# Physiological and Biochemical Zoology

## Trade-offs (and constraints) in organismal biology

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| **Abstract:** | Trade-offs and constraints are inherent to life. Studies of these phenomena also play a central role in both organismal and evolutionary biology, but conceptions of these phenomena vary among fields and are sometimes contradictory. Trade-offs can be defined, categorized, and studied in several, not mutually exclusive ways. Six common causes of trade-offs are recognized, some of which exist at different levels of biological organization. (1) Allocation constraints occur when a resource (e.g., energy, time, space, essential nutrients) is limited, such that increasing allocation to one component necessarily requires a decrease in another. When only two components are considered, this is referred to as the Y-model, and one classic example is the energy devoted to size versus number of offspring. (2) Functional conflicts occur when features that enhance performance of one task decrease performance of another. Well-known examples involve the biomechanics of bone and muscle function (e.g., relative lengths of in-levers and out-levers, force-velocity trade-offs related to muscle fiber type composition). Functional trade-offs seem to underlie much of the interspecific variation in bird beaks and bills. (3) Integrator molecules (e.g., hormones, neurotransmitters, transcription factors) often simultaneously affect multiple traits, with some effects being beneficial for one or more components of Darwinian fitness (e.g., survival, age at first reproduction, fecundity) and others detrimental. One example involves circulating concentrations of testosterone: high levels can increase growth rate, muscle mass, bone density, and territorial/aggressive behavior, but also increase parasitism and decrease paternal care. (4) Antagonistic pleiotropy describes genetic variants that increase one component of Darwinian fitness (or a lower-level trait) while simultaneously decreasing another. For example, one model for the evolution of senescence relies on the idea that alleles benefitting early reproduction will be favored by natural selection even if they have negative effects on later survival. (5) Ecological circumstances may impose trade-offs. For example, a behavior that increases energy availability (e.g., foraging) might also decrease survival (e.g., because of increased exposure to predators). (6) Sexual selection may lead to the elaboration of (male) secondary sexual characters that improve mating success but handicap survival because they impose costs (e.g., in terms of energetics, performance abilities or conspicuousness to predators). Overlap occurs among the types of trade-offs. For example, the effects of integrator molecules may be underlain |

by alleles with antagonistic pleiotropic effects. Empirical studies of trade-offs often search for negative correlations between two traits (e.g., as caused by allocation constraints) that are the expected outcomes of the trade-offs, but this will generally be inadequate if more than two traits are involved and especially for complex physiological networks of interacting traits. Moreover, trade-offs often occur only in populations that are experiencing harsh conditions (e.g., cold winters, droughts), energetic challenges, or at the extremes of phenotypic distributions, as among individuals or species that have exceptional athletic abilities. The six categories of trade-offs can be placed upon organizational axes related to their duration and causality. Proximate vs. ultimate categorizes trade-offs into mechanistic and evolutionary causes. Time durations of trade-offs range from acute that occur on the order of seconds to days to cross-generational, evolutionary processes. Trade-offs may be (partially) circumvented through various compensatory mechanisms that relate to the duration. Going forward, a pluralistic view of trade-offs and constraints, combined with integrative analyses that cross levels of biological organization and traditional boundaries among disciplines, will enhance the study of evolutionary organismal biology.

# Perspective: Trade-offs (and constraints) in organismal biology

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Key words: adaptation; allocation; evolution; genetic correlation; hormones; integrator molecules; plasticity; selection experiments

**What is already known:**

Trade-offs and constraints play a central role in both organismal and evolutionary biology. However, definitions of these concepts vary among fields and are sometimes contradictory.

**What this study adds:**

We attempt to provide a comprehensive framework for relating the concepts of trade-offs and constraints. We identify six types of trade-offs (both proximate and ultimate), illustrate them with empirical examples, and consider the various ways they can be studied in biological systems.

## Abstract

Trade-offs and constraints are inherent to life, and studies of these phenomena play a central role in both organismal and evolutionary biology. Trade-offs can be defined, categorized, and studied in at least six, not mutually exclusive, ways. (1) Allocation constraints are caused by a limited resource (e.g., energy, time, space, essential nutrients), such that increasing allocation to one component necessarily requires a decrease in another (if only two components are involved, this is referred to as the Y-model, e.g., energy devoted to size versus number of offspring). (2) Functional conflicts occur when features that enhance performance of one task decrease performance of another (e.g., relative lengths of in-levers and out-levers, force-velocity trade-offs related to muscle fiber type composition). (3) Shared biochemical pathways, often involving integrator molecules (e.g., hormones, neurotransmitters, transcription factors), can simultaneously affect multiple traits, with some effects being beneficial for one or more components of Darwinian fitness (e.g., survival, age at first reproduction, fecundity) and others detrimental. (4) Antagonistic pleiotropy describes genetic variants that increase one component of fitness (or a lower-level trait) while simultaneously decreasing another. (5) Ecological circumstances (or selective regime) may impose trade-offs, such as when foraging behavior increases energy availability yet also decreases survival. (6) Sexual selection may lead to the elaboration of (usually male) secondary sexual characters that improve mating success but handicap survival and/or impose energetic costs that reduce other fitness components. Empirical studies of trade-offs often search for negative correlations between two traits that are the expected outcomes of the trade-offs, but this will generally be inadequate if more than two traits are involved and especially for complex physiological networks of interacting traits. Moreover, trade-offs often occur only in populations that are experiencing harsh environmental conditions or energetic challenges at the extremes of phenotypic distributions, such as among individuals or species that have exceptional athletic abilities. Trade-offs may be (partially) circumvented through various compensatory mechanisms, depending on the time scale involved, ranging from acute to evolutionary. Going forward, a pluralistic view of trade-offs and constraints, combined with integrative analyses that cross levels of biological organization and traditional boundaries among disciplines, will enhance the study of evolutionary organismal biology.

*Keywords:* adaptation, allocation, antagonistic pleiotropy, biomechanics, constraint, energetics, locomotion, sexual selection.

## Introduction

Evolutionary biology has long considered trade-offs as central to the field, and many subfields within organismal biology also hold this view (e.g., see Schmidt-Nielsen 1984; Garland, Jr. and Carter 1994; Ackerly et al. 2000; Taylor and Thomas 2014; Zamer and Scheiner 2014; Martin et al. 2015; Agrawal 2020). Historically, evolutionary considerations of trade-offs did not include much effort to elucidate underlying mechanisms at the molecular, biochemical, morphological or physiological levels of biological organization, but more recent studies are often quite mechanistic (Sinervo and Svensson 1998; Flatt et al. 2011; Immonen et al. 2018). For example, biomechanical trade-offs related to feeding may be a root cause of some adaptive radiations (Grant 1986; Schluter 1995; Marroig and Cheverud 2005; Slater et al. 2009; Monteiro and Nogueira 2011).

Here, we outline a general framework for relating the concepts of trade-offs and constraints in biology, with an emphasis on the perspectives of organismal biology. We will see that some types of constraints and trade-offs are simple in origin, arising from the laws of geometry and physics (Alexander 1985; Taylor and Thomas 2014), such as those pertaining to lever arms (Aerts 1990) (but see McHenry and Summers 2011). These are relatively easy to understand, even intuitive, and sometimes straightforward to study. Others emerge unpredictably from the properties of networks and the very nature of complex biological systems, or from the idiosyncratic ways that signaling molecules interact with target tissues and with each other.

Even though both trade-offs and constraints are widely invoked in biology, precisely what these terms mean to practitioners is often unclear (e.g., see Antonovics and van Tienderen 1991; Roff and Fairbairn 2007; Bourg et al. 2019). Given their long history of usage in different fields and often in the absence of formal definitions, we do not attempt to impose rigid, unitary definitions. Instead, we highlight definitions that seem the most useful to us. In total, we recognize six general categories of trade-offs (**Table 1**). We will discuss the first four in detail but devote relatively little space to the last two because they are exceedingly broad topics that entail a voluminous literature (V. Ecological circumstances and VI. Sexual selection).

The range of trade-offs we consider is broad and diverse, and it might seem that we are trying to compare apples and oranges. We are, intentionally. Trade-offs impact all aspects of organismal biology, and they can be studied at the mechanistic level to understand proximate causes, at the population level to understand how trade-offs affect evolutionary trajectories, and everything in between. Our point in bringing together different research approaches and perspectives is to argue for a broader, synthetic view of trade-offs that cuts across different levels of biological organization (genes to molecules to species) and different conceptual goals. This is particularly important when recognizing that most trade-offs are not simple, binary, A versus B propositions. Instead, there is growing appreciation that trade-offs occur as networks of interacting processes, where, for example, the trade-off between A versus B might depend on the resolution of a prior trade-off between A1 and A2 upstream in a network that culminates in A. Accepting that trade-offs occur as networks promotes an interdisciplinary approach to studying organismal biology, because the trade-offs that govern the network will likely involve mechanisms that span disciplines (e.g., genetics, physiology, endocrinology) and selective forces that encompass yet more disciplines (e.g., behavior, ecology, sexual selection).

## What are Trade-offs and Constraints?

In biology, the simplest type of trade-off occurs when one trait cannot increase without a decrease in another (Garland, Jr. 2014; Cohen et al. 2020). The key word here is "cannot." For something to be called a trade-off, we should have evidence that it is at least difficult, if not impossible, to increase one thing without decreasing another. Furthermore, "cannot" is distinct from "does not." Specifically, trade-offs refer to the mechanistic processes that cause one trait to decrease when another increases, in contradistinction to simply an observed pattern of negative association between traits. Thus, when studying the outcomes of an evolutionary process, we think of trade-offs as a cause rather than a symptom. The observed patterns should be viewed as the results of trade-offs, rather than the trade-offs themselves (see also Cohen et al. 2020).

A constraint can be defined very broadly as "bias on the production of variant phenotypes or a limitation on phenotypic variability caused by the structure, character, composition, or dynamics" of biological systems (Maynard Smith et al. 1985). From an evolutionary perspective, one might simply say that "populations unable to evolve to selectively favored states are constrained" (Gomulkiewicz and Houle 2009, p. E218). A perhaps more tangible way to express this is simply "Physical constraints ... set the design space that evolution and behaviour are free to explore" (Taylor and Thomas 2014, p. 7). Reviews of the term "phylogenetic constraints" are available elsewhere (McKitrick 1993; Blomberg and Garland 2002).

Although the concepts of trade-offs and constraints are closely related (see also Taylor and Thomas 2014), we note that two major reviews of the role of (developmental) constraints in evolution did not mention trade-offs a single time (Maynard Smith et al. 1985; Arnold et al. 1989), nor did a paper on physical constraints on evolution (Alexander 1985). Moreover, relatively few empirical studies have tried to test alternative hypotheses of constraints versus trade-offs (Shine 1992). Cohen et al. (2020, p. 154) "contrast trade-offs, which may be modulated via organismal or evolutionary processes to adjust the balance between the mechanisms/traits in question, with constraints, which are limits on fitness or functioning that are not subject to important modulation." In this distinction, a constraint could involve a single trait, with the constraint setting a limit on the trait’s value (e.g., a constraint on maximum body size: Goldbogen 2018). Organismal biologists often view an animal's performance capacities (e.g., how high it can jump) as constraining its behavioral options (Garland, Jr. and Carter 1994; Orr and Garland, Jr. 2017). This is much the same as we argued for the trade-off definition in two paragraphs above, except a trade-off involves a constraint placed simultaneously on the functional relationship between two (or more) traits.

As a simple biological example of a constraint causing a trade-off, we can consider resources, such as energy. If the total amount of energy that is available to an organism is constrained, then increasing the amount of energy allocated to one function will necessarily mean that another function must use less energy. When only two such competing functions are involved, this is termed the Y-model (e.g., de Jong 1993; Harshman and Zera 2007; Roff and Fairbairn 2007; Careau and Garland, Jr. 2012; Lailvaux and Husak 2014; Harris 2020).

One classic example of a Y-model trade-off involves size versus number of offspring (e.g., for turtles see Fig. 2 in Iverson et al. 1993). Aside from energy, the amount of space inside the body cavity could limit the size and number of eggs a female could carry. However, resource-related constraints will only cause trade-offs if the organism is using all of the available resource. In other words, the total amount of available resource may be limited, but if the organism is not close to reaching that cap, then a trade-off will not be ineluctable (cf. Bateson 1963; Shine 1992; Speakman and Garratt 2014). For an example related to time (Kronfeld-Schor and Dayan 2003), if an organism is strictly diurnal and lives near the equator, then it will have about 12 hours within which to accomplish its normal activities, such as foraging. However, it might be able to satisfy its daily needs within far less than 12 hours. If so, then it would have the potential to increase the amount of time spent on foraging without a necessary decrease in the amount of time spent on some other daily activity (e.g., building or maintaining a nest). Indeed, time budgets show that many animals appear to have excess time available for foraging, advertising (typically by males) or shopping for mates (typically by females), and other activities (Herbers 1981). One might expand the scope of this example by wondering why an organism would be "constrained" to be 100% diurnal. For many organisms, this could have something to do with visual abilities. For ectotherms that must bask in the sun to raise body temperature (heliotherms), it would have much to do with thermoregulatory constraints.

Physiologists, morphologists, and evolutionary biologists often appeal to constraints or limits related to body size, allometry, and scaling relationships, some of which can be deduced based on first principles of physics (e.g., see Stearns 1980; McMahon and Bonner 1983; Calder 1984; Schmidt-Nielsen 1984; Lindstedt 1987; Nijhout and Emlen 1998; Kelt and Van Vuren 1999; Hein et al. 2012; Taylor and Thomas 2014; Voje et al. 2014; Rezende and Bacigalupe 2015; Bright et al. 2016; Jones et al. 2018; White et al. 2019; Downs et al. 2020; Verberk et al. 2020; Cloyed et al. 2021). For example, body size will constrain the length of time that an animal can fast, how fast it can run, and the size of prey that can be subdued.

As constraints and trade-offs pervade our everyday lives, many related concepts come to mind when we think about them, such as something being a double-edged sword (e.g., Stahlschmidt et al. 2015), you can't have it both ways, you can't have your cake and eat it too, and there's no such thing as a free lunch. Our familiarity with these sorts of concepts can be both a blessing and a curse when we try to formalize definitions and consider interrelationships among things in a rigorous, physical, mathematical, or statistical way. Sometimes metaphors help us understand biology, but other times they obfuscate (Slobodkin 2001).

## Six categories of Trade-offs

To present our perspective on trade-offs, we focus on six common categories of trade-offs that are frequently discussed in the literature (Table 1).

### 1. Allocation constraints

Allocation constraints occur when a limit exists for the total amount of a resource that is available (e.g., energy, time, space, essential nutrients), such that increasing allocation to one component necessarily requires a decrease in allocation to another. When only two components are considered, this is the Y-model, which is the easiest type of trade-off to think about, to depict graphically, to model mathematically, and to analyze statistically. Frequently in real biological systems, the situation is more complex.

For a given resource, multiple, hierarchically arranged Y-model constraints often exist. For example, energy might be devoted to the frequency versus duration of foraging bouts, so those two components of foraging behavior will trade-off if only so much energy is available for foraging in general. An organism might take energy from other activities (such as searching for mates) and thus break the trade-off between foraging bout frequency and duration, but then it will be trading-off foraging with mate searching. And so it goes among components of the overall energy budget, unless the total amount of energy available to the organism can be increased. Another strategy is to switch between resource-intensive behaviors or physiological states, which is a type of phenotypic plasticity (for an example with crickets, see Miyashita et al. 2020).

Some apparent allocation constraints are partly definitional. For example, if muscle fiber type composition is recorded in only two categories (e.g., fast versus slow) and in a way that only allows quantification as a proportion of the total muscle fibers, then the proportion of fiber types necessarily sum to unity. In practice, this is usually done because it is not feasible to measure every muscle fiber in the cross section of an entire muscle (let alone all of the muscles in, say, the thigh). Using proportions will necessarily increase negative covariation.

A real example involving interspecific variation in muscle fiber type composition of lizards (Bonine et al. 2005) is somewhat more complicated (**Figure 1A**). Different types of muscle fibers are relatively better or worse at various functions, including speed of contraction and stamina, and may differ in energetic efficiency (McGillivray et al. 2009; Schiaffino and Reggiani 2011; Brooks 2012; Blaauw et al. 2013). In the lizard example shown here, three main fiber types occur: fast-twitch glycolytic (FG), fast-twitch oxidative-glycolytic (FOG), and slow-twitch oxidative (SO) fibers. The last of these is relatively rare, but we know of no fundamental reason why lizards with a high percentage of SO fibers could not exist, even if no such species has yet been discovered. Therefore, based on the data shown in **Figure 1A**, one might conclude that the combined action of natural and sexual selection has never favored lizards with a high proportion of SO fibers.

Although the different characteristics of muscle fiber types have the potential to cause functional constraints, we must be careful when considering negative relations that occur simply because two or more components must sum to unity. **Figure 1B** illustrates this point. Data for three independent random variables (X1, X2, X3) were created with zero correlation using the Microsoft Excel function RAND(), which returns a value between 0 and 1. Then, the plotted values were created with the formulas FG = X1/(X1 + X2 + X3), FOG = X2/(X1 + X2 + X3), and SO = 1 - (X1 + X2). Thus, the values for FG, FOG, and SO are constrained to sum to unity (**Figure 1B** shows them plotted as percentages). As shown in **Figure 1C** (below the diagonal), some correlations occur simply because the parts must sum to one. If these were real data, we might be tempted to draw important conclusions about biological functions, but an important "signal" in the data only exists after accounting for correlations that must occur by construction. In the case of muscle fibers, the constraint and apparent trade-off caused by methodological/definitional issues could be avoided by use of absolute counts rather than percentages of different fiber types, but absolute counts generally are not available because it is too laborious to make complete counts for entire muscles.

## 2. Functional conflicts

Functional conflicts (or constraints) can cause trade-offs when features that enhance performance of one task decrease performance of another (Holzman et al. 2011; Shoval et al. 2012). Well-known examples involve the biomechanics of bone and muscle function involving the relative lengths of in-levers and out-levers (Alfaro et al. 2004; Arnold et al. 2011; Santana 2016). Other examples include force-velocity trade-offs related to muscle fiber type composition (Herrel et al. 2009; Schaeffer and Lindstedt 2013)], the effects of shape on swimming performance in fish (Blob et al. 2010; Langerhans and Reznick 2010), and the effects of wing shape on flight performance in birds (Taylor and Thomas 2014). The model in **Figure 2** involves a muscle fiber-type based trade-off. As another example, functional trade-offs between running and fighting appear to have emerged as greyhounds and pit bulls were being developed by artificial selection (Pasi and Carrier 2003; Kemp 2005). Functional trade-offs also seem to underlie much of the interspecific variation in bird beaks and bills species (Herrel et al. 2009; Shoval et al. 2012; Rico-Guevara et al. 2019) (but see Bright et al. 2016). Moreover, variation in bird bills affects "vocal performance" as it relates to a trade-off between rates of sound production and the frequency bandwidth of sounds, partially due to biomechanical constraints that cause trade-offs involving maximal force and velocity or torque and angular velocity (Derryberry et al. 2018).

In turtles, maximum egg width appears to be constrained by the size of the pelvic aperture. However, the pelvic girdle also functions during locomotion and limb retraction, so selection on pelvic architecture may often be complex and in opposition with respect to reproduction versus locomotion (Congdon and Gibbons 1987; see also Oufiero and Gartner 2014 on lizards).

Functional conflicts also occur at the levels of physiology (e.g., on optimal hematocrit, see Schuler et al. 2010; Stark and Schuster 2012), integrator molecules (Martin et al. 2011), and molecular biology (Somero and Hochachka 2002).

## 3. Shared biochemical pathways

Many biochemical and physiological pathways share integrator molecules (e.g., hormones, neurotransmitters, transcription factors) (Ketterson and Nolan Jr 1992; Finch and Rose 1995; Harshman and Zera 2007; Hau and Wingfield 2011; Martin and Cohen 2015; Garland, Jr. et al. 2016) that simultaneously affect multiple traits, with some effects potentially being beneficial for components of Darwinian fitness (e.g., survival, age at first reproduction, fecundity) and others having detrimental effects. One well-studied example involves circulating concentrations of testosterone: high levels can increase growth rate, muscle mass, bone density, activity levels, and territorial/aggressive behavior, but also increase parasitism and decrease paternal care (Marler et al. 1995; Sinervo and Svensson 1998; McGlothlin et al. 2007, 2010; Miles et al. 2007; Mills et al. 2008; John-Alder et al. 2009; Moore and Hopkins 2009). Glucocorticoids also influence many aspects of physiology (Sapolsky 2000) under both baseline and stressed conditions, and may underlie correlations among numerous traits at various levels of biological organization (Sinervo and Svensson 1998; Sapolsky 2000; John-Alder et al. 2009; Romero and Wingfield 2015; Garland, Jr. et al. 2016; Singleton and Garland, Jr. 2019; Harris 2020).

A human example involves use of caffeine, the most widely consumed central-nervous-system stimulant. Caffeine can increase motivation and performance in both mental and physical tasks (Grgic et al. 2019) and coffee consumption is associated with lower mortality rates (Park et al. 2017), but caffeine also increases heart rate and blood pressure (Mort and Kruse 2008), and has been associated with increased frequency of anxiety and sleep disorders (Cappelletti et al. 2015; Bertasi et al. 2021). Happily, one review concluded that "daily coffee and caffeine intake can be part of a healthy balanced diet; its consumption does not need to be stopped in elderly people" (Nehlig 2016, p. 89).

Recently, trade-offs involving integrator molecules have been placed within a network framework (Martin et al. 2011; Cohen et al. 2012). This has led to insights about the connected nature of physiological traits and insights about how molecules that mechanistically regulate a trade-off can also trigger other physiological responses that help mitigate that same trade-off (Adamo 2017). We discuss physiological networks below (see "Physiological regulatory networks").

## 4. Antagonistic pleiotropy

Antagonistic pleiotropy (Rose 1982; Austad and Hoffman 2018) occurs when genetic variants that increase one component of Darwinian fitness simultaneously decrease another, causing a negative additive genetic correlation between the two components. Antagonistic pleiotropy underlies one of the major evolutionary theories of aging, and is perhaps most commonly discussed in that context (Williams 1957; Kirkwood and Rose 1991; Finch and Rose 1995; Cohen et al. 2020). This theory of ageing posits that alleles increasing components of early-life reproductive success (e.g., age at first reproduction) may reduce late-life survival, but the latter "problem" is not so important because the strength of selection is generally stronger early in life. Indeed, antagonistic pleiotropy is sometimes virtually synonymized with trade-offs between reproduction and longevity (e.g., see Austad and Hoffman 2018).

Antagonistic pleiotropy is not a unique category under which to organize trade-offs, but rather a level of biological organization at which trade-offs can be conceptualized and studied. For example, alleles that increase foraging duration should increase energy acquisition and hence the ability to grow and reproduce, but will also increase exposure to predators and parasites, and so decrease survival (e.g., see Clobert et al. 2000). Note that in this hypothetical example the pleiotropy is rather indirect. More direct effects will occur in many cases, such as when variation in the circulating concentrations of a hormone simultaneously interact with receptors in two different tissues or organs, with both positive and negative consequences for fitness components. Whatever the context, pleiotropy occurs not magically but via ordinary biochemical pathways and physiological mechanisms, including integrator molecules, and in the context of ecological circumstances and whatever sexual selection may be occurring. Therefore, this category of trade-off is not separate from the others that we recognize (Table 1).

## 5. Ecological circumstances (selective regime)

Trade-offs under our categories of (I) allocation constraints, (II) functional conflicts, and (III) shared biochemical pathways involve proximate mechanisms that are typically internal to an individual organisms, and our category (IV) antagonistic pleiotropy places these proximate mechanisms in an explicitly genetic context. Our next category, (V) ecological circumstances, emphasizes the external context of trade-offs. Many trade-offs are driven by ecological circumstances, and when the relationship between traits and Darwinian fitness varies with environmental conditions, they will be context dependent. For example, Y-model trade-offs related to energy availability may only occur during particular seasons or years, as food availability varies. Similarly, a trade-off between time spent foraging and predation risk may not occur if predators are rare. In this case, nothing inherent to foraging reduces survival -- no direct, mechanistic connection exists.

Variation in external factors, which causes variation in the selective regime, is likely to affect the balance between the trade-off alternatives, and this variation likely occurs in regular ways, such as deserts generally imposing limits on absolute resource availability (e.g., water). For example, frogs experience trade-offs among body size, temperature regulation, and water regulation that are mediated by behavior, physiology, and environmental conditions (Tracy et al. 2010). Frogs typically require proximity to water or humid environmental conditions, because in general they have low cutaneous skin resistance to water loss (Shoemaker et al. 1992). This represents a constraint on the distribution of frogs among habitats. However, various behavioral adaptations, including nocturnal activity and selection of humid microhabitats, allow them to survive in terrestrial environments (Wells 2007; Hillman et al. 2009; Tracy et al. 2010). These adaptations turn a constraint (low skin resistance to water loss) into a set of trade-offs involving ecological circumstances. For example, there might be a trade-off between selecting microhabitats with high humidity versus those with high prey or predator abundance. Such a trade-off in microhabitat selection is likely to vary across seasons (e.g., spring vs. the heat of summer) and across regions (e.g., lower elevation vs. higher elevation sites that have lower evapotranspiration), so that studying these trade-offs necessarily involves considering ecological circumstances.

In addition, proximate trade-offs, such as functional conflicts, may come into play. For example, to live arboreally while avoiding desiccation, some frogs evolved high skin resistance to water loss and large body size, with the latter decreasing surface area-to-volume ratios and hence relative water loss. However, this combination of traits also limits their ability to elevate body temperature by basking (Tracy et al. 2010), which imposes a functional conflict trade-off between reducing water loss and elevating body temperature. To bask in dry places and be small, frogs must return to water regularly to replenish evaporated water (Tracy et al. 2013). This example for frogs illustrates how trade-offs can be interpreted at different conceptual levels. Proximate mechanisms involving trade-offs (categories I-III) limit what organisms can do, whereas ecological circumstances weight the alternatives that must be traded off by setting the selective regime.

## 6. Sexual selection

Although some researchers prefer to consider sexual selection as a type of natural selection, Darwin viewed the former as distinct enough to warrant separate consideration (Darwin 1871). Keeping them separate also facilitates empirical studies of selection (Wade and Arnold 1980; Arnold and Wade 1984a, 1984b). Sexual selection may lead to the elaboration of (male) secondary sexual characters (e.g., tails of male peacocks or swords of male swordtail fish) that improve mating success but impose energetic, performance, or other costs (Pough 1989; Oufiero and Garland, Jr. 2007; Husak and Swallow 2011; Husak and Lailvaux 2014; Mowles and Jepson 2015). From the perspective of conflicts with natural selection, many papers have considered the evolution of compensatory mechanisms for such traits (e.g., Oufiero and Garland, Jr. 2007; Husak and Swallow 2011; Husak and Lailvaux 2014). Others have considered how allocation-based trade-offs may occur even among sexually selected traits, such as song versus plumage among species of birds (Shutler 2011; Wiens and Tuschhoff 2020), or among body parts in the development and evolution of holometabolous insects (Nijhout and Emlen 1998).

Just as ecological circumstances (category V) determine the selective regime (ecological theater: Hutchinson 1965) under which mechanistic trade-offs play out (categories I-III) and influence cross-generational, microevolutionary changes, so too does sexual selection. Furthermore, the context under which sexual selection influences mechanistic trade-offs may depend on ecological circumstances. For example, some hypotheses about sexual selection theorize that females are selecting for traits that indicate how well a male reduces a trade-off. Specifically, the immunohandicap hypothesis posits that females prefer males that can maintain ornamental secondary sex characteristics in the face of parasites, specifically because these traits are subject to damage by parasites. As such, parasites act to ensure that that ornamental traits are honest traits (Hamilton and Zuk 1982). In this case, the level of parasitism in a population, and hence how honest ornamental traits are, could be viewed as an ecological circumstance surrounding sexual selection. Sexual selection will also involve mechanistic trade-offs. A mechanistic hypothesis is that androgens have the dual role of increasing expression of sexual ornaments while suppressing immune function (Owens and Short 1995). It follows that males can only have ornamental characteristics and fight parasite infections if they are of high quality. Although the immunosuppressive effects of androgens are debated (Roberts et al. 2004; Foo et al. 2017), the hypothesis built around androgens and the hypothesis built around sexual selection are not conflicting or even separate hypotheses: they are simply addressing the question of ornamentation and parasite infection from different conceptual directions.

## Some Examples of Why Trade-offs Matter

Trade-offs are fascinating, and their prevalence and diversity in organisms means that they should be targets of study in their own right. But trade-offs also shape evolutionary and ecological processes, and this makes trade-offs a central concern in any attempt to explain how organisms evolve and how the evolution-driven characteristics of organisms act to structure ecological communities. Addressing this topic in depth would be too ambitious here, so we just touch on it lightly.

Trade-offs have a key role in maintaining the genetic diversity of species. To illustrate this, we present some examples of trade-offs for pea aphids (*Acyrthosiphon pisum*). We use pea aphids not because we think they are an exceptional species, but instead because they are unexceptional; other species are likely to show similarly diverse trade-offs that maintain genetic diversity.

Pea aphids come in two colors, green and red, with the inheritance of color behaving like a single-locus, biallelic character when there is sexual reproduction (Caillaud and Losey 2010). Color involves a trade-off that depends on ecological circumstances, because green aphids are more susceptible to parasitic wasps, while red aphids are more susceptible to some species of predatory ladybeetles. Because parasitism by wasps is density-dependent, this generates frequency-dependent selection on green vs. red aphid morphs, thus maintaining the color polymorphism by balancing selection (Losey et al. 1997).

As another example, pea aphids contain facultative bacterial symbionts that are inherited between asexual generations with very high fidelity; the symbionts are part of the extended aphid genotype and phenotype. One symbiont, *Serratia symbiotica*, confers resistance to heat shocks that, when experienced by susceptible juvenile instars, give rise to adults with lower fecundity (Oliver et al. 2010). This reduction in fecundity due to heat shocks is ameliorated for aphids containing *S. symbiotica*, but there is a trade-off because *S. symbiotica*-containing aphids have reduced population growth rates under normal temperatures (Harmon et al. 2009). Variation in the frequency of heat shocks in summer, at least in part, explains the maintenance of variation in resistance (intermediate frequencies of aphid clones containing *S. symbiotica*).

As a final example, a second bacterial symbiont, *Hamiltonella defensa*, confers resistance to parasitic wasps (Moran et al. 2005), but at the cost of reduced reproduction rates, thus giving an example of a survival-reproduction trade-off (Ives et al. 2020). At a regional spatial scale, the wasps likely exert frequency-dependent selection on resistant *H. defensa*-containing aphid clones, because low prevalence of resistant clones allows for increases in the wasp population, while high prevalence causes the population of wasps to decline. This can generate ecological-evolutionary (eco-evo) dynamics and rapid evolution that maintain an intermediate frequency of *H. defensa*-containing clones in the pea aphid population. Although eco-evo dynamics can maintain diversity for resistance at the regional scale, at the local scale of individual fields the strong selection exerted by wasps can lead to high variation in the frequencies of *H. defensa*-containing clones (from 2 to 88% among fields sampled at the same time, or within the same field sampled through time). Thus, the trade-off between resistance to parasitism and reproduction can lead to eco-evo dynamics and a spatio-temporal mosaic of genotypes in the population.

Although studies often focus on the role of trade-offs in maintaining genetic diversity within a species, trade-offs can also lead to variation in a trait among species. In a classic example of natural selection in the wild, Grant and Grant (1993) showed that when seed production on the island of Daphne Major ceased due to a drought, individuals of a Darwin's finch (*Geospiza fortis*) with deeper beaks had greater survival, because they were more able to crack the hard seeds that were left after the more-easily cracked small seeds had been eaten. Bill depth is under temporally varying balancing selection, however, and mean bill depth decreased when rains and seeds returned. Thus, variation in bill depth causes a trade-off, with the optimal bill depth depending on environmental conditions. The variation in bill depth observed within this population, however, is small compared to the variation in bill depth among species of Darwin's finches, and bill depth is a trait involved in the adaptive radiation of this group of species on the Galapagos Islands. The trade-offs involving bill depth that explain some of the variation within a species likely also explain some of the variation in bill depth among species (Herrel et al. 2009; Shoval et al. 2012).

Such examples as bill depth and the adaptive radiation of Darwin's finches illustrate why trade-offs play a central role in ecological theory about the generation and maintenance of biodiversity. Robert MacArthur's (1972) paradigm of species coexistence is based on the assumption that trade-offs define the niches of species, and that the restrictions set by trade-offs ultimately explain the number of competing species that can coexist in a community. In the absence of trade-offs, a single species could potentially be better than all other species at securing resources, leading to a community with a single "superspecies." Even as ecological understanding of competition has broadened, the importance of trade-offs has not diminished. Competition occurs not only among species for shared resources, but also among species with shared predators (Holt 1977). In the absence of trade-offs, a single species might be able to develop extreme defenses against all predators and thereby become a superspecies. Coexistence among competitors can also occur by species using different strategies to cope with spatial and temporal environmental variation, with trade-offs again underlying the differences among strategies. Without the trade-offs experienced by all species, the world would likely be a biologically much less rich place.

## Proximate vs. Ultimate Causation: Mechanism vs. Evolution

Ernst Mayr (1961) introduced the concepts of proximate and ultimate causation as an organizational paradigm for understanding cause and effect in biology, and applying these concepts can aid in understanding trade-offs. Proximate causation refers to immediate mechanisms of a biological trait. For trade-offs, proximate causes include resource limitations leading to allocation constraints, functional conflicts, and shared biochemical pathways (**Table 1**). In contrast, ultimate causation refers to the evolutionary processes that shape a biological trait (Mayr 1961), including ecological circumstances that cause variation in selection regimes, sexual selection (Table 1), and other evolutionary mechanisms (e.g., founder effects, genetic drift). Thus, we can loosely associate proximate causes with processes that occur within an organism's lifetime, and ultimate causes as processes that involve Darwinian selection that spans generations. Proximate versus ultimate causation might also be separated into effects that are observed within a generation versus those observed among generations (see also below). Proximate and ultimate trade-offs are not mutually exclusive in part because ultimate trade-offs act through proximate mechanisms and those mechanisms can evolve.  For example, whether selection favors an increase or decrease in the frequency of an allele will depend on the balance of its positive and negative (antagonistic) effects on Darwinian fitness, and these effects will depend on the other genetic variants present in the population (i.e., the genetic background) (Mayr 1954; Sarup et al. 2011; Chandler et al. 2014; Taylor and Ehrenreich 2015) which change over time.  Thus, in Table 1, we have, perhaps awkwardly, tagged antagonistic pleiotropy as both proximate and ultimate.  In any case, understanding proximate causes of trade-offs can inform our understanding of ultimate causes, and vice versa (e.g., see Kirkwood and Rose 1991; Sinervo and Licht 1991; Garland, Jr. and Carter 1994; Sinervo and Svensson 1998; Feder et al. 2000; Taylor and Thomas 2014).

One example of an antagonistic proximate mechanism of a trade-off involves the pleiotropic effects of the tumor suppressor gene *TP53* on aging and cancer.  The gene *TP53* codes for the protein p53, which responds to cell damage by initiating cell cycle arrest or apoptosis (Kastan et al. 1991; Clarke et al. 1993; Lowe et al. 1993; Nelson and Kastan 1994). Mice with mutations in *TP53* that enhance activity of its associated pathway have fewer spontaneous tumors compared to wild-type littermates, but these mice also exhibit early onset of phenotypes associated with ageing (Tyner et al. 2002; Poyurovsky 2006).  At the proximate level over an individual's lifetime, this demonstrates a trade-off between ageing and incidences of cancer that are mediated by the pleiotropic effects of *TP53*.  At the ultimate level of human evolution, this also suggests the reason natural selection cannot simply act to increase activity of *TP53* to reduce cancer risk: doing so would reduce longevity.

## Proximate Causes of Trade-offs

Many internal factors underpin trade-offs.  Signaling or integrator molecules are prime examples (e.g., hormones, cytokines, neurotransmitters, transcription factors: Martin and Cohen 2015).  Integrator molecules can cause changes in energy flow to different functions even when an organism is not at its ceiling of energy availability (Ketterson and Nolan Jr 1992; Finch and Rose 1995; Harshman and Zera 2007; Moore and Hopkins 2009; Hau and Wingfield 2011; Garland, Jr. et al. 2016).  Similarly, many organisms have endogenous seasonal rhythms (Budki et al. 2012; Weil and Nelson 2016) that partly control energy allocation to growth, reproduction, the immune system, and physical activity, among various components of the overall energy budget (Carey 1996; Garland, Jr. et al. 2011b; Martin et al. 2015; Careau and Wilson 2017a).  Internally driven annual and circadian rhythms often cause changes in resource availability via their effects on both behavior (e.g., amount of time spent foraging) and physiology (e.g., digestive efficiency, amount of body fat, biochemical pathways).  In general, these effects will occur via integrator molecules.  Although the coordinated changes that depend on integrator molecules are essential for organismal function during development and reproduction, and in response to environmental conditions, they may also have "unintended consequences" that result in downstream trade-offs.

## Physiological regulatory networks

Physiological regulatory networks provide a framework for understanding the relationships among physiological functions and for identifying the consequences of changes in integrator molecules. They consist of a network of signaling molecules grouped into subnetworks, and each subnetwork regulates a particular set of physiological processes (e.g., immune defenses, reproduction) (Cohen et al. 2012). Maintaining organismal function requires both crosstalk among the subnetworks and integration of information from the external environment, which is facilitated by a limited number of molecules termed integrators (Martin et al. 2011; Cohen et al. 2012). Each integrator has numerous connections, so a change in the integrator facilitates changes in numerous subnetworks (Cohen et al. 2012). Moreover, perturbations that cause a response in one subnetwork can ripple through other subnetworks (Cohen et al. 2012). Because integrator molecules coordinate responses across physiological systems, they may often constitute proximate mechanisms underlying trade-offs. In the context of **Figure 2 or 3**, variation in the presence (e.g., concentration in the circulation) of an integrator molecule might serve as the trait that mediates the relationship between two other traits, much as variation in muscle fiber types partly determines both locomotor speed and endurance. However, integrator molecules often have much more complicated interactions than the binary ones in that relatively simple model (Zera and Harshman 2001; Moore and Hopkins 2009; Martin et al. 2011).

As an endocrine example, glucocorticoids (generally referred to as "stress hormones", although they have many additional functions) have been a focus of research because they affect numerous physiological systems and help coordinate reproduction, energy balance and use, immune defenses, and growth (Sapolsky 2000; Romero and Wingfield 2015; Garland, Jr. et al. 2016; Singleton and Garland, Jr. 2019; Harris 2020; Lattin and Kelly 2020). In insects, the stress response and immune response networks share some signaling molecules, including octopamine and adipokinetic hormone (Adamo 2017). These hormones are released during a fight-or-flight stress response and its corresponding intense physical activity (Orchard et al. 1993; Lorenz and Gäde 2009), and facilitate trade-offs with components of the immune system (Adamo 2017). For example, apolipophorin III is normally involved in immune surveillance (Zdybicka-Barabas and Cytryńska 2013). During a stress response, however, adipokinetic hormone causes the release of lipids and apolipophorin III, which acts as carrier protein for lipids to facilitate energy delivery (Weers and Ryan 2006). Apolipophorin III subsequently becomes unavailable for is normal role in immune surveillance (Adamo et al. 2008; Adamo 2017).

Within a physiological network framework, it becomes clear that not all interactions mediated by integrator molecules cause trade-offs, and that the outcome will depend on the species, the internal and external context, and the pathway involved (Adamo 2017), highlighting the need to investigate the mechanism underpinning trade-offs rather than relying on measuring negative correlations [see "Studying Trade-Offs (and Constraints)"]. Some interactions facilitate responses that help reduce the effects of trade-offs induced by the regulatory network and others cause reconfiguration of the system. (Adamo 2017). It follows that integrator molecules can help ameliorate the effects of a trade-off as well as cause a trade-off. As researchers investigate trade-offs within this framework, the complexity of the interactions among physiological systems that cause and mitigate trade-offs will be further illuminated (and the same is true for biomechanical systems, e.g., see Holzman et al. 2011). Such a perspective will help clarify why trade-offs involving integrator molecules are context dependent (Adamo 2017).

Integrator molecules also play critical roles in coordinating potential trade-offs between incompatible stages, such as wake and sleep or feeding and fasting, by helping regulate circadian rhythms in the body. For example, mammalian basal glucocorticoid concentrations are partially controlled by signals from the suprachiasmatic nuclei (SCN), the master synchronizer of the mammalian circadian timing system in the brain (Oster et al. 2006; Dibner et al. 2010). Glucocorticoids help communicate the circadian patterns from the SCN to cells throughout the body by binding with receptors on those cells and synchronizing cellular rhythms (Dibner et al. 2010). Circulating concentrations of glucocorticoids peak just before the onset of the active phase (Munck and Náray-Fejes-Tóth 1992), and prepare the organism for activity by increasing the release and production of glucose, and increasing physiological arousal (Sapolsky et al. 2000; Oster et al. 2017). In so doing, glucocorticoids help regulate a trade-off between wake and sleep stages, in relation to associated activities, such as feeding and fasting (Dibner et al. 2010; Oster et al. 2017; Riede et al. 2017).

The concept of physiological regulatory networks is one of several frameworks developed to explain why suites of physiological traits and their associated trade-offs change in tandem. Other frameworks include the concepts of allostasis/allostatic overload (McEwen and Wingfield 2003; Korte et al. 2005), the reactive scope model (Romero et al. 2009), and tolerance/resistance strategies for dealing with infections [See (Harris 2020) for a review of all hypotheses exploring the role of stress, many of which invoke trade-offs.]. Central to these frameworks is the concept that an external or internal perturbation shifts the physiological network in a manner that results in trade-offs and potentially fitness costs. Additionally, these frameworks emphasize that the magnitude and duration of the signal is important in determining the organismal phenotype expressed.

## Integrator molecules and trade-offs: examples involving immune defenses

The concept of regulatory networks also helps explain some trade-offs that arise within the immune system (Heng et al. 2008; Downs et al. 2014). The immune system is an interconnected network of molecules and pathways that includes redundancies in signaling, self-regulatory pathways, and sequential responses in which prior responses provide signals that direct later responses (Murphy et al. 2007). Early steps within a pathway include the release of signaling molecules that constrain subsequent responses and result in trade-offs, as illustrated by the integrated signaling pathways for pro- and anti-inflammatory response (Zimmerman et al. 2014). For example, the pro-inflammatory cytokine interleukin-6 (IL-6) is produced in response to bacterial components, and it initiates downstream effects including the production of acute phase proteins in the liver that then simulate an acute-phase inflammatory response (Jørgensen et al. 2000; Nakae et al. 2001; Dienz and Rincon 2009). Interleukin-6 is also part of a chemical cocktail that suppresses anti-inflammatory responses (Dienz and Rincon 2009). Similarly, a type 2 T helper cell (Th2) response by a host against macroparasites, such as helminths, inhibits the type 1 T helper cell (Th1) responses that are elicited by microparasites, leading to the possibility that infection with a macroparasite facilitates coinfection with a microparasite, and vice versa (Romagnani 1997). This cross-regulation between Th1 and Th2 responses might explain the prevalence of bovine tuberculous (TB, *Mycobacterium bovis*) infection in African buffalo (*Syncerus caffer*) (Jolles et al. 2008; Ezenwa and Jolles 2011, 2015). Prevalence of helminths and TB infections were negatively associated across herds, and within herds, buffalo with coinfections had increased mortality. Furthermore, buffalo exhibited a negative relationship between markers for Th1 and Th2 responses during the dry season, suggesting that cross-regulation may make buffalo more susceptible to coinfection and associated morbidity (Jolles et al. 2008). Although experimentally deworming buffalo did not decrease risk of acquiring TB, dewormed buffalo with TB had increased survival (Ezenwa and Jolles 2015).

A framework for thinking about classes of immunological defense mechanisms and their associated trade-offs is resistance and tolerance to infections (Romero et al. 2009). Infection *resistance* involves fighting infection to limit the number of parasites or pathogens infecting a host (Best et al. 2014; Kutzer and Armitage 2016). In contrast, infection *tolerance* emphasizes reducing the fitness costs of infections rather than control of parasite burden (Råberg 2014; Kutzer & Armitage 2016); it should not be confused with *immunological tolerance*, which is the failure to mount an immune response to an infection (Owen 1945; Schwartz 2012).

The costs of parasite resistance and tolerance are context dependent (Sears et al. 2011). Nutritional limitations are an example of extrinsic factors that can influence which class of mechanism is used in response to an immune challenge (Kutzer and Armitage 2016). For example, flies on a reduced-nutrient diet had similar bacterial loads compared with flies on a standard diet, but had higher survival and were more tolerant to the infection with *Salmonella typhimurium* (Ayres and Schneider 2009), suggesting an unseen nutrient-driven trade-off. Switches between parasite resistance and tolerance can also be mediated by integrator molecules. For example, high concentrations of glucocorticoids in red-winged blackbirds (*Agelaius phoeniceus*) are associated with higher tolerance to Haemosporidian parasites, an agent of avian malaria (Schoenle et al. 2018).

## Time Scales and Trade-off Compensation

A broad way to classify the temporal duration of trade-offs is acute, chronic, lifetime, and intergenerational. The absolute duration of acute and chronic trade-offs will depend on the life history of the species; a trade-off that lasts a week might be considered acute for an elephant but chronic for an aphid. Distinguishing between ephemeral trade-offs that arise from suboptimal investment in traits driven by limited resources and more permanent trade-offs that arise from genetics and integrator molecules is useful because it informs the biological scale at which consequences of the trade-off occurs (Ardia et al. 2011). Acute changes driven by resource limitations are going to have organism-level consequences, whereas trade-offs that are maintained across generations have consequences for ecological community function and hence underpin evolutionary patterns that are driven by trade-offs (see also Slobodkin and Rapoport 1974; Agur and Slobodkin 1986).

Humans faced with economic or engineering trade-offs usually try to overcome them or at least minimize their adverse effects. For example, a change from internal combustion engines to electric motors has changed the basic physical rules that govern relationships between power, torque, weight, and the financial cost of transport by automobile. Like humans, other organisms faced with trade-offs involving either internal or external factors are not generally passive. Rather, they respond in various ways on multiple time scales (Slobodkin and Rapoport 1974; Woods and Wilson 2015). For example, animals that have recently eaten (Garland, Jr. and Arnold 1983; Huey et al. 1984) or are pregnant (e.g., Garland, Jr. 1985; Seigel et al. 1987; Ghalambor et al. 2004) may experience reduced locomotor performance, which could hamper their ability to escape from predators. Such a reduction caused by pregnancy can be viewed as a cost of reproduction, which is a key component of life-history trade-offs. However, pregnant lizards, snakes, and fish have been shown to alter their behavior in ways that should at least partly compensate for this reduction (Bauwens and Thoen 1981; Brodie III 1989; Shine 2005; Banet et al. 2016), and some species may show physiological or biomechanical (kinematic) compensations to help maintain locomotor abilities (Scales and Butler 2007). Similarly, low body temperatures that reduce locomotor performance can lead to behavioral changes that should partly compensate for the reduced locomotor performance (Hertz et al. 1982; Crowley and Pietruszka 1983).

In this section, we explore how trade-offs are organized along a temporal scale, from acute to microevolutionary, and how the duration of a trade-off relates to the scale of the consequences and the compensation strategies employed.

### Acute trade-offs

Acute trade-offs are ephemeral, shorter than an individual's lifetime, and organisms have evolved various systems to compensate for them. Aside from plants, most organisms can alter their behavior immediately in ways that might mitigate a trade-off, and they may learn from past experiences. For instance, if a small mammal starts getting cold, it may move into the sun, and if this happens repeatedly, it may learn good places to find sun. If the cold challenge persists, then it can piloerect. With further cold challenge, non-shivering thermogenesis will likely occur, followed by shivering. All of these changes occur within seconds to minutes. Integrator molecules can promote these behaviors. For example, if a trade-off arises because of limited energy resource in a given location, then increased circulating glucocorticoids can motivate increased food consumption and increased foraging behavior, which facilitates acquisition of food, although these responses are context dependent (Dallman et al. 2007; St. Juliana et al. 2017). In anemonefish, the nonapeptides arginine, vasotocin, and isotocin mediate a trade-off in parental care behaviors on a time scale of tens of minutes (DeAngelis et al. 2020).

Physiological responses that mediate acute trade-offs can also occur within seconds to minutes to hours. In a study of humans, for example, both muscle power output and mental performance decreased when tested together compared to in isolation (Longman et al. 2017). As another example, physiological regulatory networks can lead to fairly rapid trade-offs between stress and immune responses, and they can also cause a rearrangement of immune responses to mitigate the effects of a trade-off or facilitate a relevant immune response (Martin 2009; Adamo 2017).

Acute trade-offs are often condition dependent; that is, they are facultative rather than obligate trade-offs (French et al. 2007a; Ardia et al. 2011). *Urosaurus ornatus* lizards, for example, only show reduced wound healing while reproductive when food resources are limited; the severity of the trade-off depends on the severity of the resource limitation; and the trade-off disappears when the limiting resource is restored (French et al. 2007b). These acute allocation trade-offs can be mechanistically regulated by a perturbation that shifts a physiological regulatory network into a new state (Martin and Cohen 2014; Martin et al. 2016) or changes the concentration of a physiological signal (Zera and Harshman 2001).

## Chronic trade-offs

Chronic trade-offs last longer than acute trade-offs, but for a shorter duration than life-long trade-offs. Chronic trade-offs can be adaptive or they can represent responses to a chronic internal or external perturbation that can result in dysregulation of an organismal response (Zera and Harshman 2001; McEwen and Wingfield 2003; Martin et al. 2016). Adaptive (evolved) chronic trade-offs can involve predictable changes, including those that occur in response to seasons or life-stage events. For example, a Common Eider (*Somateria mollissima*) never leaves its nest during laying and incubation, and, thus, trades off foraging in favor of reproduction (Afton and Paulus 1992). Although a chronic trade-off during reproduction, this trade-off quickly dissipates at the end of incubation.

Plasticity in organismal responses has evolved in part to mitigate and alleviate chronic trade-offs. Physiological acclimation (in the lab) and acclimatization (in the wild) occur, which are examples of phenotypic plasticity (Garland, Jr. and Kelly 2006; Piersma and van Gils 2010). This kind of plasticity occurs by many mechanisms, including epigenetic alterations of gene expression (Kelly et al. 2012; Hau and Goymann 2015; Garland, Jr. et al. 2017). Depending on the type of plastic change that occurs, it may or may not be reversible, and plasticity that occurs early in life is generally less likely to be reversible (Garland, Jr. et al. 2017).

## Developmental trade-offs

Developmental trade-offs are plastic responses that occur early in life or during critical (sensitive) periods and hence are relatively likely to have long-lasting effects, sometimes irreversible (e.g., Howie et al. 2012; Garland, Jr. et al. 2017; Dunn et al. 2018; and references therein). These trade-offs can arise because a signal during a critical developmental window leads to irreversible change to a phenotype; this type of phenotypic plasticity is known as developmental plasticity (West-Eberhard 2003). One mechanism by which this can occur is canalization, whereby a trait's developmental pathway is pushed down one of many possible developmental pathways by a signal early during development (Waddington 1942). For example, *Daphnia ambigua* respond to predator cues during development by shifting their life histories so they mature more slowly and produce fewer offspring relative to those raised without predator cues (Walsh et al. 2015).

## Transgenerational effects

Transgenerational trade-offs can be caused by maternal effects or other processes in which the factors generating the trade-off, or the effects that the trade-off has, are transmitted to the next generation(s). Thus, transgenerational trade-offs may involve plasticity in which the environment experienced by parents alters the phenotypes of subsequent generations (Fox and Mousseau 1998). For example, parthenogenic pea aphid (*Acyrthosiphon pisum*) females determine whether their offspring develop wings; when population densities are high, females are more likely to produce winged offspring (Johnson 1965; Müller et al. 2001). Having wings or not is a trade-off, because pea aphids without wings have greater fecundity, whereas offspring with wings can disperse to habitat that (hopefully) has better resources. Similarly, when cues indicate high densities or a deteriorating environment, parthenogenic *Daphnia* produce male offspring, and mated sexual females produce resting eggs (Hobaek and Larsson 1990; Kato et al. 2011). Here, the trade-off is between asexual reproduction and sexual reproduction with a resting stage to wait out possibly poor environmental conditions.

Cabbage loopers (*Trichoplusia ni*) exhibit a trade-off between transgenerational immune priming and transfer of nutritional stress tolerance (Shikano et al. 2015) when they are simultaneously exposed to a nutritional stress and sublethal immune challenge by the bacterial pathogen *Bacillus thuringiensis.* Parents could only transfer resistance to pathogens, but not nutritional stress tolerance (Shikano et al. 2015). Three mechanisms by which transgenerational effects can occur are via the environment created by the mother for the offspring (Fox and Mousseau 1998), by direct passing of resources or hormones to the offspring (Schwabl 1993; Mousseau and Fox 1998; Groothuis and Schwabl 2008), and by epigenetic alterations of gene expression (Badyaev and Uller 2009). These changes do not entail changes in the genetic code, and thus are not fixed for as long as microevolutionary trade-offs.

## Microevolutionary trade-offs

Microevolutionary trade-offs are based on genetic variation within populations, and they persist on an evolutionary time scale, i.e., across generations. In contrast to acute and chronic trade-offs, they cannot be broken by behavioral or physiological responses within an individual organism. Rather, breaking cross-generational (microevolutionary) trade-offs requires "solutions" on an evolutionary timescale. Hence, trade-offs that occur within populations may relate to speciation in some cases, thus crossing into the realm of macroevolution (Schluter 1995; Herrel et al. 2009).

Genetic correlations that underlie trade-offs are caused by internal, proximate mechanisms, including (i) linkage disequilibrium between two or more loci and (ii) pleiotropic gene action (Lande 1982). Trade-offs that arise from linkage disequilibrium can occur because genes that control two traits are located closely to each other on a chromosome and/or because of non-random mating, and they will persist if favored by selection (Lande 1984; Falconer and MacKay 1996). Breaking these trade-offs requires a cross-over event during meiosis or relaxation of selection (Lande 1981; Kirkpatrick 1982). "Pleiotropy" denotes the effects of a single gene on multiple traits, and this results in a trade-off when these effects are antagonistic with respect to fitness or some lower-level traits (see previous discussion of TP53). Mutations, including gene duplications, are needed to break pleiotropy-based trade-offs. In general, cross-over events that break trade-offs caused by linkage disequilibrium are thought to be more common than mutations that break trade-offs caused by pleiotropy (Falconer and MacKay 1996; Sinervo and Svensson 1998).

Genetic correlations might be more ephemeral than expected because they can be affected by environmental factors; that is, there may be genotype-by-environment interactions (Sgrò and Hoffmann 2004; Rose et al. 2005). For example, exposure to a novel environment can cause the expression of a previously unexpressed gene (hidden or cryptic genetic variation), which might break the negative correlation between two traits (Rose 1984; Sgrò and Hoffmann 2004). Alternatively, a correlation could be plastic and expressed differently in different environments (Service and Rose 1985; de Jong 1990; Stearns et al. 1991; Sgrò and Hoffmann 2004). For example, when reared at 25°C, *Drosophila melanogaster* from populations selected for cold resistance exhibited a trade-off between development time and body size, but the direction of the correlation reversed when reared at 14°C (Norry and Loeschcke 2002).

Correlational selection occurs when particular combinations of two or more traits are associated with Darwinian fitness (Endler 1986). For example, anti-predator behavior and aspects of coloration are correlated within populations of the garter snake *Thamnophis ordinoides* (Brodie III 1992). Striped patterns inhibit detection of motion by some predators and are associated with direct locomotor escape movements, whereas unmarked or blotched patterns are associated with frequent direction shifts during escape from a human predator (Brodie III 1992). Within our classification scheme (Table 1), correlational selection is an external source of potential trade-offs (V. Ecological circumstances (selective regime)). These trade-offs can be broken when the selection regime changes and no longer favors previously favored suites of traits (Lande 1984) or when compensatory mutations occur. One model suggests that most adaptive signatures detected in genome scans could be the result of compensatory changes, rather than of progressive character adaptations (Pavlicev and Wagner 2012).

The proximate genetic mechanisms of microevolutionary trade-offs (linkage disequilibrium and pleiotropy) are in some cases associated with ultimate causes of microevolutionary trade-offs, including correlational selection regimes (Sinervo and Svensson 1998). Correlational selection can occur on traits that are genetically correlated and act to maintain linkage disequilibrium for pairs or sets of loci. If selection relaxes, then linkage disequilibrium can disappear (Lande 1981; Kirkpatrick 1982). Alternatively, selection regimes can lead to genetic correlations by favoring pleiotropically acting alleles. Thus, in these ways and others, genetic architecture can evolve in response to selection (Falconer and Mackay 1996; Lynch and Walsh 1998; Sinervo and Svensson 1998; Walsh and Lynch 2018). These processes occur on an evolutionary time scale.

In all cases, the evolutionary resolution of trade-offs will be shaped by costs and benefits with respect to the components of Darwinian fitness. Among many other types of costs, costs of reproduction, in a broad sense, are the subject of a substantial literature in both evolutionary and organismal biology (e.g., Stearns 1976, 1980, 1989, 1992; Partridge and Harvey 1985; Reznick 1985; Seigel et al. 1987; Brodie III 1989; Sinervo and Svensson 1998; Reznick et al. 2000; Harshman and Zera 2007; Speakman 2008; Flatt and Heyland 2011; Speakman and Garratt 2014; Martin et al. 2015; Banet et al. 2016; Andrew et al. 2020). In the most general terms, costs of current reproduction are viewed as being paid through reduced future reproduction and/or survival, but many studies take a more granular or mechanistic focus.

## Network Perspectives on Trade-offs

Discussion of trade-offs in the literature, and to some extent by us in this Perspective, leans towards examples in which trade-offs occur between pairs of traits; trade-offs that are binary are easier to explain both at the proximate level of mechanisms and the ultimate level of the responses of species to natural selection that are molded by the trade-offs. In reality, however, for any species and question studied, there will likely be many inter-related trade-offs that affect multiple traits. One of our main goals in this Perspective is to champion the need for a broad view of trade-offs to encompass simultaneously both multiple proximate mechanisms and ultimate drivers of evolution. Here, we illustrate the complexities that emerge in networks of trade-offs using an explicit numerical model related to locomotor performance. [We would also direct readers to the book by Taylor and Thomas (2014), which provides an interesting analysis of constraints related to different walking gaits, and to Cloyed et al. (Cloyed et al. 2021), who review constraints related to body size.]

### Simple binary trade-offs

The focus in the literature on binary trade-offs can lead researchers to miss important trade-offs or to misinterpret the nature of a trade-off (Speakman and Garratt 2014). To illustrate some of the issues involved in conceptualizing and defining trade-offs that may involve multiple traits, as well as testing for them with data, we present simulations of functionally (causally) related traits. We begin with a simple system in which a trade-off might occur: one trait affects two others in opposite directions. Specifically, we simulated random data from a normal distribution to represent individual variation in the percent fast-twitch fibers in hindlimb muscles, which had a positive effect on maximal sprint speed but a negative effect on locomotor endurance (for an example with bird displays, see Miles et al. 2018). We intentionally ignored other traits that would affect these two performance metrics. As shown in the Online Supplemental Material, we then added some random error to both speed and endurance so they would not be perfectly correlated with fiber-type variation or with each other.

**Figure 2** shows the bivariate relations for 40 simulated data points. The negative correlation between speed and endurance (Fig. 2A) is highly statistically significant ($r = -0.636$, 2-tailed $P < 0.001$). However, a path analytic model (Fig. 2B) indicates no correlation between speed and endurance ($r = -0.015 \pm 0.078$ [estimate ± standard error]): this is the correlation of variation in speed and endurance that is not explained by muscle fiber variation. Similar results are obtained by partial correlation analysis or by correlating the residuals from simple linear regressions of each performance trait on percent fast fibers, whereas a principal components analysis is unclear regarding the speed-endurance correlation (Online Supplemental Material).

What do we learn from this simple example? First, the "trade-off" is the joint effect of one trait on two others, whereas the consequence of that trade-off is observed in the simple bivariate correlation of the two dependent traits. In much of the literature, that bivariate correlation would be called a trade-off, rather than the consequence of a trade-off. As a shorthand, we will sometimes refer to a negative relationship between two traits simply as a trade-off, keeping in mind that it is actually the result of a trade-off. In any case, the negative relationship between speed and endurance can be accounted for entirely by the joint causal effects of percent fast fibers, and once this is controlled for statistically, the negative relationship disappears.

### A trade-off network of seven traits

Complex traits require complex causal models (e.g., see Zaman et al. 2014; Melo and Marroig 2015; Orr and Garland, Jr. 2017; Lightfoot et al. 2018; Sella and Barton 2019), and trade-offs within a network are more complex than depicted in the 2-trait model of Figure 2. **Figure 3** presents a network that expands the example in Figure 2 to seven morphological, performance, and fitness traits (Online Supplemental Material). For simplicity, we did not perform genetic simulations to obtain values for the morphological/physiological traits. Rather, we began by creating uncorrelated random normal variables for hypothetical hindlimb lengths and percent fast-twitch fibers in the hindlimb muscles. Simulations that began with genes or genetic parameters would also allow exploration of antagonistic pleiotropy as a basis for trade-offs at higher levels of organization (Table 1).

The network model is a caricature that might apply to a generalized terrestrial vertebrates. For quadrupeds (or bipeds), maximum sprint speed will be the product of maximum stride length and stride frequency. These two traits would be affected by more than just leg length and contraction speed, respectively, but other causal traits (e.g., degree of pelvic rotation, length of the airborne phase) are viewed as unmeasured variables absent from the model. Thus, for this model, speed is computed as the product of leg length and percent fast fibers.

Endurance, on the other hand, was computed as a positive function of leg length, which should have a positive effect on locomotor efficiency, and a negative function of percent fast fibers, which should reduce muscle efficiency. This is the first functional trade-off built into the model. It can also be viewed as an allocation trade-off because muscle fibers are given as a percent rather than an absolute amount, and we do not have a variable of muscle size in the model (see the section on Allocation constraints and Figure 1). Again, many more lower-level traits affect muscle function and running endurance capacity, including hormones and probably signals from the central nervous system (e.g., see Garland, Jr. 1984, 1993; Bramble and Lieberman 2004; Noakes 2012; Tobiansky et al. 2020), but we have tried to keep the model simple.

We modeled endurance as having a positive effect on both probability of survival to reproductive age and reproduction, which can be taken as the number of offspring produced if the individual survives (otherwise zero). Speed, however, was modeled as having a positive effect on survival (e.g., via better ability to escape from predators when chased) but a negative effect on reproduction (e.g., perhaps because it is tied to display frequency in a way that is not attractive to potential mates, thus bringing in sexual selection, which we identify as another source of trade-offs in Table 1). This is the second built-in trade-off, one that would likely be modified by ecological circumstances in our classification scheme (Table 1). Finally, fitness is the product of survival probability and reproduction. We present one representative simulated data set for 40 hypothetical individuals (Figs. 3 and 4).

## Analysis of the entire network

For the simulated data, the bivariate relationships between the lower-level traits and performance (e.g., leg length with speed and endurance) are as one would expect from basic knowledge of physiology and biomechanics, and as are built into the model (**Figure 4**). However, the expected negative relationship between speed and endurance does not exist (r = -0.062), nor is there a negative relationship for the two fitness components, survival and reproduction. These bivariate relationships are confirmed by a path analysis that estimates all of the coefficients simultaneously (**Figure 3**). Thus, this relatively simple simulation model illustrates how unexpected results can emerge from a network perspective on trade-offs. In this particular case, the surprises involve an absence of two negative relationships (speed vs. endurance and survival vs. reproduction). The trade-off that might be expected to generate the negative relationship between speed and endurance -- the trade-off involving fast muscle fibers -- is counterbalanced by the positive effect of leg length on both speed and endurance. The absence of a negative relationship between survival and reproduction is more complex to explain, because it occurs further along in the trade-off network. A negative relationship between survival and reproduction might be expected from the positive effect of speed on survival and the negative effect of speed on reproduction. However, fast muscle fibers increase speed and decrease endurance, and this acts to counterbalance the opposing direct effects of speed on survival and endurance. Specifically, the [fast fiber] -> [speed] -> [reproduction] pathway and the [fast fiber] -> [endurance] -> [reproduction] pathway are negative, but the pathways to survival have opposite signs, with the [fast fiber] -> [speed] -> [survival] pathway being positive. There is also a counterbalancing effect of leg length, for which three of the four pathways to survival and reproduction are positive. The overall patterns observed in networks such as this cannot be deduced from only the signs of interactions between network components; the magnitudes of the interactions are also needed.

Relationships at higher levels of biological organization may be very difficult to predict from those involving lower-level traits, due to the detail of information that is needed (see also Agrawal 2020). We encourage readers to explore other models with the code provided (Online Supplemental Material), as they may provide both biological and statistical insights, and serve as the basis for teaching materials.

## Analysis of subsets of the network

Few studies would include all seven traits in the model (**Figure 3**). A functional biologist, for example, might measure both of the morphological/physiological traits (A, B) and the performance traits (C, D). A behavioral ecologist might wish to measure performance, the two fitness components, and fitness itself. Using the same set of simulated data as discussed above, bivariate correlations (**Figure 4**) and path analysis (**Figure 3**) would indicate a positive effect of both performances on survival, but one negative and one positive effect on reproduction. In spite of these relations, the two fitness components do not show a negative relationship that might have been expected based on much life history theory and empirical examples.

Since a resurgence of interest in bringing quantitative genetics more strongly into evolutionary biology that began in the late 1970s, attempts to measure selection in the wild have become increasingly common (Hoekstra et al. 2001; Kingsolver et al. 2001; Irschick et al. 2007, 2008; Moiron et al. 2020). Following the seminal methods papers by Lande and Arnold (1983) and Arnold (1983), a likely approach would be a path analysis including the lowest-level traits, performances, and fitness, i.e., testing the Morphology --> Performance --> Fitness paradigm (**Figure 5**). This path model indicates positive effects of both speed and endurance on fitness, although the effect of speed is marginally non-significant (Likelihood Ratio Test, P = 0.0581). Note that the analysis of pairwise correlations would suggest that endurance (r = 0.623) but not speed (r = 0.190) affects fitness (**Figure 4**).

## Lessons from network simulations

Although the simulation model that we present is only a caricature of real-life complexities, it nonetheless illustrates a fundamental lesson: *To fully understand the role of trade-offs in the functioning and fitness of organisms, we need to integrate across disciplines and explore trade-offs in the context of causal networks rooted in mechanism.* A corollary is that multiple types of trade-offs generated by different biological processes must be considered. Another corollary is that unexpected functional properties may emerge even from relatively simple systems (e.g., see Alfaro et al. 2004). The diversity of types of trade-offs that interact with each other (**Table 1**) underscores our goal of trying to integrate and synthesize the range of trade-offs that researchers investigate. Studying trade-offs is inherently an interdisciplinary endeavor that encompasses both proximate and ultimate causation and factors both external and internal to the organism, at multiple levels of biological organization.

## Studying Trade-offs (and Constraints)

### General Considerations

Trade-offs are studied in numerous ways, spanning all of the approaches in evolutionary biology, behavioral ecology, and the many subfields of organismal biology (e.g., functional morphology, physiological ecology, comparative physiology). A thorough review and critique of the many alternatives is beyond the scope of this perspective. Instead, we offer brief comments and highlight a few examples for each of several approaches. Readers interested in more detailed discussions are directed first to the extensive literature concerning life-history evolution (e.g., Stearns 1976, 1980, 1989, 1992; Reznick 1985; Kirkwood and Rose 1991; Partridge and Sibly 1991; Sibly 1991; Roff 1992, 2002; Charlesworth 1994; Sinervo and Svensson 1998; Migliano et al. 2007; Flatt and Heyland 2011; Lemaître et al. 2015; Lawson and Borgerhoff Mulder 2016; Cohen et al. 2020).

Regardless of the level at which trade-offs are examined (e.g., within-individual plasticity, among individuals within a population, among species), empirical studies often search for negative correlations between two traits, but this will generally be inadequate for networks of interacting traits (Pease and Bull 1988; Mills et al. 2008; Blows and Walsh 2009; Walsh and Blows 2009). Failing to include traits that play a key role in a particular trade-off is another common problem in empirical studies. Moreover, trade-offs often occur only at the extremes of distributions, as in animals that have exceptional athletic abilities (Poole and Erickson 2011; Sharp 2012; Wilson et al. 2013; Irschick and Higham 2016; Lailvaux 2018) or live in extreme environments (Garland, Jr. 1994; Holzman et al. 2011). Therefore, the choice of individuals, populations or species to study can have a large effect on the ability and statistical power to detect trade-offs.

Whereas a trade-off involving only two traits implies a negative relationship between them, the problem becomes much less clear with more than two traits (Pease and Bull 1988; Charlesworth 1990) (see also Figure 1). Suppose, for example, that an animal could engage in three types of foraging behavior, A, B and C, that together must sum to the number of daylight hours. If C is held constant, then the trade-off appears as a negative relationship between A and B. But if all three vary, then a positive relationship could be observed between A and B provided there are negative relationships between A and C, and between B and C. As the number of traits involved increases, using negative (genetic) correlations to identify trade-offs becomes more problematic (Pease and Bull 1988). Moreover, finding holes in the conceivable morphospace (e.g., see Raup 1966; Frankino et al. 2009) does not discriminate among the alternate hypotheses that (1) some internal constraint keeps organisms from occupying that space, (2) insufficient time has elapsed for organisms to fill the space, or (3) selection has simply never favored organisms that would occupy the space because such a niche has not existed (i.e., lack of ecological opportunity) (see also Weber 1990). A more prosaic issue is incomplete sampling of the organisms in question, including a failure to consider extinct forms that may, for example, have been significantly larger or smaller in body size than occurs among living forms (e.g., see Gearty et al. 2018).

## Comparative studies of differences among species

Trade-offs are often studied by comparing species (or populations). One example involves the possible trade-off between speed and stamina that would be predicted based on muscle physiology and biomechanics (a Category II trade-off, caused by functional conflicts, but also possible involving Category I. Allocation constraints). Studies of lizards have found mixed support for the existence of such a trade-off (Vanhooydonck et al. 2001, 2014; Albuquerque et al. 2015) (see also Toro et al. 2004; Goodman et al. 2007). In male manakin birds, testosterone implants increase the twitch speed of a dorsal wing muscle but reduce its endurance, which affects their courtship display (Tobiansky et al. 2020). Thus, this example of a functional trade-off involves an integrator molecule (Table 1).

Another example is the trade-off between offspring size and number, which is a core component of life-history theory (Stearns 1976, 1992; Roff 2001) and has been documented in various animals, including among species of mammals (Walker et al. 2008), birds (Christians 2000), turtles (Elgar and Heaphy 1989), and lizards (Warne and Charnov 2008). The offspring size-number trade-off is not absolute. In turtles, for example, the correlation is -0.7 across genera, meaning that some species are rather far off of the line that describes the relationship (Elgar and Heaphy 1989).

In addition to trade-offs, constraints can also be recognized in comparative data where they appear as a limit to the range of a given phenotype or by a gap in phenotypic space. Consider a single trait, such as body mass, for which a large data base exists (e.g., Okie et al. 2013). If we have data that include all living species, and we find that, say, no adult mammal is smaller than about two grams, then we might infer that two grams represents the lower limit (constraint) on body size for mammals. A recent comparative analysis of body masses of both fossil and living mammals used phylogenetically based statistical methods and an energetic model to reach the conclusion that, for aquatic mammals, thermoregulatory limitations on feeding efficiency constrain maximum size, whereas energetic costs constrain minimum size (Gearty et al. 2018).

## Individual variation

Differences among individuals in locomotor performance has received considerable attention, especially in squamates, since Bennett's (1980) pioneering paper. Bennett (1980, p. 760) did not use the phrase "trade-off" but tested for correlations between maximal sprint speed and distance-running capacity, and found that "these data do not indicate a specialization of individual animals into sprinters and distance runners." Since 1980, several studies of individual variation in lizards and snakes have measured sprint speed and stamina, generally finding little evidence for trade-offs (e.g., Garland, Jr. 1984, 1988; Garland, Jr. and Else 1987; Sorci et al. 1995; Robson and Miles 2000; Perry et al. 2004; Lailvaux et al. 2019).

Studies of elite human athletes have found evidence for weak trade-offs in performance of different events (Van Damme et al. 2002; Walker and Caddigan 2015; Careau and Wilson 2017b). These analyses involve more sophisticated statistical approaches and much larger sample sizes than those of the squamate studies, and also sampling from the extremes of human variation, which, as noted above, may increase the likelihood of observing trade-offs (Garland, Jr. 1994; Holzman et al. 2011).

## Physiological correlations and manipulations

Physiologists study natural variation among individuals, populations, and species (Bennett 1987; Garland, Jr. and Adolph 1991; Garland, Jr. and Carter 1994; Hayes and Jenkins 1997; Spicer and Gaston 1999; Williams 2008; Gaston et al. 2009), but they spend most of their time performing manipulations, and sometimes they address trade-offs and constraints with such experiments. One example is determining optimal blood hematocrit levels, which can be manipulated in various ways, including by administration of erythropoietin (Kolb et al. 2010; Schuler et al. 2010). Higher hematocrit increases hemoglobin concentration and hence blood oxygen carrying capacity, and is generally associated with higher maximal rates of oxygen consumption and aerobic exercise performance ($VO_2max$). However, very high concentrations of red blood cells increase blood viscosity to an extent that limits aerobic exercise capacity. In a selection experiment that targeted swim-induced aerobic metabolic rate in voles, an evolved 60% increase in $VO_2swim$ was associated with decreased hematocrit (Lipowska et al. 2019). Another example of physiological manipulations involves use of surgical reductions in yolk and alterations of follicle-stimulating hormone in *Uta stansburiana* lizards to examine constraints and trade-offs involving egg size, shape, and number (Sinervo and Licht 1991), and subsequently relations with many other traits at multiple levels of biological organization (Mills et al. 2008).

Obviously, physiological studies that involve ablations, pharmacological manipulations, dietary alterations or hormone supplementation can tell us about constraints and trade-offs that act within the lifespan of an individual. However, controversy has existed concerning the value of such manipulations for elucidating evolutionary trade-offs (e.g., see Rose et al. 1996). As argued by Sinervo and Svensson (1998), they can be informative if the mechanisms altered by physiological manipulations are the same ones that are affected by genetic variation and pleiotropy. They can also serve as a bridge for understanding proximate versus ultimate causation.

## Genetic correlations

In combination with knowledge of how selection is acting on traits, genetic correlations (or, more properly, the additive-genetic covariance matrix [G-matrix]) can be used to predict the rate and direction or evolutionary changes (e.g., see Lande and Arnold 1983; Arnold 1987, 1992; Schluter 1996). In the same type of analysis, several methods have been used to measure the extent of genetic constraints on the response to selection (assuming that the G-matrix remains constant) (Blows and Walsh 2009; Walsh and Blows 2009).

According to Conner (2012), "quantitative genetic approaches, especially genetic correlations among traits, have been the dominant empirical methods for studying constraint on adaptation." Nevertheless, for various reasons, Conner (2012, p. 3313) concluded that "genetic correlations are not very useful for studying constraint" (see also Houle 1991). One reason of particular relevance to our Perspective is that estimates of genetic correlations, in and of themselves, are completely amechanistic "black boxes" that provide no insight regarding the biology underling trait correlations. This is not to say that quantitative genetic approaches are not valid, but instead that they are most useful when tied explicitly to knowledge of the biological mechanisms that drive phenotypic (co)variation (see also Houle 1991; Sinervo and Svensson 1998; Careau and Garland, Jr. 2012).

## Selection in the wild

If the ecological circumstances that might cause trade-offs are of interest, then studies of selection in the wild are the method of choice. Such studies can be purely observational or can involve experiments, such as field introductions or transplants (Endler 1986; Travis and Reznick 1998; Biro et al. 2006; Irschick and Reznick 2009; Kingsolver and Diamond 2011), or modification of the characteristics of individual organisms (Curio 1973; Mills et al. 2008; John-Alder et al. 2009).

Reznick and colleagues (Reznick and Travis 2019) have documented a complex evolutionary trade-off between the evolution of reproductive allocation and swimming performance in guppies (*Poecilia reticulata*) from natural populations in Trinidad. They have studied replicated populations from communities with or without abundant predators (Reznick and Travis 2019). Guppies adapted to life with predators allocate more resources to reproduction and, as a consequence, have larger burdens to carry when pregnant (Reznick and Bryga 1996; Reznick et al. 1996). When startled, they also have faster C-start responses and more rapid acceleration thereafter (Ghalambor et al. 2004). Independent studies show that faster startle responses increase the odds of escaping attack from predators (Walker et al. 2005) and that guppies from high predation localities are significantly more likely to survive attacks by predators (O'Steen et al. 2002). The trade-off between speed of the alarm response and reproductive allocation is manifested as a significant interaction between the speed of the response and the stage of development of the developing young. The young increase in wet mass and volume as development progresses. Guppies from high predation environments are faster when their litters are in early stages of development, but progressively lose this advantage as development proceeds. Response speeds are not different when females are carrying litters in advanced stages of development, which is when the differences between high and low predation guppies in the volume and mass of developing young is maximized (Ghalambor et al. 2004).

More recently, Blob and colleagues have studied another fish, the Hawaiian stream goby (*Sicyopterus stimpsoni*), in which juveniles may face conflicting selective regimes related to avoiding predators in the lower reaches of a stream versus climbing waterfalls to reach the habitats occupied by adults (Blob et al. 2010). Laboratory experiments intended to mimic these different types of selection point to trade-offs based on ecomorphology and locomotor abilities (Schoenfuss et al. 2013; Moody et al. 2017).

These studies highlight the advantages of addressing trade-offs in multiple ways: focusing on populations that differ in selective regimes; analyzing trade-offs through development using well-defined performance metrics; including multiple types of trade-offs simultaneously. Ongoing work will also incorporate explicit genetic information designed to give greater insight into the mechanisms underlying trade-offs.

## Selection experiments and experimental evolution

Selection experiments of various types in both laboratory and field settings have been used to address trade-offs and constraints in a variety of organisms (Bell 2008; Garland, Jr. and Rose 2009; Kassen 2014). Correlated responses to selection indicate genetic correlations, many of which will represent functional relationships among traits, including trade-offs and constraints (Chippindale et al. 1996; Dunnington and Siegel 1996; Rauw et al. 1998; Rose et al. 2005; Garland, Jr. et al. 2016). Here we will give three examples.

As of 2017, Lenski and colleagues (Lenski 2017a, 2017b) had maintained 12 populations of *E. coli* in a simple laboratory environment for more than 25 years and 60,000 generations. Among various results, they discovered a trade-off between growth on glucose and acetate involving two metabolic "ecotypes" that can stably coexist. Each ecotype has a competitive advantage when rare, which it loses when it becomes more common. This represents a classical trade-off of the form that makes interspecific competition less than intraspecific competition, and facilitates the coexistence of species in nature.

Weber (1990) used artificial selection to test hypotheses about constraints on wing shape in *Drosophila*. He noted that flies captured from wild populations and from lab populations subjected to environmental manipulations all fell along the same line for the relationship between two linear wing measurements. One hypothesis for such a pattern is that fundamental developmental constraints (Maynard Smith et al. 1985) that disallow other wing shapes, i.e., resist selection that would act to move them off of theregression line in morphospace. The alternative hypothesis is that organisms are held in morphospace by stabilizing selection. When he artificially selected on wing shape, the populations diverged rapidly from the ancestral form, thus disproving the developmental constraint hypothesis. This simple and direct -- but elegant -- approach deserves to be used more often (e.g., see Beldade et al. 2002; Frankino et al. 2009)!

A selective breeding program for voluntary exercise in mice was begun in 1993, with four replicate High Runner (HR) lines bred for wheel-running behavior on days 5 and 6 of a 6-day period of wheel access and four non-selected control (C) lines (Swallow et al. 1998). One observed trade-off involves the two components of daily wheel-running distance that can be quantified: the number of minutes per day that include at least one revolution and the average speed of running (RPM) computed by dividing total distance by minutes per day. In the base population, these two traits were positively correlated both phenotypically and genetically (Swallow et al. 1998; V. Careau and T. Garland, Jr., unpublished animal model analyses extending from Careau et al. 2013). By generation 43, however, the line means for speed and duration of running were significantly negatively correlated for both males and females in the HR lines, and at the level of individual variation the speed-duration correlation was, on average, lower (less positive) in the HR lines as compared with the C lines (Garland, Jr. et al. 2011a). Cross-generational analyses with a quantitative genetic "animal model" clearly demonstrate the evolution of a negative genetic correlation between speed and duration of running in the HR mice (V. Careau and T. Garland, Jr., unpublished). These results are consistent with the idea that trade-offs may only occur in organisms that are near some sort of limit. In addition, the additive-genetic variance-covariance matrix for running across all six days of the tests used to select breeders each generation evolved in a way that exacerbated genetic constraints and limited future adaptive response to selection (Careau et al. 2015).

Mechanisms underlying the evolution of a trade-off between average speed and duration of daily wheel-running behavior could involve motivation and/or ability. Operant conditioning studies found that the motivational system of HR mice has evolved in a way that reduces the reinforcing value of shorter running durations (Belke and Garland, Jr. 2007) and several pharmacological, neurobiological, and gene expression studies elucidate mechanisms underlying motivational changes (Rhodes et al. 2005; Keeney et al. 2012; Saul et al. 2017; Thompson et al. 2017). With respect to ability, the HR lines have higher endurance (Meek et al. 2009) and maximal aerobic capacity ($VO_2max$) (Cadney et al. 2021) as measured during forced exercise, but have not suffered a general decline in maximal sprint speed (but see

Dlugosz et al. 2009). Changes in endocrine function, such as increased circulating concentrations of the "stress hormone" corticosterone in the HR lines, may contribute to changes in both their motivation and ability for sustained, aerobically supported running (Malisch et al. 2007; Garland, Jr. et al. 2016; Wallace and Garland, Jr. 2016). At the level of fitness components, neither litter characteristics at birth or weaning, nor aspects of maternal care, seem to have suffered general declines in the HR lines (Girard et al. 2002; Keeney 2011). However, lifespan may have declined (Vaanholt et al. 2010) (but see Bronikowski et al. 2006). No trade-off between activity levels and immune function seems to have evolved (Malisch et al. 2009; Downs et al. 2012; Dlugosz et al. 2013).

These selection studies show the power of manipulating the "ecological" circumstances of populations in ways that are explicitly designed to reveal trade-offs at the mechanistic level. As such, they make it possible to understand how the integration of multiple trade-offs determines the evolutionary trajectories of populations.

## Theoretical models

Trade-offs and constraints are often studied with models of various types, including mathematical formulations and computer simulations. Optimality models (e.g., Alexander 1981, 1996; Taylor and Thomas 2014) are commonly used, based on costs versus benefits, and all of them assume some sort of constraint (limit) that causes a trade-off; otherwise, they would always predict "more is better" for all fitness components (e.g., survival, fecundity) and for many subordinate traits (Shoval et al. 2012). Typically, the assumed allocation constraints involve limits on available energy, time or some other resource (Rosen 1967; Maynard Smith 1978; Pierce and Ollason 1987; Parker and Smith 1990; Sibly 1991; Jørgensen et al. 2016). Alternatively, many more-abstract models define trade-offs or constraints as simple functions that set conditions on what combinations of trait values are possible (Schaffer 1974; Charlesworth 1990). Optimization models with trade-offs are structurally similar to quantitative genetic models in which a genetic covariance matrix describes how changing multiple genetic traits together affect fitness (Pease and Bull 1988; Charlesworth 1990; Arnold 1992). Optimality models and the trade-offs they assume may not give insight into the trade-offs per se, but they may nonetheless give insight into the logical outcomes that the trade-offs impose. "The role of optimization theories in biology is not to demonstrate that organisms optimize. Rather, they are an attempt to understand the diversity of life" (Maynard Smith 1978, p. 52).

We believe that theoretical models and corresponding statistical models -- with strong ties to real-world empirical examples -- will be needed to understand the mechanisms and outcomes of trade-off networks like the one we used for our heuristic demonstration (Figs. 3 and 4). Statistical analyses of networks will often involve path analysis, which started with Wright (1921, 1934). Theoretical models can also be used to ask, for example, when will antagonistic pleiotropy itself evolve (Guillaume and Otto 2012). Finally, we see a need for models that explicitly include genetics and mechanistic networks of physiological and morphological traits, all under natural selection, in order to better understand how patterns of trait correlations emerge in real populations, and how we can find them in real data.

### Physical models

Physical models can also be used to study trade-offs. For example, aerodynamics have important effects on the performance of planes and automobiles, so the design process includes empirical testing of scale or full-size models in wind tunnels. In high-performance cars, aerodynamic downforce can help them stick to the road during high-speed cornering, but it also causes drag that hurts fuel economy and reduces top speed. This trade-off can be circumvented by use of spoilers (usually rear-mounted "wings") that pivot with speed.

Aerodynamics are also key to understanding the evolution of gliding behavior and of flight, and how body size, body plan, and body shape may affect flight performance (e.g., see Evangelista et al. 2014; Taylor and Thomas 2014). As one example, Emerson and Koehl (1990) used tests of live animals in the wild and wind tunnel models to study the effects on flight performance of the morphology and behavior of "flying" frogs, which have evolved multiple times from nonflying ancestors. They found that the posture and morphology associated with "flying" behavior decreases horizontal traveling distance but improves maneuverability, implying a trade-off.

## Concluding Remarks

Trade-offs are foundational to understanding the evolution of, plasticity of, and constraints on an organism’s phenotype, and they are superficially intuitive to the point that the concepts underpin popular idioms. Trade-offs in biology, however, are diverse and defy a single, precise definition because of their pervasiveness and because of the interconnectedness of trade-offs among levels of organization and levels of causality. Rather than try to give a precise, unitary definition of trade-off, we have instead described six categories of trade-offs that span a range of biological levels of organization, and that encompass both proximate and ultimate causes. Similarly, we discussed the durations of trade-offs as a way to think about what "strategies" are available that allow individuals to break trade-offs, and the consequences of trade-offs for an organism’s fitness and a species' evolution. Throughout, we emphasized the need to measure mechanisms of trade-offs to distinguish trade-offs from observed negative correlations. Finally, we have attempted to provide a synopsis of different perspectives on trade-offs to show how they give complementary conceptual tools for understanding both mechanisms and drivers that underlie evolution. Our hope is that scientists with different perspectives talk to each other about trade-offs and thus improve our understanding of both how organisms work and how they evolve.

## Online Supplemental Material.zip

For Figure 2:
SPSS Syntax_Figure_2.sps
reads in this as a starting file:
Random_Numbers_Path_4_all_SPSS.xlsx
and creates:
Random_Numbers_Simple_Trade-off_4.sav
which is read in by:
Onyx_Simple_Trade-off_4.xml

For Figures 3, 4, 5:
SPSS Syntax_Random_Path_7_UPLOAD.sps
reads in this as a starting file:
Random_Numbers_Path_4_all_SPSS.xlsx
and creates:
Random_Numbers_Path_1.sav
which is read in by:
Onyx_7.xml

Table 1. Six ways trade-offs are recognized in the literature.

| Category of Trade-off | Proximate vs. Ultimate Causation | Biological example | Human societal or cultural example |
|---|---|---|---|
| I. Allocation constraints | Proximate | competition between energy devoted to size vs. number of eggs; reproduction vs. somatic maintenance and repair | paying the rent vs. feeding the family |
| II. Functional conflicts | Proximate | speed vs. force in biological lever arms; body shape in relation to swimming performance of fish | speed vs. force in mechanical lever arms; speed and power vs. fuel economy in automobiles |
| III. Shared biochemical pathways | Proximate | testosterone increases territoriality but decreases parental care | caffeine increases motivation but also anxiety; SSRIs reduce depression but also lower sex drive (with potential fitness consequences) |
| IV. Antagonistic pleiotropy | Proximate/Ultimate | aging, explained by alleles that increase early-life reproductive success but reduce late-life survival, and so will be favored by selection | aging in the broadest sense, which may occur via many physiological, cellular, biochemical, and molecular mechanisms |
| V. Ecological circumstances (selective regime) | Ultimate | increased foraging effort brings more energy and fecundity, but also increases predation risk and hence lowers survival | managing ecosystems for one service may come at the expense of another (King et al. 2015) |
| VI. Sexual versus natural selection | Ultimate | male secondary sexual characters associated with displays (e.g., peacock tail feathers) increase | trade-offs when choosing mates (Waynforth 2001; Vigil et al. 2006) |

| | | | |
|---|---|---|---|
| | | reproductive success but decrease survival; different types of signals are negatively correlated among species in some lineages (Wiens and Tuschhoff 2020) | |

## Figure Legends

Figure 1. Example of definitional/methodological constraint and trade-off, illustrated with a tri-plot (ternary diagram). A) Values are means for 24 species of lizards (Bonine et al. 2005). Fiber types of the iliofibularis muscle were recorded in a way that only the percentage of total fibers (counts) are available for fast-twitch glycolytic (FG), fast-twitch oxidative-glycolytic (FOG), and slow-twitch oxidative (SO) fibers. SO fibers are relatively rare, so a negative relationship between the %FG and %FOG fibers will generally exist. Interspecific variation in lizard fiber types is even more complicated than shown here because some species (not included in this study) can have as many as 50% of a fourth fiber type, tonic fibers (Abu-Ghalyun et al. 1988; Mutungi 1992). B) Simulated data for three random variables (see text). Even here, statistically significant negative correlations are observed, implying trade-offs. Hence, the appropriate null expectation is not necessarily zero correlation, and this must be considered when searching for biological trade-offs (see text).

Figure 2. Analysis of simulated data for a simple trade-off. The % fact-twitch muscle fibers in hindlimb muscle have a positive effect on maximal sprint speed, but a negative effect on endurance (left panel), which causes the two performance measures to be negatively related in a simple bivariate scatterplot (right panel: Pearson's r = -0.505). The left panel shows a path diagram; by convention (Wright 1921, 1934; von Oertzen et al. 2015), single-headed arrows indicate causal relations and double headed arrows indicate correlations (for examples, see Foster et al. 2015; Collins and Higham 2017; Hiramatsu and Garland, Jr. 2018). All variables were standardized to unit mean and standard deviation prior to analysis with maximum likelihood estimation in Onyx (von Oertzen et al. 2015). Values next to black arrows are path coefficients (standardized regression coefficients) and their standard errors; values next to gray dashed arrows are estimated correlations and their standard errors. Numbers above or below the boxes indicate the amount of unexplained variance for a given trait. Speed and endurance are uncorrelated based on the path analysis (left panel), which incorporates the effect of % fast fibers on both traits.

Figure 3. Path analysis to illustrate trade-offs in a network of causally related traits. Using SPSS, uncorrelated random data were simulated for the two morphological/physiological traits (leg length, % fast fibers) and then used to compute the downstream traits to the right (Online Supplemental Material includes the SPSS syntax). Survival was converted to a categorical variable (0 = no, 1 = yes). Plus and minus signs indicate the direction of the relationship that was simulated. A set of 40 data points was analyzed. Values next to black arrows are path coefficients (standardized regression coefficients) and their standard errors estimated by maximum likelihood in Onyx. Values next to gray dashed arrows are estimated correlations and their standard errors. Numbers above or below the boxes indicate the amount of unexplained variance for a given trait. The fit of the overall model is acceptable, based on RMSEA of 0.0 and no significant lack-of-fit ($\chi^2$ = 1.94 with restricted degrees of

freedom = 8), and all of the causal relations are statistically significant (as expected by construction of the model), including the two trade-offs built into the model (antagonistic effects of % fast fibers on the two performance metrics and antagonistic effects of maximal sprint speed on the two fitness components). These trade-offs affect the relationships between the two performance traits and between the two fitness components; however, neither of those relationships are significantly negative, because the effects of the built-in trade-offs are counterbalanced by effects of other traits.

Note that other paths could have been estimated, such as one from leg length directly to fitness, but this model follows the tradition of the original Morphology --> Performance --> Fitness paradigm (Arnold 1983; Garland, Jr. and Losos 1994; Figure 2 in Ackerly et al. 2000).

Figure 4. Bivariate scatterplots for all traits from the model shown in Figure 3. Asterisk indicates $P < 0.05$ for a 2-tailed test with no correction for multiple comparisons.

Figure 5. Natural selection quantified by path analysis following the Morphology --> Performance --> Fitness paradigm (Arnold 1983; Lande and Arnold 1983), based on a subset of the traits shown in Figure 3. This path model indicates positive effects of both speed and endurance on fitness, although the effect of speed is marginally non-significant (Likelihood Ratio Test, $P = 0.0581$). In contrast, pairwise correlations suggest that endurance ($r = 0.623$) but not speed ($r = 0.190$) affects fitness. Analyses were performed with Onyx (von Oertzen et al. 2015).

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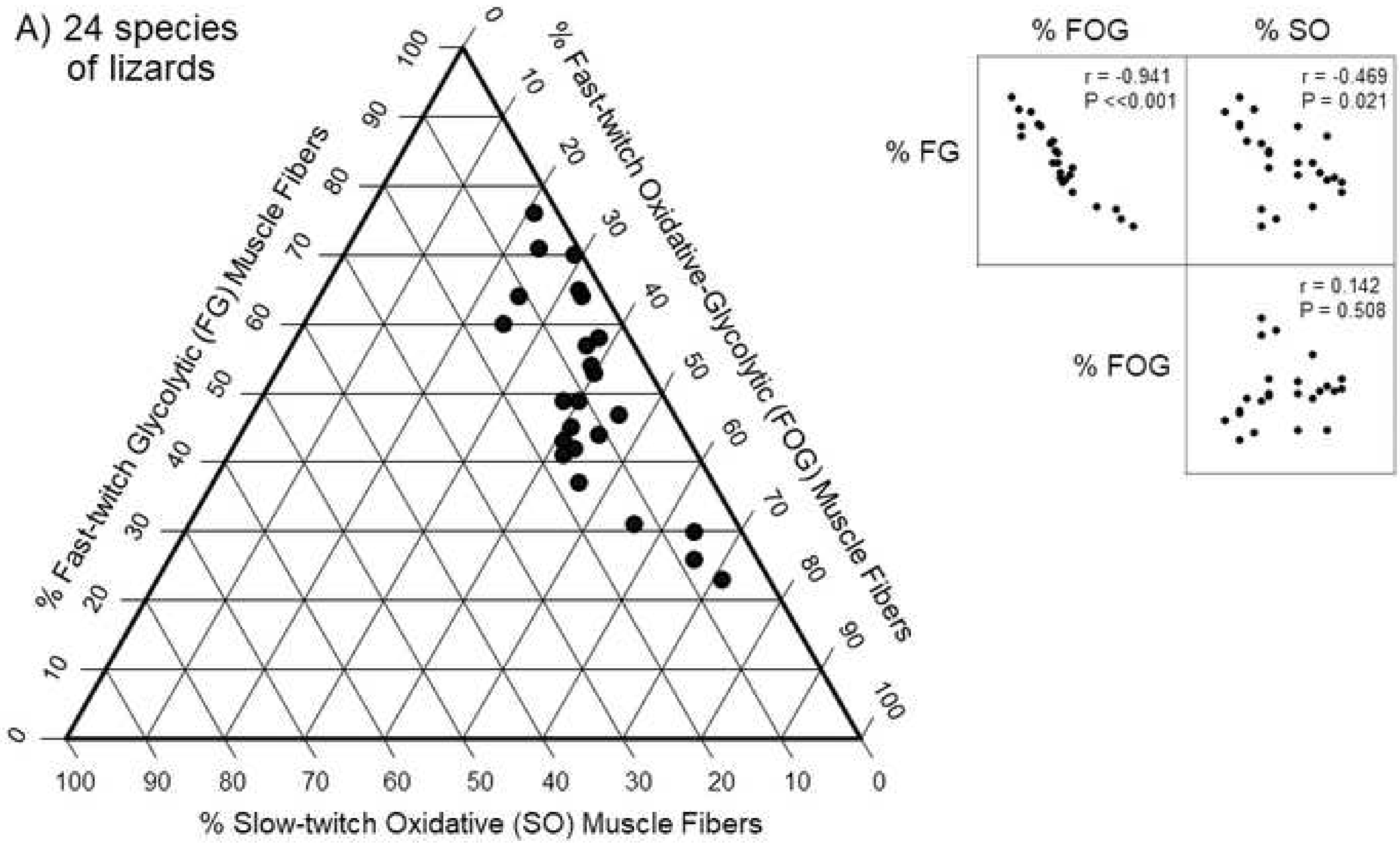
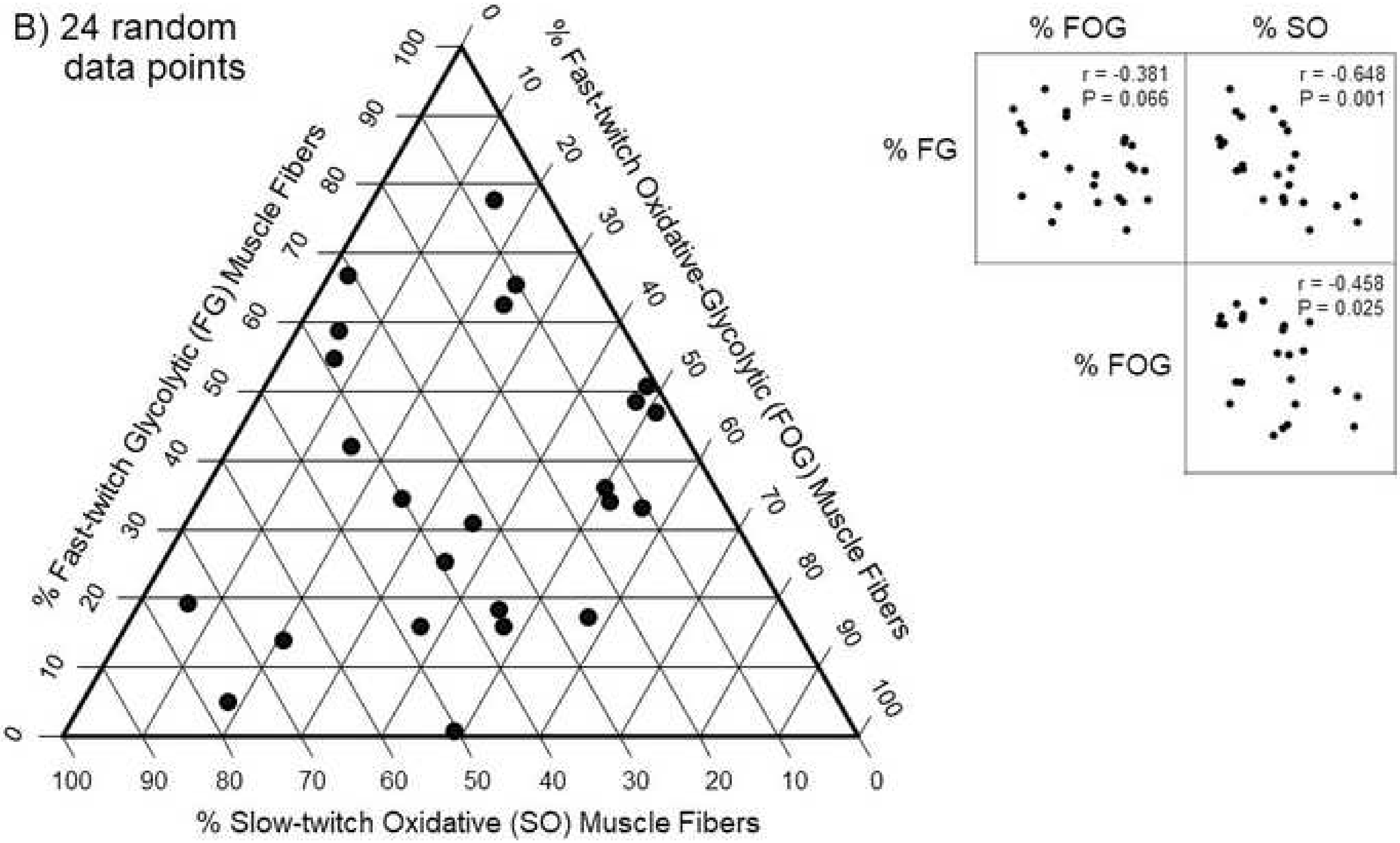
Figure 1a



A) 24 species of lizards
% Fast-twitch Glycolytic (FG) Muscle Fibers
0
10
20
30
40
50
60
70
80
90
100
% Fast-twitch Oxidative-Glycolytic (FOG) Muscle Fibers
0
10
20
30
40
50
60
70
80
90
100
% Slow-twitch Oxidative (SO) Muscle Fibers
100
90
80
70
60
50
40
30
20
10
0
% FOG
% SO
% FG
r = -0.941
P <<0.001
r = -0.469
P = 0.021
% FOG
r = 0.142
P = 0.508

Figure 1b



B) 24 random data points
% Fast-twitch Glycolytic (FG) Muscle Fibers
% Fast-twitch Oxidative-Glycolytic (FOG) Muscle Fibers
% Slow-twitch Oxidative (SO) Muscle Fibers
0
10
20
30
40
50
60
70
80
90
100
% FOG
% SO
% FG
% FOG
r = -0.381
P = 0.066
r = -0.648
P = 0.001
r = -0.458
P = 0.025

Figure 2

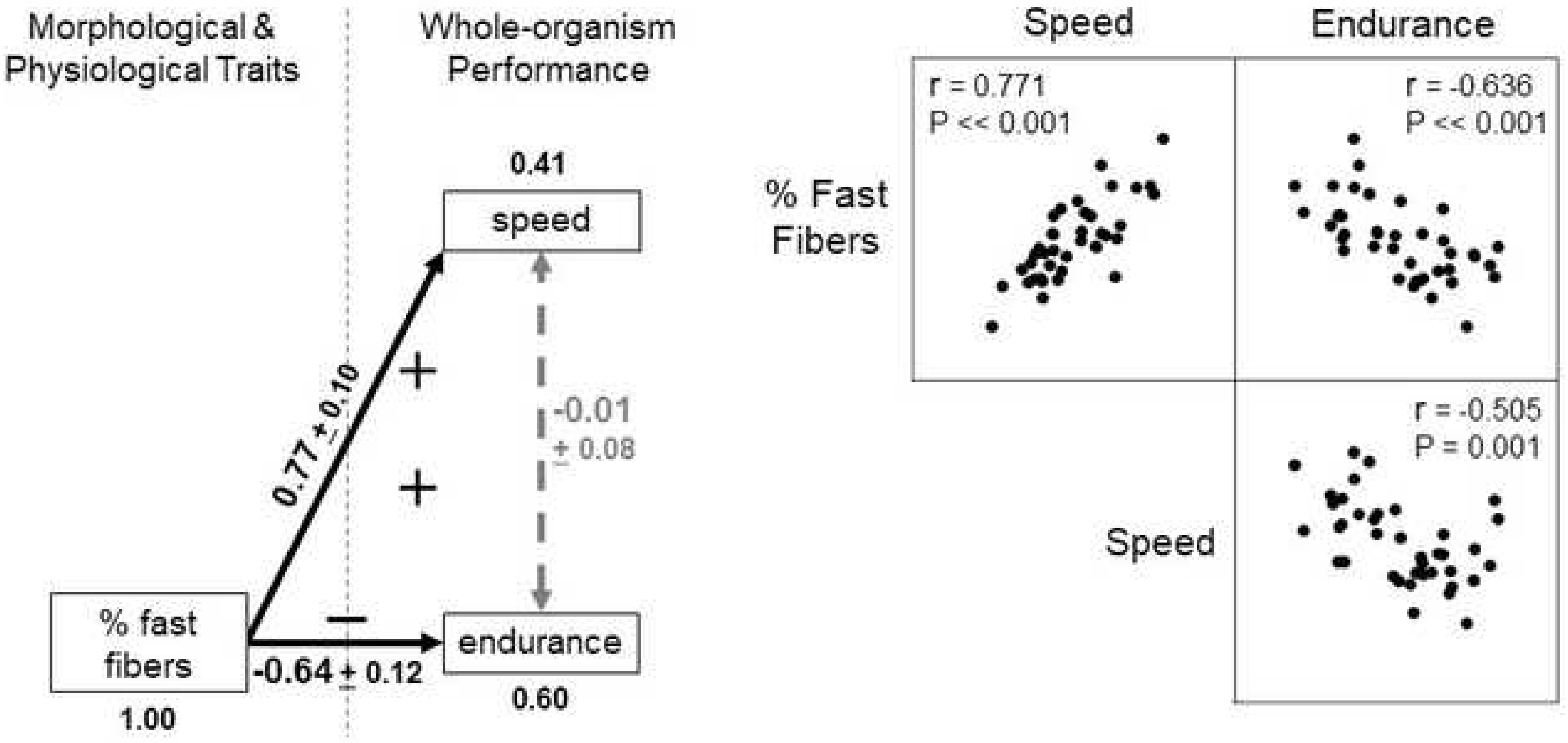

Morphological & Physiological Traits
Whole-organism Performance
0.41
speed
0.77 ± 0.10
+
+
-0.01 ± 0.08
% fast fibers
1.00
−
-0.64 ± 0.12
endurance
0.60
Speed
Endurance
% Fast Fibers
r = 0.771
P << 0.001
r = -0.636
P << 0.001
Speed
r = -0.505
P = 0.001

Figure 3

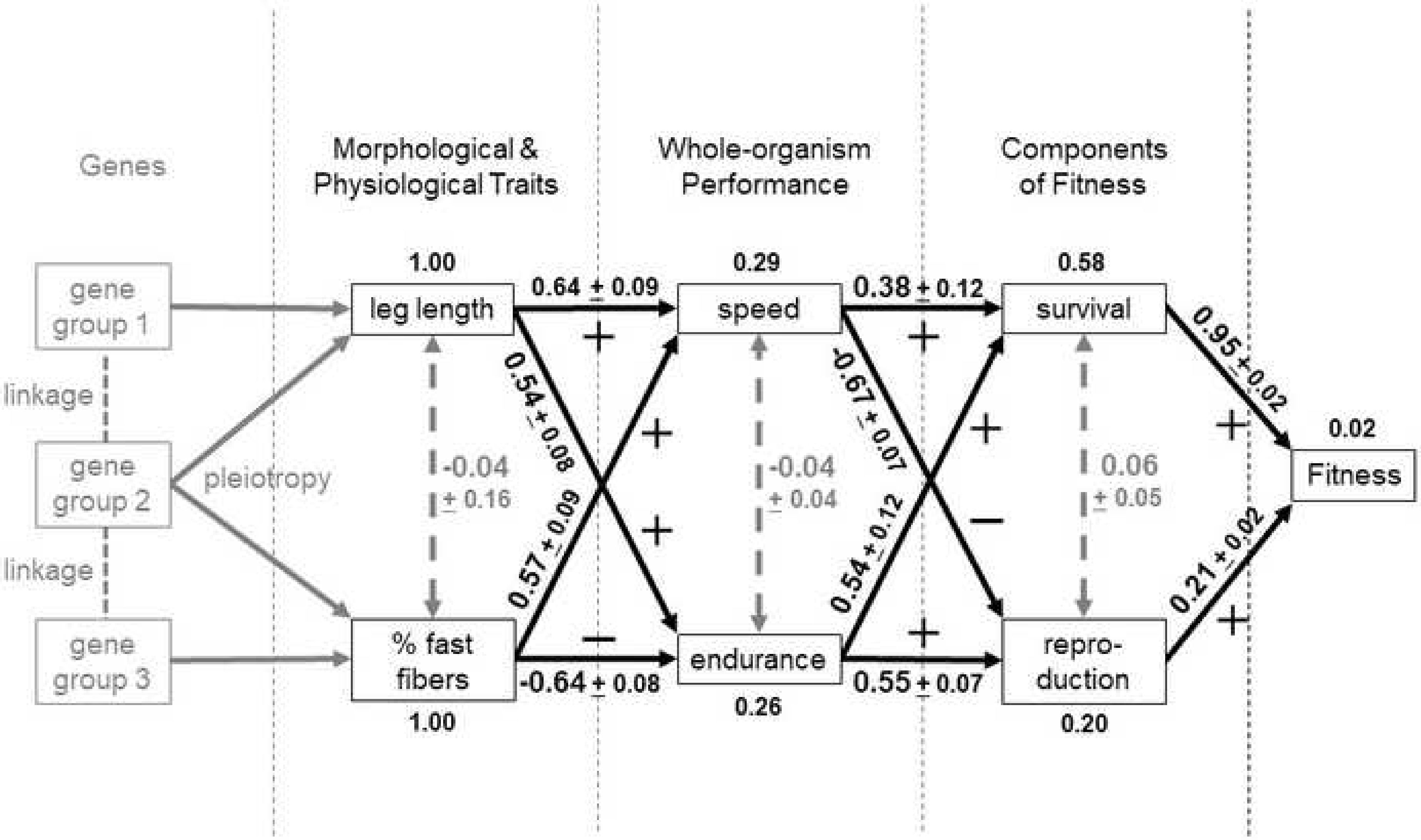
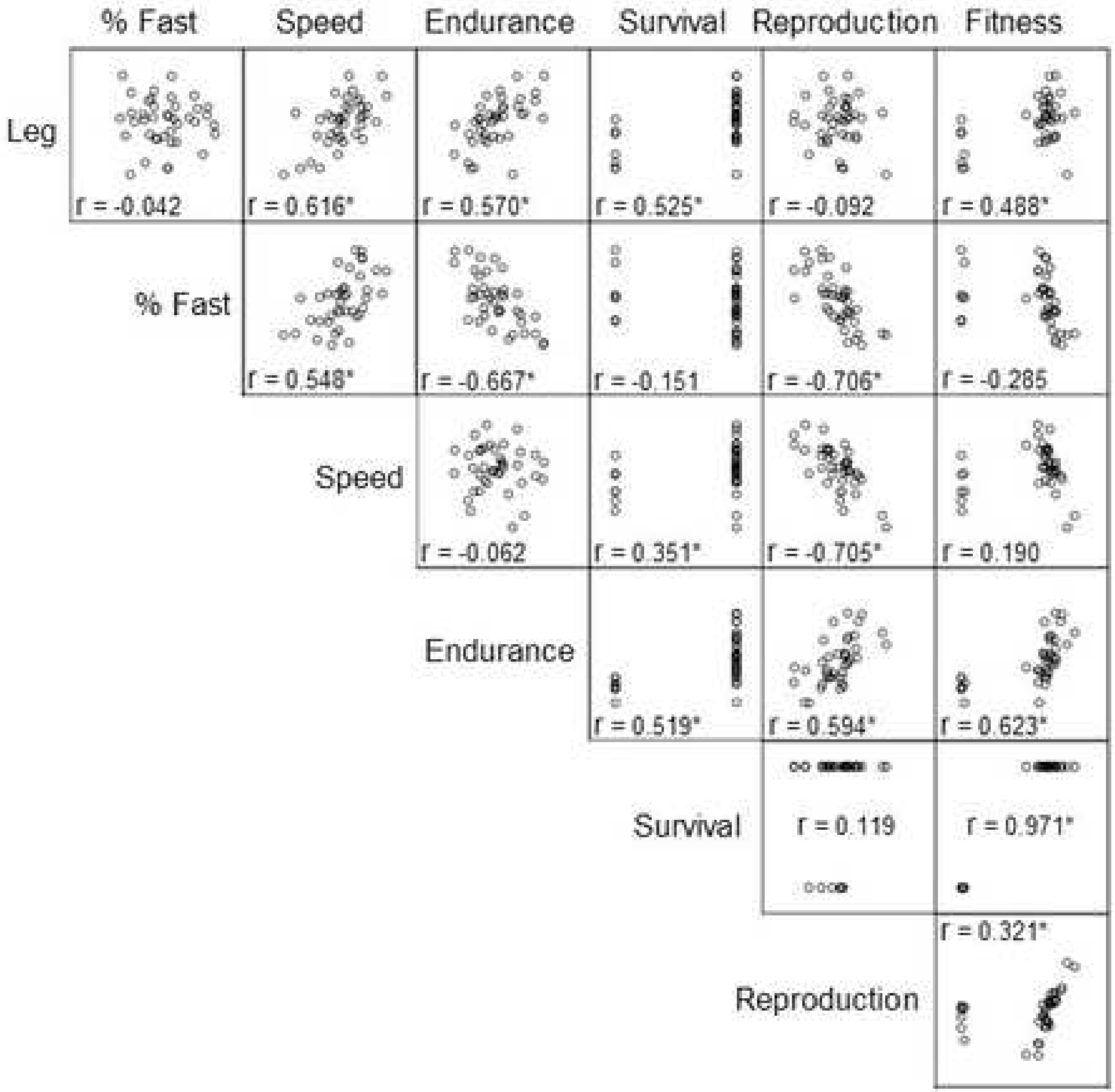
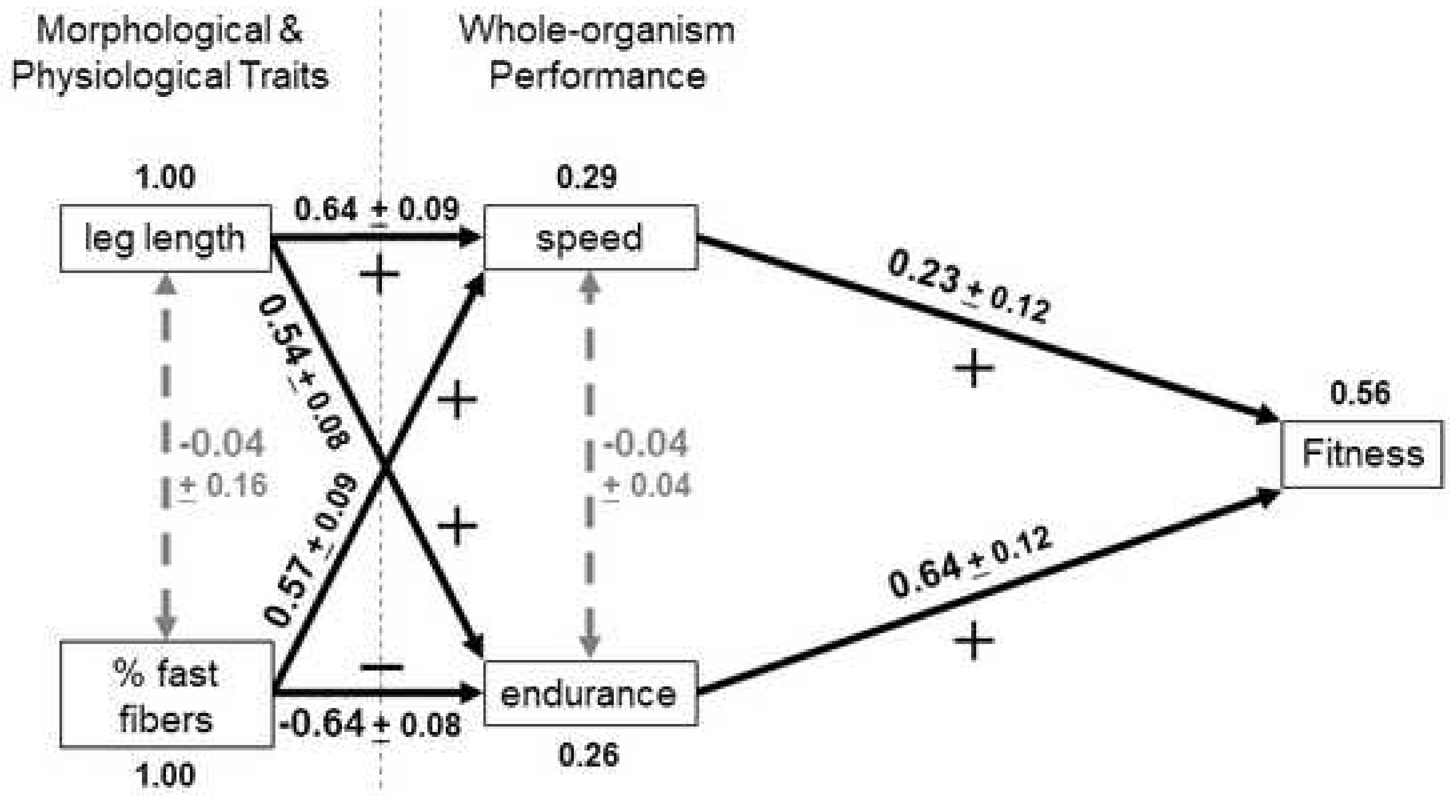

Genes
Morphological & Physiological Traits
Whole-organism Performance
Components of Fitness
gene group 1
gene group 2
gene group 3
linkage
linkage
pleiotropy
1.00
leg length
% fast fibers
1.00
-0.04 ± 0.16
0.64 ± 0.09
0.54 ± 0.08
0.57 ± 0.09
-0.64 ± 0.08
+
+
+
−
0.29
speed
endurance
0.26
-0.04 ± 0.04
0.38 ± 0.12
-0.67 ± 0.07
0.54 ± 0.12
0.55 ± 0.07
+
+
−
+
0.58
survival
repro-duction
0.20
0.06 ± 0.05
0.95 ± 0.02
0.21 ± 0.02
+
+
0.02
Fitness

Figure 4



% Fast
Speed
Endurance
Survival
Reproduction
Fitness
Leg
r = -0.042
r = 0.616*
r = 0.570*
r = 0.525*
r = -0.092
r = 0.488*
% Fast
r = 0.548*
r = -0.667*
r = -0.151
r = -0.706*
r = -0.285
Speed
r = -0.062
r = 0.351*
r = -0.705*
r = 0.190
Endurance
r = 0.519*
r = 0.594*
r = 0.623*
Survival
r = 0.119
r = 0.971*
Reproduction
r = 0.321*

Figure 5


Morphological & Physiological Traits
Whole-organism Performance
1.00
leg length
0.64 ± 0.09
+
0.29
speed
0.23 ± 0.12
+
0.54 ± 0.08
+
-0.04 ± 0.16
+
0.57 ± 0.09
-0.04 ± 0.04
0.56
Fitness
0.64 ± 0.12
+
% fast fibers
−
endurance
-0.64 ± 0.08
1.00
0.26

## Reviewer 1 Comments

General comments:

The authors have done a nice job with the revision.  I look forward to sharing this paper with my mentees and colleagues!  I have a few remaining suggestions for clarity.

REVISION:  Thank you!

Specific comments:

Lines 273-296:  I agree that this example of how the % fiber type in a muscle must result in negative correlations among 2 or 3 fiber types.  But I am confused by this section, because these do seem to be lovely examples of allocation trade-offs, even if there is a statistical problem with the analysis of percentage data.  It would be good to inform the reader how to "account for correlations that occur by construction".  One approach would be to not use % data, but rather counts of fibers of the different types, I think.  You later address this issue to some extent in the paragraph beginning on line 941, so perhaps these two sections could be better integrated.

REVISION:  We have edited this section and also added a sentence pointing to the use of absolute counts, rather than percentages.

Lines 319-321:  I think that this section would be improved by adding some explanation of the examples.  For example, high hematocrit increases the amount of oxygen carried per ml of blood, but increases blood viscosity.  Enzymes that are highly flexible function well at low temperatures but denature easily at high temperatures.

REVISION:  We have added explanation and further discussion of the hematocrit example.  (We do  not have an enzyme example in this part of the manuscript.)

Lines 340-342:  It would be good to give a reference for the varied effects of caffeine on humans.

REVISION:  Great idea.  References added.  Happily, one review concluded that "daily coffee and caffeine intake can be part of a healthy balanced diet; its consumption does not need to be stopped in elderly people" (Nehlig 2016, p. 89).

Line 678:  add comma after "within herds"

REVISION:  Done.

Line 697:  change "driving" to "driven"

REVISION:  Done.

Line 784-5: This is a little confusing: "… generates a trade-off of responses to a stressor with both a physiological and behavioral immune defense". I'd suggest, …with both physiological and behavioral components".

REVISION: We have deleted this whole second half of this paragraph.

Paragraph beginning at line 1169: I think aerodynamic models can be both physical and mathematical, so perhaps this section should be labeled as "organismal modeling approaches to study tradeoffs". Also, such models are not limited to aerodynamics, but can apply to a variety of biomechanical systems, and biochemical networks. Also, this section could use a concluding sentence, along the lines of "modeling approaches have been used to mathematically describe how changes in structure and physiology can alter the capacity of an animal to perform different functions".

REVISION: OK. First, we moved this section to the bottom of the larger section ("Studying Trade-offs (and Constraints)"), so it now follows "Theoretical models." We then added a new introductory sentence.

Line 1271 is confusing: "on days 5 and 6 of a 6-day period of access when young adults and four non-selected control…". I'm not sure what this means.

REVISION: We edited this for clarity.

Line 1312: To distinguish with the organismal modeling section (line 1169), consider calling this section "theoretical evolutionary models".

REVISION: Well, this would not necessarily be restricted

Table 1, row IV, column 3 is missing some words.

REVISION: Fixed.

**Reviewer 2 Comments**

This article was an enjoyable read on a sunny Sunday afternoon. This is an important topic that needs more coverage and this editorial is very timely. The coverage is comprehensive, and I found the article to be very well written. I do have a few comments.

REVISION: Thanks for the encouragement! Sorry to intrude on your weekend!

This is an editorial, not an interpretation of original data. It is an opinion piece. As such there are going to be statements with which others may disagree. My view is - so much the better. Not only will this open dialogue, it should increase interest in the Journal. This is a round-about way of saying it probably won't please all reviewers either but as long as we are open to accepting opinion pieces, they too can have a kick at the can. The section on Network Perspectives on Trade-offs makes a transition from scholarly review to theoretical model. The article then transitions back to discussion in the following section. In some ways, this disrupts the flow of the article. It might be better presented as an addendum, but I leave this to the authors discretion.

REVISION: We have moved the theoretical model section Network Perspectives on Trade-offs to c couple of different places as we worked on the manuscript and responded to previous reviews. Probably no placement is perfect. We view it as an important part of the manuscript, and so we don't want to move it to an addendum, where it would likely be overlooked.

I would change the title of the last section to simply read "Studying Trade-Offs (and Constraints)". The moment you put "How to" in the tile you raise the backs of many readers. It come across as dictatorial which is not the case.

REVISION: Good idea. Change made.

Finally, when it comes to models, my personal view is that models are only useful if they are predictive of outcomes that can subsequently be tested (If the model is an accurate explanation of the data then this should happen when this occurs….). Making a model that simply explains existing data, to me, is of very little use. I am reminded of a quote from James Watson to the effect "No model can explain all of the data for some of the data is bound to be wrong". I'm not suggesting this be added to the editorial - just taking the opportunity to express my views.

REVISION: Duly noted.

In short, I found this to be an excellent article that definitely belongs in the Journal.

REVISION: Thank you!