 2005; Giuliani et al., 2014; Kirschner, 2005; Kitano, 2002; Milo et al., 2002; Prill et al., 2005). This perspective contrasts with the reductionist view of biology, which focuses on singular parts of a larger process (Strange, 2005). Although systems thinking is generally

employed in the disciplines of molecular genetics (Conant and Wagner, 2003; Draghi and Wagner, 2009), development (Davidson, 2011; Hinman et al., 2003), cellular biology (Ravasz et al., 2002; Wagner and Fell, 2001; Westerhoff and Palsson, 2004), neuroscience (Driver and Balakrishnan, 2021; Sinha et al., 2020), and ecology (Newman, 2006; Proulx et al., 2005), it can be applied to topics outside of these fields (Araya-Ajoy and Dingemanse, 2014; Baran et al., 2017; Hebets et al., 2016; Hidalgo et al., 2009), including behavioral neuroendocrinology, as we outline here (Hofmann et al., 2014; Martin et al., 2011). Modes of systems thinking have a lengthy history of application to the fields of ethology (e.g., Tinbergen, 1951), making the case for its application described here using the most contemporary forms of analysis involving modern physiological and genomic approaches.

Components of a system are often called **modules**, tightly integrated suites of characters (Aderem, 2005; Wagner et al., 2007). The interactions among modules are called **edges**, and when relevant modules and edges are put together, they are called a **network** (Cohen and Havlin, 2010; Kitano, 2002). Network size can vary, as can the number of edges among modules. Some networks may contain a few modules with a few edges among them, whereas other networks may contain hundreds of modules with hundreds of edges. As we allude above, system thinking is agnostic to the grain size of these modules—they can be genes, proteins, cells, tissues, organs, morphological traits, behaviors or social systems (Aderem, 2005; Kirschner, 2005). In a similar vein, factors such as hormones and cytokines create an edge between or among modules, depending on the modules that make up the network.

An important property of a biological network is robustness (Kitano, 2002, 2004), which is the persistence of a given trait under perturbation (Félix and Wagner, 2008). Perturbations can vary and can involve stochastic noise, environmental changes, or genetic variation (Félix and Wagner, 2008). We use the term 'trait' liberally, as its definition must scale appropriately to the network under consideration. Robustness itself arises from functional redundancy and distribution (Fig. 2; Félix and Wagner, 2008; Wagner, 2005). These are two intrinsic features of a

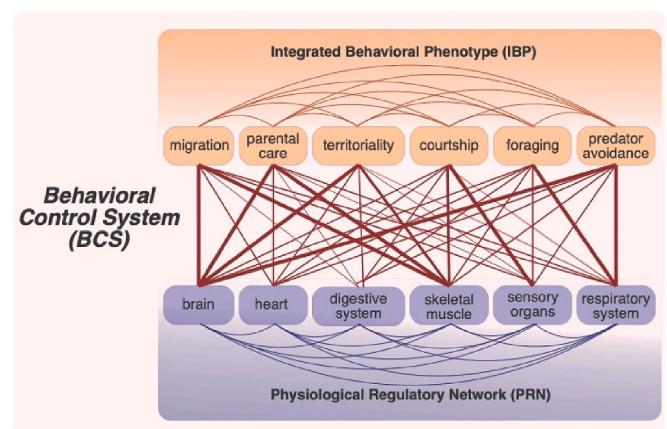


Fig. 1. Schematic representation of the behavioral control system, or BCS. The integrated behavioral phenotype (IBP) is hierarchically integrated with the physiological regulatory network (PRN), with the former arising as a manifestation of the latter. Strength of connection (edges) between modules can vary among individuals or species, as shown by differences in the thickness of the lines. Edges are maintained by endocrine, molecular, and neural systems (see text).

network that arise when more than one module supports a given process (redundancy, Fig. 2A), and when a process is governed by multiple paths (distribution) in the network (Fig. 2B). These effects can endow an organism with functional stability and elasticity (Félix and Wagner, 2008). For example, functional stability arises through a network's ability to absorb effects of perturbation without diminishing or crumbling, whereas functional elasticity arises through the presence of multiple network solutions to address a given problem (Alon, 2003).

3. A systems approach to physiology and behavior

3.1. Physiology

Physiologists previously incorporated system principles into their conceptualization of organismal form and function (Bartsch et al., 2015; Bashan et al., 2012). Organs do not work in isolation, but rather operate in concert with other organs to form systems that support homeostasis and behavioral responses to biotic and abiotic stimuli. Thus, efficient organismal functioning results from an active physiological regulatory network (Cohen et al., 2012; Martin and Cohen, 2014). A consequence of such organization is that biological challenges are in part managed through physiological robustness, which develops from the interplay between functional redundancy and distribution. These two properties of the physiological regulatory network result from the configuration of its edges and are defined by the action of integrating network modules. Often integrators are signaling molecules, such as hormones, that mediate connectivity among tissues and/or organs (Cohen et al., 2012; Cox et al., 2016; Martin and Cohen, 2014). We must recognize that the effects of these integrators are plastic, changing in response to facets of social experience and/or environmental context (Fuxjager et al., 2010; Fuxjager and Marler, 2010; Goymann et al., 2019; Hirschenhauser and Oliveira, 2006; Hirschenhauser et al., 2008). This implies that edges within a physiological regulatory network likely vary spatio-temporally in terms of their strength and/or presence, which in turn suggests that emergent properties of the physiological network are intrinsically dynamic.

Physiological regulatory networks are also labile from an evolutionary perspective, as they can change through time in response to a wide range of evolutionary forces (selection, drift, etc.). Many studies explore the ways that molecular systems underlying discrete physiological process are modified, resulting in substantive changes to cellular, tissue, and organ performance (Carroll, 2005; Carroll et al., 2013; Galván et al., 2022; Hoekstra and Coyne, 2007). Such evolution can occur through mutations to specific genes that modify proteins

necessary for physiological performance (Aminetzach et al., 2009; Ding et al., 2016; Kullander et al., 2003), or through changes to regulatory promoters that alter expression of genes throughout the body (Abzhanov et al., 2006; Ogura et al., 2004; Pease et al., 2022). Integrator systems are similarly labile. For example, whereas steroid hormones are conserved molecules (Schuppe et al., 2020), the machinery that underlies steroid metabolism, detection, and transduction can also evolve (Fuxjager and Schuppe, 2018; Hau, 2007; Schlenger et al., 2022). Hormone system functionality is also well known to evolve through divergence in regulation, which often manifests through the emergence of species differences in tissue-specific receptor expression (Anderson et al., 2021; Fuxjager et al., 2015; Johnson et al., 2018; Mangiamele et al., 2016). In some cases, these differences can be traced back to behavioral variation across taxa, supporting the view that modifications to the integrator systems within a PRN are associated with phenotypic evolution.

The mechanisms by which physiological regulatory networks evolve are only just beginning to be understood. Changes to the molecular genetics of physiological traits can arise all at once or in a layered manner across a species' history (Hoekstra and Robinson, 2022). In this latter model, specializations to a physiological system or process would accrue over time, with ancestors of specialized taxa sharing some underlying molecular features to these traits, including integrators that connect nodes within a physiological regulatory network. We have obtained evidence for this idea in manakins, as it relates to various molecular underpinnings of physiology and behavior (Pease et al., 2022, to be discussed below).

3.2. Behavior

Animal behavior can also be conceptualized through systems biology. Most biologists recognize that an animal's behavioral repertoire consists of a collection of traits that make up an integrated behavioral phenotype. One illustration of this concept comes from work that explores behavioral syndromes, or correlated suites of behavior that are expressed across different contexts (Moiron et al., 2020; Sih et al., 2004a; Sih et al., 2004b). Syndrome theory also implicitly recognizes that certain behavioral suites are unrelated to each other, which in turn points to discrete behavioral modules. Yet, one limitation to the syndrome framework is that it does not necessarily describe how these modules are related to each other. Some modules, for example, may be expressed in a mutually exclusive manner. This is most apparent when the produce of one or more behavioral traits precludes the expression of other behavioral traits (Halfwerk et al., 2014; Johnson and Candolin,

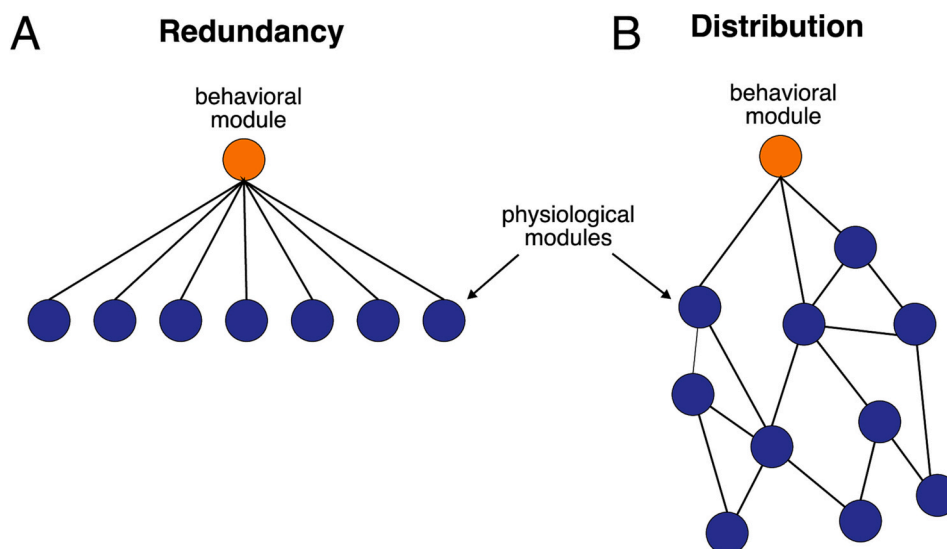


Fig. 2. Illustration of system robustness as defined through concepts of network (A) redundancy and (B) distribution. Note that networks have been simplified to clarify the concepts. A behavioral module (e.g., behavioral trait) is depicted by an orange circle, whereas the physiological modules contributing to the behavior's expression are depicted by blue dots. For redundancy, several physiological modules independently contribute to behavioral expression. For distribution, fewer physiological modules directly contribute to behavioral expression, but those modules that do are highly interconnected with other modules. This figure is adapted and modified from Félix and Wagner (2008).

2017; Ketterson et al., 1992; Magnhagen, 1991; Marler et al., 1995; Podhorna et al., 2018; Skelly, 1995; Verdolin, 2006). These findings imply that sometimes disparate behaviors are in fact connected to one another and cannot be viewed as independent actions. Nonetheless, systems thinking can provide us with a glimpse into how modules interconnect, while simultaneously describing the phenomenological nature of these connections (Wilkins et al., 2015).

Physiological and behavioral systems together are hierarchically integrated into a singular behavioral control system (Fig. 1). This concept is important because it unites our understanding of physiological regulatory networks with our understanding of integrative behavioral phenotypes, creating one cogent model that reflects the diverse ways in which physiology influences behavior. Some of these connections are obvious; for example, we know that behavioral traits are controlled by neural and muscular systems. However, less intuitive are the ways that other physiological processes (and their interactions) might influence behavior, behavioral adaptation, and diversification, and these mechanisms should not be ignored (see below). Accordingly, by superimposing the physiological regulatory network over the integrated behavioral phenotype, we can clearly see that edges connect multiple modules to define the behavioral control system.

To explore the utility of the behavior control system approach, we examine three different ways that connectivity among modules influence behavior. First, behavioral modules may be connected to each other by edges that run through one or more physiological modules. The brain clearly illustrates this point as it controls nearly all behavioral traits, many of which are governed by common sets of neuronal nuclei (a straightforward and intuitive example). Thus, if selection on a particular behavioral trait occurs through the evolution of a specific brain region, then other behavioral traits controlled by this same area may also change. Consider, for example, these behavioral byproducts may become targets of selection well after they initially evolve. In this way, the BCS sets up a situation in which certain behavioral traits may originate as contingencies, but then provide new substrates on which selection can act to promote behavioral diversity (Blount et al., 2018). Uncovering such behavioral byproducts is challenging, particularly because selection can recursively act on them to give rise to new traits. One recent example of how this might work comes from a large-scale analyses of woodpecker territorial displays (drumming) (Miles et al., 2020). Early in the process of signal evolution, selection appears to drive the evolution of display rhythm (how display speeds change through time); yet the emergence of certain rhythmic patterns can constrain how other elements of the display evolve later in a species' history in response to sexual selection by male-male competition. By contrast, some rhythms create opportunities for sexual selection to exaggerate drumming down the line. Thus, thinking about behavior through the lens of systems biology provides a platform on which we can conceptualize *how* certain behavioral contingencies—and their physiological basis (not explored in the woodpecker example)—arise in the first place and help shape additional changes to the behavioral repertoire.

Second, behavioral modules may be connected to each other through multiple physiological modules. Consequently, seemingly disparate behavioral traits may be linked to seemingly unrelated physiological systems, whereby selection on any one physiological module may unexpectedly change a given behavioral trait. The well-established relationship between the brain and gut clearly illustrates this concept (Aiello and Wheeler, 1995; Kotschal et al., 2019), as trade-offs in the phenotypes of these organs have consequences for cognitive behavior and nutrient acquisition. Artificial selection experiments confirm as much, with selection for enhanced learning abilities depressing gut-mediated absorption of fuel needed for reproduction (Kotschal et al., 2013). This research sets the stage for thinking about the unintuitive consequences of selection for certain traits, when seemingly unrelated behaviors are linked through physiological processes that we ordinarily consider unrelated. The example highlighted above points to a trade-off between two physiological processes—cognition and nutrient

acquisition. One might also imagine the situation in which unexpected connections among physiological modules potentiate certain behavioral traits. Few have examined behavioral evolution through this lens, particularly with respect to physiology.

Third, behavioral traits might arise as an emergent property of physiological connectivity. In such cases, we might predict that removing one physiological module and/or its connectivity with other physiological modules would abolish the existence of the behavioral trait. Deep sea diving in some mammals is a behavior that occurs only through the coordinated action of multiple specialized organ systems working in tandem, many of which are highly specialized (Blix, 2016; Butler and Jones, 1997; Williams and Ponganis, 2021). If the ability of this system to integrate performance breaks down, then so too does the capacity to dive to extraordinary depths. Thus, behavioral adaptation occurs through a synergy among physiological modules and their connections to each other. This example is relatively straightforward and intuitive, but emergent properties from physiological connectivity might arise in unexpected ways to influence an animal's behavioral abilities, skills, and propensities. Consideration of this point by researchers who study endocrine mechanisms underlying behavioral evolution may produce new insights, with unexpected findings that expand how we imagine that selection shapes animal behavior.

The three ways of thinking outlined above suggest that behavioral control is a phenomenon that is best understood by evaluating an organism's broader physiological landscape, as opposed to singular physiological systems that appear to be most relevant (Martin and Cohen, 2014; Martin et al., 2011). Of course, thinking about behavior and its mechanistic basis in this manner immediately reveals the incredible complexity of how animals generate interactions within their physical and social environments. So, how might the behavioral control system work to influence neuroendocrine processes underlying behavioral adaptation and diversification, or vice versa? We explore this question below, with a focus on a particular group of birds, the manakins.

4. A case study in manakin birds

Studies of species of manakins illustrates the concept of a behavioral control system and how it sheds light on neuroendocrine function and behavioral evolution. Manakins (Aves: Pipridae) are a family of neotropical Passeriform birds. They radiated within the last 10 million years, and the identification and study of their diverse phenotypes underscore the role by which sexual selection has shaped phenotypic diversity (Kirwan et al., 2011; Leite et al., 2021; Prum, 1997). It is within this framework that we see how numerous anatomical and physiological modules, spanning diverse organ systems, form networks upon which sexual selection produces these incredible phenotypes.

In the paragraphs below, we describe a series of general behavior and physiological traits that each constitute modules within a behavioral control system. We also illustrate these modules in Fig. 3A-F to help readers imagine how they manifest in an animal system. As such, each paragraph references one trait in the figure. At the end of this section, we attempt to combine these traits and build a behavioral control system for an adult male manakin, who is in reproductive condition and actively courting females. This behavioral control system clearly shows the integrating role of androgens, the topic covered in the final section.

4.1. Trait A—Diet and feeding

Manakins acquire their nutrition largely by consuming small fruits (Cestari and Pizo, 2013; Loiselle and Blake, 1990; Rosselli, 1994). Although this may seem irrelevant to ideas about sexual selection, it may be central to this thesis. Small fruits are generally abundant in tropical forests, are a good source of energy, and often are available year-round. By having such readily available food, there is less pressure on males to help provision young (Bradbury, 1981; Foster, 1977; Snow, 1976).

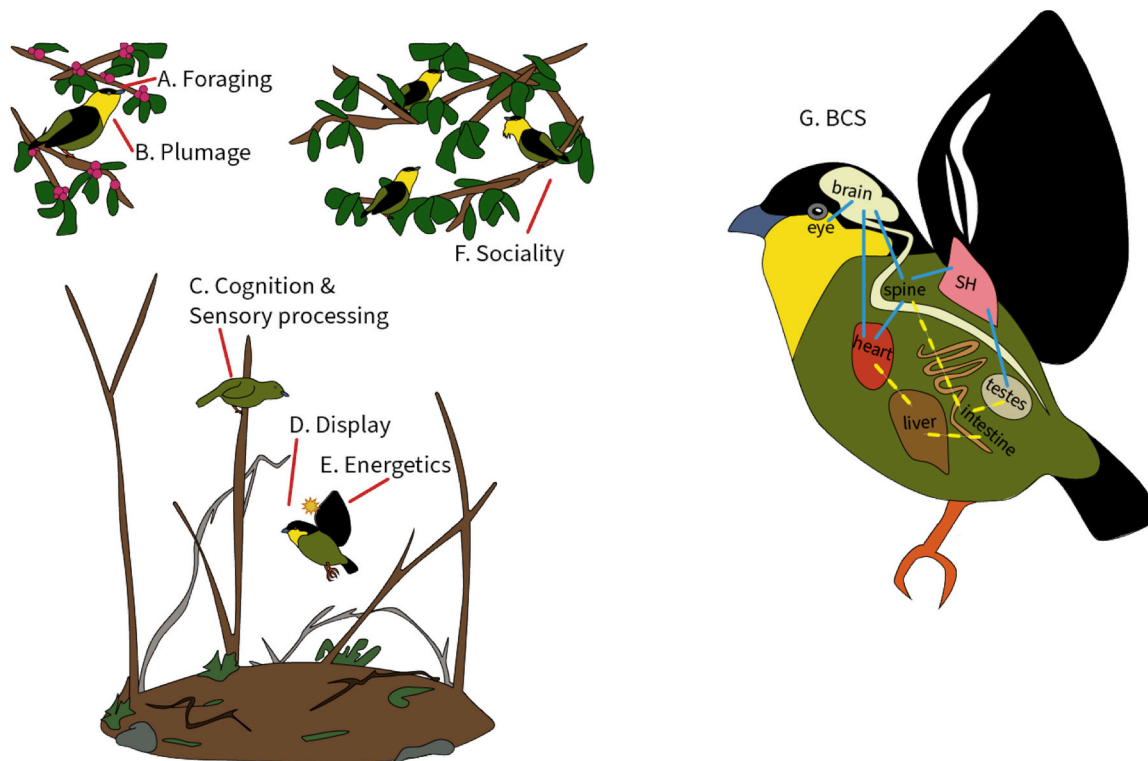


Fig. 3. Illustrative depiction of the golden-collared manakin reproductive systems. Schematic shows the birds (A) feeding on brightly colored fruit with its (B) brilliant and extravagant plumage. (C) A female of the same species (with drab green plumage) visually and auditorily evaluates a male as he produces his elaborate (D) elaborate jump-snap display. This behavior involved leaping among saplings over the forest floor, while snapping his wing together in mid-air to produce a loud and conspicuous popping sound. (E) These displays elevate heart rate and undoubtedly incur an energetic expense, even if a male's daily energy budget is similar to other tropical frugivorous birds. (F) Many manakin species are also highly social, and thus individuals navigate complex social dynamics. In the species shown here (golden-collared manakins), males interact at leks and while feeding in fruiting trees. In other species, males are even more social and perform elaborate cooperative dance routines for courtship. (G) preliminary construction of a behavioral control system (BCS) associated with courtship behavior. Blue lines represent edges in the system that researchers have begun to connect by way of androgenic hormone action as the primary integrator. Dashed yellow lines represented edges that have not been connected into the system, but that we suspect play a role given our systems view of physiology.

Moreover, because fruits are often widely dispersed, males have no need to guard one fruit-rich resource, liberating them from the need to establish and protect territories. Such conditions are thought to have led to the evolution of polygyny in manakins, creating a context for strong selection to act on the birds' phenotype. It is easy to imagine that these effects have wide-ranging consequences for behavioral evolution. Indeed, frugivory (as opposed to insectivory) requires specialized sensory systems to detect when fruit is ripe and ready to be eaten (Driver and Balakrishnan, 2021; Osorio and Vorobyev, 1996). Frugivory also requires gastrointestinal adaptations to effectively digest nutrients (Worthington, 1989), although manakins do likely also eat insects for protein at some point in their lives, such as during their nestling phase (Boyle, 2010). In this way, the manakin's dietary focus on predominantly ripe tropical fruits requires adaptations by different physiological modules with wide ranging implications for how the bird's behavior—particularly reproductive behavior—evolves (Piersma and Drent, 2003).

4.2. Trait B—Plumage ornamentation

Manakins often exhibit striking sexually dimorphic plumage colors, with females (and often juvenile males) appearing drab green and males appearing highly colorful (Chapman, 1935; Kirwan et al., 2011). Females are best served by being inconspicuous, whereas males evolve bright colors because of the fitness advantages that such plumage traits create by attracting mates (Stein and Uy, 2006), with plumage color being a target of selection in these birds (McDonald et al., 2001; Parsons et al., 1993). Plumage color arises from modified biochemical pathways

in the integument that produce pigments for deposition into feathers (Gazda et al., 2020). Some pigment substrates are obtained from colorful fruits consumed by the birds, again a result of gustatory and GI-tract adaptations (McGraw et al., 2002). Thus, specialized biochemical modules have become incorporated into the broader physiological regulatory network that contributes to the integrative behavioral phenotype. If we link the aforementioned systems together (i.e., points A and B), then we might predict that manakin visual systems are especially tuned for certain wavelengths so that both males and females can detect colorful fruits and conspecific plumage. Retinal pigmentation and neural centers responsible for processing visual information are therefore likely physiological modules that effect behavioral evolution (Day et al., 2011; Endler et al., 2005).

4.3. Trait C—Cognitive and perceptual abilities

Evidence also implies that manakins have striking cognitive and perceptual abilities. For instance, research in golden-collared manakins (*Manacus vitellinus*) reveals that individuals disperse in search of food during the rainy season, when they are not breeding. As the dry season nears, males quickly establish or return to their display courts, which they build to perform courtship dance displays (Chapman, 1935). We do not know how far the birds travel, but the male's remember the location of their leks and navigate back to these through dense forest. Such orientation and navigation behavior likely involves significant cognitive skill, presumably arising from parts of the brain that underlie learning and memory, such as the hippocampus (Day et al., 2011). These males also maintain a clean court, removing debris using unique strategies

depending on the size and shape of the debris (Chiver and Schlinger, 2017a). Males of many manakin species perform similarly complex behaviors (Johnsgard, 1994). The neural circuits that underlie these behaviors undoubtedly involve substantive sensory and cognitive-based regions, such as the hippocampus, as well as brain areas that mediate and integrate properties of sexual motivation (Chiver and Schlinger, 2017a; Schlinger and Chiver, 2021). Past work on similar behaviors of bowerbirds shows involvement of both cognitive and motivational neural modules in the activation of sexual behavior (Madden, 2001), and such effects certainly have wide-ranging ramifications of the other diverse behavioral processes that are related to these important brain-level systems.

4.4. Trait D—Display behavior

To attract females for mating, many male manakins perform extraordinary behavioral displays (Fuxjager and Schlinger, 2015; Prum, 1990, 1998). These elaborate forms of physical courtship require specialized neural systems that motivate behavioral performance and then coordinate the downstream motor components of displays (Fusani et al., 2014a; Fuxjager et al., 2012). This again highlights the importance of the brain in terms of both behavioral control and display perception. For the former, there may be a range of specialized brain regions that help mediate the production of manakin display maneuvers. Recent studies outside of the manakin family bolsters this idea by suggesting that species—in this case woodpeckers—have evolved specialized brain regions to mediate the production of drumming displays (Schuppe et al., 2022), which are complex gestural signals in their own right (Miles et al., 2018b; Schuppe et al., 2021). Equally intriguing is that these specialized brain regions are anatomically and morphologically similar to nodes of the song control system in oscine songbirds, opening the possibility that antecedent neural systems are modified across birds to support the further diversification and elaborate of display behavior. Of course, specialization of other CNS systems involved in motor control are likely involved, including neural circuits that string together behavioral routines into larger programs (Schwark et al., 2022).

From the sensory processing side, manakins must be able to detect the elaborate displays that conspecific produce for courtship or competition. Such displays are multimodal, in that they incorporate signals that stimulate two or more sensory modalities in the receiver (Higham and Hebets, 2013; Mitoyen et al., 2019). Male manakins, displays tend to function through visual and acoustic sensory realms (Prum, 1990; Schlinger and Chiver, 2021), meaning that these systems must detect and process salient components of these stimuli (Day et al., 2011; Endler et al., 2005). Although we already mentioned that the bird's visual systems may have adapted to fully capture wavelengths of fruits and elaborate male plumage, this system may also be modified to detect especially rapid movements and gestures. This is because many manakin courtship displays are performed with exceptional speed, while behavioral studies suggest that individuals can discriminate elements of display routines that differ by 10s of milliseconds (Barske et al., 2011). Of course, birds are well known to have exquisite visual abilities. Future studies will need to explore more thoroughly the extent to which visual (and possibly acoustic) systems are modified to facilitate display perception in these birds.

Finally, another important component of manakin physiology that evolved alongside display behavior is the skeletal muscular system. As the main effectors of display performance, skeletal muscles must evolve to support performance attributes required for the production of certain display maneuvers, such as rapid wing gestures and prolonged aerial acrobatics (Fuxjager et al., 2016a; Fuxjager et al., 2016b; Lowe, 1942; Miles et al., 2018a; Schlinger et al., 2001). Comparative studies reveal that these muscular adaptations are accumulated through a layered evolutionary process, where changes in gene expression occur at multiple nodes within the manakin phylogeny leading up to the point at which spectacular display performance arises (Pease et al., 2022).

4.5. Trait E—Energetics and homeostasis

For some manakins, despite their performance of vigorous courtship displays, males do not use more energy than other similar-sized birds that do not display in this manner (Barske et al., 2014). This raises the possibility that manakins might possess metabolic adaptations. Avian muscle transcriptomics supports this latter conclusion by revealing elevated and androgen-dependent expression of genes that promotes energetic efficiency in the principal manakin muscle involved in elaborate display behavior (Fuxjager et al., 2022; Fuxjager et al., 2016b; Pease et al., 2022). This adaptation is supported by work in other species outside of the manakins that show how evolutionary labile a species' metabolic machinery can be (Biro and Stamps, 2010), with numerous studies suggesting that evolutionary changes to metabolic systems can profoundly impact a wide range of physiological and behavioral processes (Książek et al., 2004; Naya et al., 2009; Steyermark et al., 2005).

The manakin cardiovascular system is also likely adapted to support whole body energy demands of reproduction (Barske et al., 2019). In some species, male heart rates briefly accelerates to >1000 beats min^{-1} —one of the highest heart rates on record in birds and mammals (Barske et al., 2014). This occurs during bouts of display, with males producing such behavior as many as 450 times per day over a 7-month breeding season (Barske et al., 2011). This places unusual demands on cardiovascular function, and thus the heart of some manakin species is $>0.93\%$ of its body weight. For context, the hearts of other avian species are decidedly smaller ($<0.85\%$) (Hartman, 1955). At the same time, manakins have lower-than-average resting heart rates compared to other tropical birds, but they simultaneously maintain similar levels of oxygen consumption (Barske et al., 2014). This suggests that these birds have higher cardiac output, in that that heart pumps more blood with each heartbeat than other birds of the same size.

4.6. Trait F—Social dynamics

Many manakins are solitary during the non-breeding season, but in the breeding season individuals (males) gather in leks for courtship (Kirwan et al., 2011; Prum, 1990, 1994). More than just gathering in leks, males of some species are exceptionally cooperative and coordinate their elaborate movements in group displays to attract the attention of females (DuVal, 2007). Neural systems linked to sociality are likely targets of sexual selection (e.g., Horton et al., 2020), including features of the vertebrate social behavior networks that serves to drive the animal's aggression toward, or affiliation with, conspecifics (Goodson et al., 2005; Newman, 1999; O'Connell and Hofmann, 2011; O'Connell and Hofmann, 2012). How this part of the brain is changed to accommodate lek breeding—let alone its seasonal changes—remain a mystery.

With these six modules in mind, we can create a BCS that guides perspectives on the evolution of manakin display behavior (Fig. 3G). Doing so reveals not only how different behaviors are likely connected via physiological modules, but also how these connections undoubtedly influence evolution of the birds' complex behavioral repertoires. These connections lead to predictions similar to some previous work. Frugivory, for example, results in sensory and/or perceptual biases that may explain the evolution of elaborate plumage phenotypes (Endler and Basolo, 1998; Ryan and Cummings, 2013). Likewise, the evolution of extreme muscle speed may influence other adaptive performance abilities related to behavioral traits that have little or nothing to do with sexual displays (Rome et al., 1999; Rome and Lindstedt, 1998). Many of the connections may lead to unexpected endpoints with respect to manakin evolution. There are also several connections that we know likely exist, but it is unclear what their effect might be on behavior. Consider the liver, for example. Hepatic function can greatly influence the performance of other organs, such as heart and skeletal muscles (Møller and Bernardi, 2013; Nachit and Leclercq, 2019); thus, in theory, evolutionary changes to the liver's functionality could have effects on diverse physiological systems, and thereby have an unrecognized impact

on behavior. Indeed, relatively little work has explored a role for the evolution of the liver alongside behavioral traits in the natural world.

5. Androgenic hormones as edges in the system that regulates manakin courtship behavior

Diverse modules must be functionally interconnected to one another. As we described above, hormones serve as major integrators in this regard, creating edges between or among modules. The notion that hormones act as integrators aligns with endocrine research, which recognizes that a principle function of hormone action is to coordinate physiological responses with intrinsic and extrinsic stimuli that animals experience (Adkins-Regan, 2005). Neural systems also act as integrators, as they not only hold together the diverse neural networks that underlie all aspects of performance (motivation, arousal, affect, movement, sensory processing, etc.), but they also communicate with diverse organ systems of the body, often using hormones as signals. Indeed, many organ systems provide feedback to their neural inputs through a variety of chemical and molecular signals.

For manakins, network edges and their ability to influence display behavior have been studied primarily through sex hormones, specifically androgens. The main bioactive hormones in this class of steroid include testosterone and dihydrotestosterone. Studies show that androgenic hormone signaling machinery evolves within and across species, often changing in response to accommodate the adaptation of reproductive behavior (although, neutral evolutionary forces like drift seem similarly capable of contributing to species differences in androgenic systems) (Fuxjager et al., 2018; Fuxjager and Schuppe, 2018). We therefore believe androgenic signaling takes the lead in forming the BCS network that has emerged in manakins in response to sexual selection. While androgenic signaling has been shown in males of other species to provide the primary inter-organ signaling function to drive the evolution of phenotypes favored by females, manakins appear to have taken this process to new levels.

As described above, testosterone is the principal circulating androgen in most vertebrates. It acts throughout the brain and body to organize systems that it will target in adulthood to regulate the production of reproductive behavior (Nelson and Kriegsfeld, 2015). Testosterone comes primarily from the testes, where it is synthesized to govern gametogenesis while also being released into the bloodstream. Testosterone then helps facilitate behavioral, anatomical, and physiological traits that are required to attract mates for fertilization. There is good evidence that, in many cases, adult levels of testosterone are designed to achieve threshold levels to activate the numerous states required of adult males (Goymann et al., 2007; Nelson and Kriegsfeld, 2015; Wingfield et al., 1990). However, in some cases those levels of testosterone are adjusted by social or environmental conditions to mediate appropriate phenotypic traits (Goymann et al., 2007; Goymann et al., 2019; Hirschenhauser and Oliveira, 2006; Wingfield et al., 1990).

Free testosterone enters cells passively but then may undergo conversion to the more potent androgen dihydrotestosterone by actions of the enzyme 5 α -reductase. Thus, the presence of this enzyme can effectively locally upregulate androgen action, thereby increasing the sensitivity of that cell or tissue to testosterone in the bloodstream (Russell and Wilson, 1994). As long as cells within those tissues have androgen receptor (AR) (see below), expression of 5 α -reductase can assist a tissue entering an androgen-dependent network. We have evidence in golden-collared manakins, that the enzyme 5 α -reductase is transcribed in skeletal muscle and spinal cord at levels sufficient to promote formation of dihydrotestosterone to better capture these tissues into such a system (Feng et al., 2010; Fuxjager et al., 2016c).

Upon testosterone or dihydrotestosterone binding, AR becomes a molecular complex that acts as a transcription factor to regulate the expression of genes with androgen response elements in their upstream promoter regions. Several genetic and cellular processes must occur to establish a cell as an androgen target, and, hence, a part of the overall

androgen-dependent network. Those events include the evolution of androgen response elements in the promoters of genes that are important to the cell's contribution to the male-typical phenotype (Chang, 2002). Additionally, cells must naturally express AR, when the testes are secreting testosterone. This could involve constitutive expression of AR, or regulated expression at the right time for reproductive function. These cells must also simultaneously express co-factors that appropriately regulate androgen-dependent gene transcription. With this appropriate milieu of proteins and nucleotide sequences, a cell may then be fully responsive to circulating testosterone and become a member of the larger androgen-dependent network.

Although most studies are not designed to assess the presence of all these cellular factors, it seems that identifying the presence of AR itself stands as an excellent marker for an androgen target cell (and thus potentially a tissue that is evolving in response to sexual selection for reproductive behavior; e.g. Fuxjager et al., 2015). Manakins stand out in this regard because studies of this group of birds show that several manakin species naturally express AR at unusually high levels in a variety of tissues that function within many of the six modules previously described. This makes these tissues/organs as nodes of an androgen-dependent network (Feng et al., 2010; Fuxjager et al., 2015; Fuxjager et al., 2012; Schultz and Schlinger, 1999).

The focus of numerous studies of manakin physiology and hormonal control of physiology and behavior has been the golden-collared manakin (*Manacus vitellinus*). At the onset of the dry season in Panama, adult males of this species gather in leks where they remain active for six or more months into the wet season (Schlinger et al., 2013). Each male claims a court, a patch of ground kept clear by the male, which is surrounded by three or more upright small saplings. Around this court, the male performs an elaborate display with acrobatics and both visual and acoustic signaling. Acoustic signals include vocalizations, but also loud snapping sounds produced through a rapid hammering of the wings together above the back (Bodony et al., 2016; Bostwick and Prum, 2003). All this behavior (and more) is activated by T in males; some of these behaviors can even be activated by experimentally administered T in females (Chiver and Schlinger, 2017b; Chiver and Schlinger, 2019; Day et al., 2007).

With this background, we can begin to describe how androgens serve as the link between the various physiological modules described above.

5.1. Module A

As both male and female manakins are frugivorous, it is doubtful that androgens play any role in the sensory or digestive modules that support frugivory, and thus potentially influence the evolution of polygyny. Nevertheless, we cannot exclude the possibility that seasonally, androgens optimize metabolic pathways that improve acquisition of food resources that sustain plumage color development or energetic demands (e.g., Nolan et al., 1992). We have no data regarding a role for androgens in forging connections between these and other modules. Moreover, because pigmentation of male plumage appears to be hormone-independent, at least with respect to promoting plumage acquisition in older juvenile males (Day et al., 2006), this set of traits may indeed represent a separate network from those modules that now follow.

5.2. Module B

The male golden-collared manakin courtship display is produced at astonishing speed, by colorful males in a background of specific rainforest hues. All of this is observed by both males and females, so whether androgens play any specific role in the male's visual system is unknown. Nevertheless, the seasonal shift from foraging in the rainy season to courtship in the dry season might require adaptation of the visual system of both males and females with sex hormones playing a role. Novel high AR expression is seen in the breeding male manakin nucleus rotundus (Fusani et al., 2014b), part of the pathway that connects the retina to the

optic tectum, and a region shown to process color, patterns, and brightness in birds (Hodos, 1969; Hodos and Karten, 1966). It is not clear whether this part of the brain similarly changes in AR sensitivity across the year; nonetheless, this work indicates that androgens may act on nucleus rotundus to enhance visual requirements for courtship display performance.

Female golden-collared manakins do have elements of their visual systems that are enlarged relative to males (Day et al., 2011) providing justification for placing the eye and/or visual processing modules as nodes within our current network. Female sensory systems can be seasonally optimized by estradiol to enhance reception of male signals in other species (Sisneros et al., 2004). Whereas traditional androgen and estrogen receptors are not conspicuous in adult female manakin visual processing circuits, estrogens can also rapidly modulate sensory processing systems in the avian brain, presumably via membrane receptors (Vahaba and Remage-Healey, 2018), so a non-canonical role for estrogens in the manakin retina or central visual processing systems cannot be ignored. Certainly, neural connectivity provides some of the linkage between sensory systems and our network even if androgens play no role. These processes bear on other sensory systems as well, discussed more fully below.

5.3. Module C

In many species, including in birds, androgens and/or estrogens promote function of the hippocampus to enhance spatial memory processes (Taxier et al., 2020). Many male manakins relocate their individual display courts requiring skills in navigation and memory likely involving the hippocampus. Estrogen receptors are expressed in the male manakin hippocampus (Fusani et al., 2014b), a region that also expresses the estrogen synthetic enzyme aromatase (Saldanha et al., 2000). Thus, androgens may promote cognitive function by actions on the hippocampus via the conversion of testosterone to estradiol. While we know that androgens can drive some behavior that requires significant cognitive skill (Chiver and Schlinger, 2017a), more work is needed to assess the role of steroids in manakin cognitive function.

5.4. Module D

There is considerable evidence that androgens promote a variety of the neural, muscular, and metabolic nodes that are required for full performance of male courtship (Fuxjager et al., 2022; Fuxjager et al., 2018; Fuxjager and Schuppe, 2018; Schlinger et al., 2013). Studies examining the distribution of AR in the golden-collared manakin show that they are expressed quite abundantly in all skeletal muscles that have been examined, including muscles known to be involved in courtship as well as in the syrinx (Feng et al., 2010), the avian vocal organ, and in the heart (Barske et al., 2019). Moreover, other manakins also express AR in muscle to a relatively high degree, as compared with non-manakin species (Fuxjager et al., 2015). Across all manakin species studied, muscle AR expression positively correlates with courtship display complexity pointing to a key role for androgens in connecting muscular nodes. Other work shows that androgenic activation of AR increases muscle twitch speeds, and thus allows the tissues to contract and relax at frequencies that are nearly three times the normal wingbeat frequency that powers flight for a bird of this size (Fuxjager et al., 2017). In doing so, androgens trigger a trade-off with muscular endurance, which in turn may constrain divergence in display behavior (Miles et al., 2018a; Tobiansky et al., 2020).

From an evolutionary perspective, comparative transcriptomic studies have attempted to map out when in the manakin phylogeny high levels of muscular AR evolve, and whether other parts of the androgenic signaling apparatus have changed alongside innovations in male courtship (Pease et al., 2022). This work suggests that evolutionary increases in AR expression within key display muscles arises after changes to the molecular machinery that helps regulate AR expression.

In fact, some manakin species without elevated muscular AR still likely maintain the ancestral machinery that regulates AR (e.g., increased expression of genes linked to AR turnover and/or genes associated with amplifying AR's effect on gene expression). In this way, we can think of elevated androgenic sensitivity in muscles adapted for display performance as more recent or derived modifications, which in turn are built upon more conserved alterations to molecular processes related to androgenic signaling.

In addition, AR are expressed in neurons that are positioned to actuate muscles used in courtship. This includes AR expression in both spinal motor and sensory neurons of the wing (Fuxjager et al., 2012), as well as in conserved and novel sites of androgen action in brain (Fusani et al., 2014b). For example, as is the case with most vertebrates, the manakin medial pre-optic nucleus, is a site of AR expression, as well as estrogen receptors and the enzyme aromatase (Fusani et al., 2014b; Saldanha et al., 2000). Here, androgens (and estrogens derived from circulating androgens) can drive the performance of male copulatory and other reproductive behaviors. AR are also expressed at significant levels in mid- and hind-brain structures, which in other species are regions where pre-motor and motor neural circuits drive avian vocal production (e.g., Brown, 1965). AR are expressed at high levels in the manakin cerebellum, where they likely function to control motor components of courtship (Feenders et al., 2008). Finally, the arcopallium, a complex region that contains premotor circuitry (Feenders et al., 2008), is also a site of extensive AR expression in the manakin brain (Fusani et al., 2014b), suggesting that this region may be a site for androgenic control of courtship.

Many manakins also claims courts for individual or groups displays, and males physically alter some of the characteristics of these courts. In golden-collared manakins, only males perform gardening behavior and testosterone activates this behavior (Chiver and Schlinger, 2017a). We suspect that effects of testosterone on such court gardening occurs through modulation of several neural sites related to motivation and arousal, many of which are androgen sensitive.

Androgens may help regulate modules of the physiological network related to the skeletal system. Androgens are important regulators of vertebrate bone growth and turnover (Clarke & Khosla, 2009; Vander-schueren et al., 2004). With respect to manakins, we know that several species show altered bone morphologies that are likely related to their ability to generate display behavior (Bodony et al., 2016; Friscia et al., 2016; Bostwick et al., 2012). How hormones might work during development and during adulthood to maintain these traits are not currently understood, but this issue merits further study.

5.5. Module E

Androgens may have potent actions on a variety of tissues to enhance metabolic systems. In skeletal muscle, androgens promote expression of genes that boost lipid and glycogen metabolism (Fuxjager et al., 2016b). The manakin heart expresses AR at high levels, at least compared to zebra finches, and commensurate with the high levels expressed in their skeletal muscles (Barske et al., 2019). Androgens increase myocardial expression of SERCA, a gene that, by facilitating intracellular Ca²⁺ re-uptake in the sarcoplasmic reticulum, can increase heart rate. Improved cardiovascular function assists in rapidly removing CO₂ and delivering O₂ to all the body's tissues, presumably permitting the male manakin's performance of energetically costly displays. Additional studies to explore potential androgen actions on pulmonary systems and neural cardiopulmonary control centers, as well as metabolic functions in other tissues might yield substantial new information on hormonal control of metabolic and homeostatic systems underlying elaborate courtship.

5.6. Module F

Less is known about the role of androgens in the social-relations between males in a golden-collared manakin lek. Other manakins,

however, form complex social relationships. For example, male lance-tailed manakins (*Chiroxiphia lanceolata*) perform cooperative displays, in which alpha and beta males together perform leap-frog displays. Alpha males maintain higher levels of circulating androgens than males of any other social status (DuVal and Goymann, 2010). Similarly, male wire-tailed manakins (*Pipra filicauda*) perform cooperative displays that are associated with differences in circulating androgen levels (Dakin et al., 2021; Ryder et al., 2020; Ryder et al., 2011). While territorial males have higher testosterone levels than floaters (Ryder et al., 2011), there are also differences between the relationship between cooperative behavior and testosterone levels within each group. For floaters, testosterone levels are positively related to the likelihood of a display being cooperative, but the opposite is true for territorial individuals. In addition, for territorial individuals, experimentally elevated levels of testosterone via implants results in a decrease in cooperative displays. Thus, the relationship between circulating hormone levels and behavior is opposed between these two social classes, perhaps a reflection of the heterogeneous patterns of gene expression observed in the manakins across their social behavior network (Horton et al., 2020).

6. Conclusions

In this paper, we present the case that systems biology provides a compelling lens through which researchers can study behavior, and how its underlying physiological mechanisms might shape behavioral adaptation and diversification. Network thinking has been brought to studies of physiology and behavior before (Hofmann et al., 2014; Martin and Cohen, 2014; Martin et al., 2011), and thus we echo these calls. Specifically, we emphasize that researchers must not only better appreciate the interconnectedness among physiological systems, but they must also begin to explore how such interconnectedness in and of itself impact the way behavior is produced. System thinking provides a framework for this perspective. For example, one might explore the behavioral effects of physiological redundancy and/or distribution, or the way connectedness of physiological systems trigger emergent behavioral properties. This specific focus has the potential to reveal exciting new insights on a vast array of extraordinary organisms.

Manakin birds are a nice exemplar of how systems biology can help us better understand behavior and its evolution. Here, we highlight how interconnections among different facets of the manakin's biology influences their dramatic and showy reproductive behavior, as well as its evolution. We then describe in detail how androgenic hormones serve as powerful integrators among physiological and behavioral control modules. Through this synthesis, several important unanswered questions are becoming more tractable, and these questions have the potential to shed a light on the process of behavioral evolution more broadly. Of particular importance is the issue of robustness, or a trait's persistence in response to a perturbation. One's view of behavioral robustness likely depends on the perturbation they are considering; for instance, some change in a selective pressure may create an evolutionary perturbation, whereas changes in the environment might create an ecological perturbation. Regardless, systems properties arising from a behavioral control network might reveal how and/or why certain behavioral traits persist through time (or not) and/or function. In other words, the nature of a behavioral control system's intrinsic design (i.e., how physiological and behavioral modules are connected to each other) might profoundly influence the degree to which certain behavioral tactics and strategies could change through time and work to enhance survival and reproduction.

Systems biology is one of many ways to better understand the physiological basis of behavior. We hope our manuscript highlights, as others have begun to do, this point—namely, that systems thinking can provide a unique perspective on how behavioral traits might arise as an emergent property of physiological connectedness. We also hope that our application of this idea to the elaborate behavior of manakins help clarify the perspective. Indeed, systems biology has been applied with

great success to other fields, such as developmental biology and genomics. When behavior and its physiological basis are viewed in this context, major advances to our conceptualization of behavioral routines and their evolution will likely expand. So too will our appreciation for the powerful role that endocrine systems can play in the processes by which behavioral evolution unfolds through time.

Acknowledgements

We thank the Manakin Genomics Research Coordination Network (RCN) that made this work possible, specifically Bette Loiselle, Mike Braun, and Emily DuVal. This project was funded by a National Science Foundation grant (DEB-1457541) to support the RCN, as well as IOS-1947472 (to M.J.F.), IOS-0646459 (to B.A.S.), and IOS-1353085 (to T. B.R.).

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