Why are animals conspicuously colored? Evolution of sexual versus warning signals in land vertebrates

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Conspicuous colors (e.g., red, yellow, blue) have evolved numerous times across animals. But the function of this coloration can differ radically among species. Many species use this coloration as a sexual signal to conspecifics, whereas others use it as a warning signal to predators. Why do different species evolve conspicuous coloration in association with one function as opposed to the other? We address this question in terrestrial vertebrates (tetrapods) using phylogenetic approaches, and test whether day-night activities of species help determine these patterns. Using phylogenetic logistic regression, we found that conspicuous, sexually dimorphic coloration is significantly associated with diurnal lineages (e.g., many birds and lizards). By contrast, the evolution of warning signals was significantly associated with large-scale clades that were ancestrally nocturnal (e.g., snakes, amphibians), regardless of the current diel activity of species. Overall, we show that the evolution of conspicuous coloration as warning signals or sexual signals is influenced by the ecology of species, both recently and in the ancient past.

KEY WORDS: aposematism, color, diel activity, macroevolution, mimicry, sexual selection, vertebrates.

Many animals have striking coloration (Fig. 1) that can appear highly conspicuous (depending on the exact background, viewer, and lighting conditions). However, the ultimate reasons why this type of coloration has evolved can differ dramatically from species to species (Cuthill et al. 2017). One important function of this coloration is to warn potential predators that the individual is toxic or otherwise unpalatable (Ruxton et al. 2004). Examples include poison frogs, coral snakes, some nudibranch mollusks, and monarch butterflies (Ruxton et al. 2004). Avoidance of these aposematic species by predators has led other species to evolve similar coloration as mimics (Ruxton et al. 2004). Yet, many other species have evolved conspicuous coloration as a sexually selected signal, with male colors often being targets of female choice (Andersson 1994; Wiens and Tuschhoff 2020). These include numerous birds, lizards, spiders, and insects (Andersson 1994; Wiens and Tuschhoff 2020). Many important studies have now helped elucidate the evolution of conspicuous colors as warning signals (Pfennig et al. 2001; Maan and Cummings 2012; Davis Rabosky et al. 2016), sexual signals (Hill 1991; Maan and Sefc 2013; Olsson et al. 2013; Dale et al. 2015), and their intersection (Jiggins et al. 2001; Maan and Cummings 2009).

Nevertheless, a fundamental question about color evolution has gone largely unaddressed: why have some species evolved conspicuous coloration as a warning signal, whereas others evolved this coloration as a sexual signal? Is there something about the ecology, physiology, or genetics of species that predicts whether their conspicuous coloration is used for signaling to potential mates or potential predators?

We suggest that the simplest explanation for this basic dichotomy in signal evolution is that sexual signals require that conspecifics can see these conspicuous colors, whereas warning signals do not. For example, some aposematic species have limited visual systems (e.g., nudibranch mollusks; Lederhendler et al. 1980; Serb and Eernisse 2008). However, warning signals have also evolved in groups with well-developed visual systems (e.g., vertebrates). In these groups, we suggest that species may
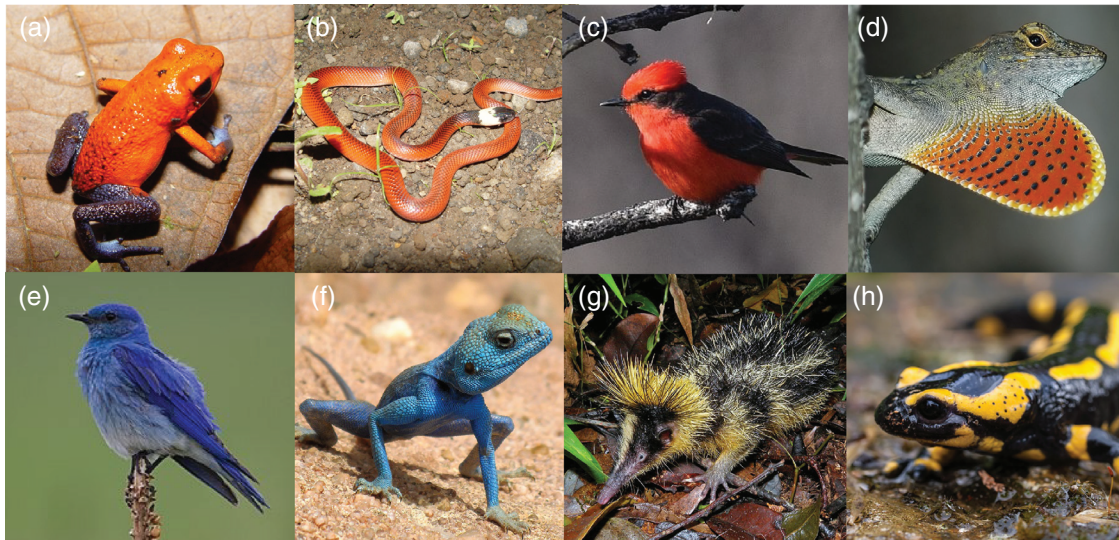
Figure 1. Conspicuous coloration can serve multiple functions. Red coloration serves as warning signal in the aposematic strawberry poison frog (a; *Oophaga pumilio*) and the mussurana snake (b; *Clelia clelia*), in which juvenile coloration (shown here) mimics that of venomous coral snakes (*Micrurus*). Red coloration functions as a sexual signal in males of the vermilion flycatcher (c; *Pyrocephalus obscurus*) and brown anole (d; *Anolis sagrei*). Blue coloration functions as warning signal in *Oophaga pumilio* (a), and as a sexual signal in the mountain bluebird (e; *Sialia currucoides*) and the Sinai agama lizard (f; *Pseudotrapelus sinaitus*). Similarly, yellow can serve as a sexual signal in some species, and as a warning signal in others, including the tenrec (g; *Hemicentetes semispinosus*) and fire salamander (h; *Salamandra salamandra*). Note that red coloration in *Oophaga pumilio* may also serve as a sexual signal. Photo credits (a and b) John J. Wiens, (c) Andy Reago and Chrissy McClarren, (d) Ianaré Sévi, (e) Andy Witchger, (f) Ester Inbar, (g) Frank Vassen, and (h) William Warby.

be more likely to evolve conspicuous coloration as a warning signal if they are primarily nocturnal, or belong to ancestrally nocturnal clades. Although color vision has been documented in some nocturnal animals (Roth and Kelber 2004; Kelber and Roth 2006; Gomez et al. 2009), it is almost certainly harder to detect color differences in full darkness. Thus, nocturnal animals may be less likely to use conspicuous coloration as a sexual signal than diurnal species (Wiens and Tuschhoff 2020). Because sexually selected coloration may be less likely to evolve in nocturnal species, warning signals may be more likely to arise instead. Moreover, the benefits of warning signals are not necessarily limited to when a species is active (e.g., if individuals are found by predators when sleeping during the day). Nevertheless, conspicuous colors used as warning signals may be less visible to potential predators at night. Given this, warning signals might be especially likely to arise in species of ancestrally nocturnal clades that have recently evolved diurnality, such as aposematic poison frogs (Dendrobatidae; Fig. 1) within the ancestrally nocturnal amphibians (Anderson and Wiens 2017). In such species, conspicuous coloration may not be widely used as a sexual signal (because of their nocturnal history), but their conspicuous coloration can function as a warning to diurnal predators. The evolution of warning signals and sexual signals may depend on a plethora of other behavioral, physiological, ecological, and genetic factors (Cuthill et al. 2017). However, day-night

activity patterns can be strongly conserved over time (Anderson and Wiens 2017) and thus may help explain signal evolution at relatively deep phylogenetic scales and among large numbers of species (Chen and Wiens 2020).

Here, we test the hypothesis that diel-activity patterns help determine whether conspicuous coloration evolves as a warning signal as opposed to a sexual signal. We use a phylogenetic approach in land vertebrates (tetrapods). Tetrapods offer an excellent system in which to address these questions because they have many well-documented cases of aposematism (Maan and Cummings 2012), mimicry (Pfennig et al. 2001; Davis Rabosky et al. 2016), and sexually selected coloration (Hill 1991; Andersson 1994; Olsson et al. 2013; Dale et al. 2015; Wiens and Tuschhoff 2020). Furthermore, their ecology and phylogeny are well studied overall. We integrate data on coloration, diel activity, and phylogeny for ∼1824 species.

We primarily test whether diel activity influences the evolution of warning and sexual signals, but we also use these data to test several related, novel questions. These questions include: (i) are the same conspicuous colors associated with both warning and sexual signals, and are certain colors more frequently associated with warning signals versus sexual signals, or vice versa? (ii) Do warning signals and sexual signals evolve independently of each other, or does one tend to give rise to the other? (iii) Do both types of signals show phylogenetic conservatism, such that

Table 1. Numbers of species sampled in each clade and in each coloration category. Coloration categories include species with dimorphic (dichromatic) conspicuous coloration, warning coloration under liberal coding, warning coloration under conservative coding (cons.), and both dimorphic coloration and warning coloration. Liberal coding used broad evidence that a conspicuously colored species was potentially toxic (or a mimic), including if the species was odorous, mildly venomous, unpalatable in some instances, and had toxic congeners. Conservative coding required clear evidence that the species was unpalatable (or a mimic).

Clade	Sampled Species	Dimorphic Only	Dimorphic + Warning (Liberal)	Dimorphic + Warning (Cons.)	Warning (Liberal) Only	Warning (Cons.) Only
Amphibians	514	14	4	3	67	57
Mammals	236	3	0	0	1	1
Lepidosaurs	507	89	1	1	43	29
Turtles	16	1	0	0	0	0
Crocodilians	1	0	0	0	0	0
Birds	549	108	11	4	15	2

more closely related species will be more likely to share warning coloration versus sexual coloration? To our knowledge, these three questions (i.e., comparing sexual and warning coloration) have not been addressed in the literature, despite decades of intense interest in the (mostly) separate evolution of warning and sexual signals.

Methods

SELECTION OF SPECIES

We used an existing dataset (Anderson and Wiens 2017) to identify species with matched phylogenetic and diel-activity data. We then obtained coloration data for these 1824 species (see below). In this dataset, species were sampled in proportion to their richness among major clades (Table 1). Within major clades, species were selected to represent higher taxa (e.g., orders, families, genera), in proportion to their richness. However, representation of higher taxa and proportional sampling of species within these taxa can be in conflict (i.e., including every genus vs. including more species of larger genera). Our sampling represents a compromise between these potentially conflicting goals. Furthermore, the dataset was large enough to potentially obtain significant results, but small enough to be computationally tractable. Species were initially sampled for a study of diel activity, and their sampling should be unbiased with respect to color.

PHYLOGENETIC INFORMATION

We initially used two trees previously generated for these species (Andersson and Wiens 2017), but we also assembled phylogenies based on more recent estimates (Appendix S1). These four trees are in Datasets S1–S4. We performed analyses on all four phylogenies, but our primary tree (Tree 1) used the more recent estimates within each major group. Trees 1 and 2 contained 1823 species and Trees 3 and 4 contained 1824 species. Other alternative phylogenies are or will be available for one or more groups.

However, our comparison of trees should reveal which conclusions are sensitive to changing topologies and which are more robust. We note that a distribution of trees across all these tetrapods is not presently available. Furthermore, such a distribution would not necessarily address the sensitivity of the results as well as considering the robustness of the results to trees from entirely different phylogenetic studies.

CODING OF SPECIES FOR COLOR, DICHROMATISM, AND WARNING COLORATION

Species were initially scored as having potentially conspicuous coloration (or not) using data from the literature. However, our main focus was on whether species had warning coloration, sexually dichromatic coloration, both, or neither. Thus, the main focus was not on distinguishing conspicuous and inconspicuous colors.

We treated five focal colors that humans perceive as red (including pink), orange, yellow, blue, and violet as potentially conspicuous. We treated violet and purple as equivalent (we generally use "purple" hereafter). We excluded green (which could be cryptic given some backgrounds). These five focal colors have their own wavelength characteristics irrespective of human perception (i.e., violet = 380−430 nanometers, blue = 430−500, yellow = 565−580, orange = 580−625, red = 625−740; Malacara 2011). Conspicuousness depends on the background (Endler 1990), among other factors. However, we think that very few species live on backgrounds consisting of any of these five colors (especially among the species sampled here). Other colors could be conspicuous given certain backgrounds. For example, bright green coloration may be cryptic among live leaves but conspicuous on rocks. Similarly, black and white coloration may be aposematic or sexually selected in some taxa (Caro 2009). To be conservative, only some colors were considered potentially conspicuous across all taxa (e.g., many tetrapods have white venters, presumably as countershading, not as a warning or sexual signal). We focused here on the evolution of these five focal colors

as potential warning and/or sexual signals (not on every color that might be conspicuous against some background). Moreover, even these five colors could be inconspicuous depending on the visual system of the viewer, the lighting conditions, and other factors. Potentially inconspicuous colors included green, black, white, brown, and gray.

The color data were initially assembled for most species based on verbal descriptions of their coloration in the literature. Nevertheless, images of all species were then rechecked by one of us (JJW) for all species. We emphasize that we focused on coding dichromatism and aposematism/mimicry, and not conspicuous coloration per se. A species was considered to have a focal color if it was reported in at least some individuals (i.e., even if polymorphic) in some part of its body (i.e., from a small patch to the entire body). Even for these focal colors, there can still be a continuum between potentially conspicuous and more cryptic coloration. In species in which the focal colors were mentioned but were described as being relatively cryptic (e.g., dull yellow, red-brown), these colors were not coded as potentially conspicuous. We do not think that there is a perfect way to subdivide this continuum, and our species-level categorizations depend on dichromatism and toxicity/mimicry, not color alone. Furthermore, our main results on warning signals are robust to two methods of coding aposematic species and our main results on sexual dichromatism are robust to randomly subsampling only 10% of the species (see below). Therefore, our conclusions should be insensitive to the coding of species with marginal coloration.

Human perception (and depiction) of color can potentially bias studies of animal color (Endler 1990; Cronin et al. 2014). However, analyses in birds (Bergeron and Fuller 2018) suggest that assessments of color based on field guides and digital images are strongly correlated with those from quantitative analyses of museum specimens. Furthermore, human perception of sexual dichromatism in birds may offer a reasonable proxy for avian perception of dichromatism (Seddon et al. 2010). Of course, there are many aspects of color that our analyses will miss, including UV, far-red, and polarized light (Bergeron and Fuller 2018). Those aspects are not our focus here.

Sexually dichromatic coloration

Species were considered sexually dichromatic if these potentially conspicuous colors were reported as showing generally consistent differences of any type between the sexes (e.g., presence or absence of a conspicuous color, the size or coloration of a conspicuous color patch). If conspicuous coloration was reported only in some individuals (without regards to sex), it was not considered sexually dichromatic unless this variation was described as sex related (i.e., confined to individuals of one sex). We did not score a species as sexually dichromatic if their dichromatism was unrelated to their conspicuous coloration (e.g., dichromatism

in brown coloration). We did not discriminate as to the extent of dichromatism: a species was considered dichromatic regardless of whether a small color patch differed between the sexes or the entire body coloration differed. All color data are given in Dataset S5, along with associated references and coding of species as dichromatic or not.

For our analyses of separate colors, some species had more than one conspicuous color and at least one color was dichromatic. We coded these species in two ways. First, for our main analyses, we assigned all conspicuous colors in that species as potential sexual signals. Second, we only assigned the dichromatic conspicuous color as a potential sexual signal. These analyses gave similar results, and most of our analyses did not analyze each color separately.

The presence of sexual dichromatism does not guarantee that conspicuous colors are sexually selected. However, there are numerous precedents for the idea that many conspicuous dichromatic colors are used as sexual signals, especially in birds, lizards, and primates (Andersson 1994; Olsson et al. 2013; Dale et al. 2015; Wiens and Tuschhoff 2020).

Warning coloration

To estimate whether species had warning coloration or not, we first assigned species as being venomous, toxic, or otherwise unpalatable based on previous reviews (see below). Species that were considered mimics of aposematic species were also considered as having warning signals. Species that were not reported to be venomous, unpalatable, or mimics were coded as nontoxic. We use "toxic" as shorthand to include all venomous, toxic, and unpalatable species. We recognize that many species may be toxic but unreported as such. However, this need not strongly impact our results, given our focus on warning coloration, and not toxicity alone. We then considered species with potentially conspicuous coloration that were toxic or mimics as having warning coloration (but see additional details below).

For frogs, an extensive database (Ferreira et al. 2019) was used to assign species as toxic or having odoriferous secretions. For salamanders and caecilians, we used a review of noxious secretions (Daly et al. 1987). However, that study presented data at the genus level. For most salamander and caecilian genera, we included only one species per genus, and a species was coded as having noxious secretions if these were recorded in the genus overall.

For lepidosaurs, species were assigned as being dangerously venomous (to humans) or not. Venom that only impacts prey may be irrelevant to whether their coloration warns potential predators (which are presumably larger vertebrates). Among lizards (Vitt and Caldwell 2009; Pough et al. 2016), the only species generally considered dangerously venomous to humans are helodermatids and one varanid (*Varanus komodoensis*). Among

snakes, only some species in Colubroidea are generally considered venomous (Vitt and Caldwell 2009; Pough et al. 2016). Therefore, all non-colubroids were considered nonvenomous. For colubroids, relevant literature was reviewed for each sampled species, and species were considered dangerously venomous (i.e., potentially lethal to humans), mildly venomous (affecting humans, but not causing fatalities), or nonvenomous (having no known effects on humans).

For birds, we used an extensive review (Dumbacher and Pruett-Jones 1996) to assign species as being toxic (very few) or more broadly unpalatable or malodorous. Species not reported as being toxic, unpalatable, or malodorous were considered nontoxic.

For mammals, we used a review of venomous species (Ligabue-Braun et al. 2012). Caro (2009) reviewed aposematism in mammals, but focused on species with contrasting black-and-white coloration. Nevertheless, we also used that review to identify species with malodorous secretions (e.g., skunks) and other defenses that can make species unpalatable (e.g., spines for porcupines).

The number of toxic species is almost certainly underestimated for all groups (e.g., unpalatable mammals, squamates with noxious secretions). Nevertheless, our survey still found numerous species that were both defended and conspicuously colored, and our goal was to understand the evolution of these species. Our final assessments are given in Dataset S5.

We used both a conservative and liberal approach when coding species as having warning coloration or not. For both approaches, if a species was not considered to have potentially conspicuous coloration (i.e., red, yellow, orange, blue, purple), then it was not considered aposematic, even if toxic. For the liberal scheme, if there was any evidence found that a conspicuously colored species was toxic (or a mimic), then it was considered to have warning coloration. This included species that were odorous, mildly venomous, unpalatable in some instances (e.g., after eating berries), and when congeners were known to be toxic (but it was unknown for the species sampled). For the conservative scheme, we required clear evidence that the species being coded was unpalatable (e.g., venom, noxious secretions, quills, poison glands). Thus, conspicuously colored species that are unpalatable only sometimes (e.g., after eating berries) were not considered aposematic under this coding scheme, nor were odoriferous or mildly venomous species. For species that had more than one conspicuous color (e.g., red and orange), all conspicuous colors were considered potentially aposematic. Mimics of aposematic species were considered as having warning coloration under both coding schemes. Species were considered to have warning coloration or not, regardless of the location or extent of that coloration on the body. Coding for each species is given in Dataset S5.

STATISTICAL ANALYSES
Diel activity and color functions

To determine whether sexual signals (dichromatic conspicuous coloration) were associated with diel activity, we used two approaches. First, we conducted phylogenetic logistic regression (Ives and Garland 2010) using the R package *phylolm* version 2.6.2 (Ho and Ané 2014). This was our primary approach, given that the association between states is straightforward to interpret. Phylogenetic logistic regression tests whether the transitions to a given state in one variable are dependent on a particular state of another character. We also conducted analyses of correlated evolution using Pagel's (1994) likelihood test with the R package *phytools* version 0.7-70 (Revell 2012). This test addresses whether transitions in one character are related to changes in another character (but without addressing the association between particular states of each character). Both methods incorporate phylogeny and allow for both the dependent and independent variables to be binary.

For logistic regression, diel activity (diurnal = 0, nocturnal = 1) was the independent variable and dichromatism (absence = 0, presence = 1) was the dependent variable. Thus, a negative coefficient indicated that dichromatism is associated with diurnal activity, whereas a positive coefficient indicated that dichromatism is associated with nocturnality.

Most sampled tetrapod species (Table 2) were either nocturnal (primarily active at night) or diurnal (primarily active by day), whereas far fewer species were crepuscular (active at dawn and/or dusk) or arrhythmic (active by both day and night). Furthermore, the tests used here generally required that each character have only two states. Therefore, we either treated all crepuscular and arrhythmic species as diurnal (maximum-diurnal coding) or nocturnal (maximum nocturnal).

For the correlation test (Pagel 1994), we compared models in which sexual dichromatism is dependent upon diel activity to models in which both traits are independent of one another. Differences in model fit were assessed using a likelihood-ratio test. We also compared models in which diel activity is dependent upon dichromatism and when both traits are co-dependent upon one another.

Before conducting these correlation tests, we identified the best-fitting model of evolution for each character (Table S1). We compared the equal rates (ER) model (equal rate for 0-to-1 and 1-to-0 transitions), to the all-rates-different (ARD) model (different rates for each transition). We used the "fitDiscrete" function in *geiger* version 2.0.7 (Pennell et al. 2014) to obtain the Akaike information criterion (AIC; Akaikae 1974) for each model. ARD had the best fit (lowest AIC) for coloration variables, but ER often had the best fit for diel-activity variables. Therefore, we conducted all correlation analyses using both ARD and ER models.

Table 2. Distribution of diel-activity patterns among sampled tetrapod species within each major clade and their association with species with different color functions. Liberal coding used broad evidence that a conspicuously colored species was potentially toxic (or a mimic), including if the species was odorous, mildly venomous, unpalatable in some instances, and had toxic congeners. Conservative coding required clear evidence that the species was unpalatable (or a mimic).

Clade	Overall Diel Activity	Dichromatic	Warning (Liberal)	Warning (Conservative)
Amphibians	11.7% diurnal 8.0% arrhythmic 0.6% crepuscular 79.8% nocturnal	11.1% arrhythmic 88.9% nocturnal	33.8% diurnal 8.4% arrhythmic 57.8% nocturnal	38.3% diurnal 6.7% arrhythmic 55.0% nocturnal
Mammals	17.8% diurnal 11.4% arrhythmic 3.0% crepuscular 67.8% nocturnal	33.3% diurnal 66.7% nocturnal	100% nocturnal	100% nocturnal
Lepidosaurs	58.2% diurnal 6.7% arrhythmic 1.6% crepuscular 33.5% nocturnal	96.7% diurnal 3.3% nocturnal	25.0% diurnal 18.2% arrhythmic 56.8% nocturnal	20.0% diurnal 23.3% arrhythmic 56.7% nocturnal
Turtles	37.5% diurnal 25.0% arrhythmic 12.5% crepuscular 25% nocturnal	100% nocturnal		
Crocodilians	100% nocturnal			
Aves	90.0% diurnal 2.0% arrhythmic 1.1% crepuscular 6.9% nocturnal	100% diurnal	96.2% diurnal 3.8% crepuscular	100% diurnal

To determine whether warning coloration was associated with diel activity, we used the same two statistical approaches described above. For these logistic regressions, diel activity (diurnal = 0, nocturnal = 1) was the independent variable and warning coloration (absence = 0, presence = 1) was the dependent variable. Thus, a positive association would indicate that warning coloration is associated with nocturnality.

We also tested whether warning coloration and dichromatism were associated with ancestral diel activity. We conducted logistic regression and likelihood correlation tests as described above for current diel activity. We considered major clades to be those with stem ages >100 million years (Myr) old. Species within each major clade were then coded as ancestrally nocturnal or ancestrally diurnal based on likelihood reconstructions (Fig. 1 of Anderson and Wiens 2017). Thus, we coded all amphibians, mammals, crocodilians, and turtles as ancestrally nocturnal, and three major clades within Lepidosauria (Serpentes, Rhynchocephalia, and Gekkota). All other species were considered to belong to ancestrally diurnal clades (i.e., birds and other lepidosaurs). Turtles were reconstructed as nocturnal under maximum-nocturnal coding but ambiguous under maximum-diurnal coding (Anderson and Wiens 2017): these were treated as nocturnal here.

We acknowledge that 100 Myr is an arbitrary cutoff. However, an older cutoff (e.g., 200 Myr) would make almost every clade ancestrally nocturnal, and therefore uninformative. Similarly, a much younger cutoff would cause the coding of individual species to converge on those based on present-day activity, making this analysis redundant. An intermediate value (e.g., 150 Myr) would make birds ancestrally nocturnal, snakes ancestrally diurnal, and otherwise have no impact (relative to the 100 Myr cutoff). Note that our goal here was to determine whether ancestral diel activity could help explain color evolution (not to find a cutoff that would yield negative or redundant results). Importantly, using the 100 Myr cutoff, the ancestral state of each major clade corresponds to the most common state among its species (except for turtles, where different states are at similar frequencies; Table 2).

Associations between functions and specific colors

Some colors might only be used for specific functions. For example, blue coloration might (hypothetically) be used only as a sexual signal and not as a warning signal. Therefore, we also tested whether each color (individually) was associated with sexual and/or warning signaling using phylogenetic logistic regression. Each color was the dependent variable and signaling

function was the independent variable. This might seem tautological: we are testing whether specific conspicuous colors are associated with warning coloration and sexual dichromatism, which are defined based on these same conspicuous colors. However, if blue coloration is only associated with sexual signals and not warning signals (for example), then there should be no association between blue coloration and warning signals. We conducted these tests prior to testing associations between diel activity and warning and sexual signals, to address whether it was appropriate to analyze all conspicuous colors together for each function, or only some.

We also calculated the frequency with which each color was used for each function. We counted the number of species that potentially use a specific color (e.g., red) for a given function (e.g., warning signal). We then divided this number by the total number of occurrences of all five focal colors for the same function among species. We used this latter number, not the overall number of species with that function, because some species had more than one color (e.g., an aposematic species with both red and yellow). This allowed the proportions for all five colors used for a given function to sum to one. We then tested whether proportions for each color differed significantly between functions. We used Pearson's Chi-squared analyses (in R), unless these were problematic due to small sample sizes ($n < 5$ species for a category). In these cases, Fisher's exact tests were conducted instead. We acknowledge that this test does not include a phylogenetic correction, but such a correction would not be straightforward given that many species each have multiple conspicuous colors.

Relationships between warning and sexual signals

We also tested whether warning coloration and dichromatism were associated with one another, whether one gives rise to the other, and whether these traits show phylogenetic signal or are randomly distributed. We tested whether warning coloration and dichromatism were associated with one another using logistic regression and the likelihood correlation test, as described above.

To analyze transition rates between states, each species was assigned as having either a warning signal (liberal coding scheme), a sexual signal, both signals, or neither signal. We give details of these analyses in Appendix S2 and Tables S2–S6.

Phylogenetic signal in warning and sexual coloration

To test whether warning coloration and dichromatism showed phylogenetic signal, we first estimated Pagel's (1999) λ using the fitDiscrete function in *geiger* (Pennell et al. 2014). Values closer to 1 indicate strong phylogenetic signal, whereas λ values closer to 0 indicate weak phylogenetic signal. We also assessed the *D*-statistic (Fritz and Purvis 2010) using the function "phylo.d" in the R package *caper* version 1.0.1 (Orme et al. 2013). *D*-statistics

values closer to 0 indicate that the trait is highly conserved, whereas values closer to 1 indicate that the trait is not.

Effects of taxon sampling

Finally, we tested whether our main conclusions were robust to incomplete taxon sampling. The sampled 1823 species represent only a tiny fraction of all ~37,000 tetrapod species. To investigate the degree to which incomplete sampling may have influenced our results, we subsampled 10% of the species in the primary tree 10 times (182 species), yielding 10 new trees and 10 corresponding datasets (following Moreira et al. 2021). We then reran our main analyses on each tree. Subsampling was conducted by randomly dropping 1641 tips from the tree with 1823 species (1823 − 1641 = 182) using the drop.tip() function in the R package *ape* (version 5.4; Paradis and Schliep 2019). The subsampled trees are given in Dataset S6 and the subsampled datasets are given in Dataset S7. The prediction from simulations (Ackerly 2000) and similar subsampling analyses (Moreira et al. 2021) is that limited taxon sampling may reduce statistical power (e.g., yielding fewer significant results), but should not generate significant, positively misleading results (e.g., significant results that contradict those based on more complete taxon sampling). R code for these and all other statistical analyses are given in Dataset S8.

Results

Using phylogenetic logistic regression, we found that all potentially conspicuous colors considered here are each significantly associated with both the presence of sexually dichromatic coloration and with warning coloration (Table S7). Therefore, we generally treated these colors collectively (i.e., presence of any conspicuous color) rather than individually (e.g., presence of red). We also found that these colors are generally used in similar proportions among species for each function (with yellow, orange, and red used most frequently), across tetrapods and within major groups (Fig. 2; Tables S8 and S9). The major exception is blue coloration, which is more frequently used as a sexual signal than a warning signal, both across tetrapods and in lepidosaurs (Fig. 2).

Conspicuous dichromatic coloration and warning coloration are both relatively widespread across tetrapods, but only rarely occurred together (4.5% or 2.5% of all sampled species with dichromatic and/or warning coloration, depending on how warning coloration is coded; Figs. 3, S1; Table 1). In amphibians, conspicuous warning coloration is widespread and dichromatic coloration is uncommon. In lepidosaurs, dichromatic coloration is widespread in lizards and warning coloration is relatively frequent in snakes (Fig. 3). Both dichromatic and warning colorations are uncommon and/or absent in mammals, turtles, and
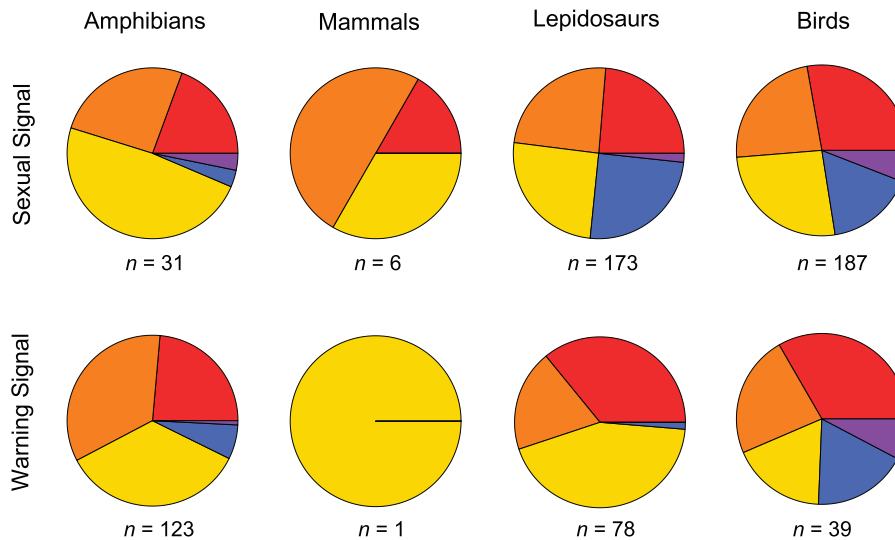
Figure 2. Estimated distribution of potentially conspicuous dichromatic and warning colors among major tetrapod clades. Pie charts show the proportion of specific colors (red, orange, yellow, blue, and purple) that are used as warning signals and sexual signals in each group. For a given function (sexual vs. warning signal), the number of species with a given color is divided by the total number of occurrences of all five colors among species with that function. The number below each pie chart is the total number of occurrences of all five focal colors in that group. This number allowed the proportions for all five colors used to sum to one because some species had multiple conspicuous colors (e.g., red and yellow warning signal). In this figure, species were coded for warning signals based on the liberal coding scheme.

crocodilians. In birds, dichromatic coloration is widespread and warning coloration is uncommon (Fig. 3).

Phylogenetic analyses confirmed that dichromatic coloration and warning coloration were not consistently associated with one another. They were only significantly related using some trees and coding schemes (for warning coloration and diel activity), for both logistic regression (Table S10) and correlation tests (Tables S11–S14). Moreover, phylogenetic analyses revealed that transitions only rarely occurred directly between warning coloration and dichromatism (Appendix S2).

The phylogeny (Figs. 3, S1) showed the repeated evolution of warning coloration among amphibians and snakes, and the repeated evolution of dichromatic coloration among birds and lizards. Yet, both dichromatic and warning colorations had relatively strong phylogenetic signal. Using Pagel's (1999) λ (Table S15), values for dichromatism ranged from 0.796 to 0.828 across trees, and warning coloration ranged from 0.813 to 0.891 (across trees and coding schemes). Moreover, the D-statistic (Fritz and Purvis 2010) was always significant (Table S16). Thus, in addition to repeated origins of each of these two types of signals, there was also sharing of signals of the same type among closely related species.

The distribution of diel activity (Table 2) showed that amphibians, mammals, and crocodilians are predominantly nocturnal, whereas most lepidosaurs and birds are diurnal. Previous analyses (Anderson and Wiens 2017) showed that amphibians, mammals, and crocodilians are ancestrally nocturnal, birds are

ancestrally diurnal, and within lepidosaurs most major lizard clades are ancestrally diurnal (except Gekkota), whereas snakes are ancestrally nocturnal. We found here that dichromatic amphibians are predominantly nocturnal as are those with warning coloration. However, among amphibians with warning coloration, the frequency of diurnal species is roughly three times higher than the overall frequency of diurnal amphibians (Table 2). In mammals, the frequency of dichromatic species that are diurnal is roughly twice as high as the overall frequency of diurnal species, and the single sampled species with warning coloration is nocturnal (Table 2). In lepidosaurs, almost all dichromatic species are diurnal lizards and most species with warning coloration are nocturnal snakes. In birds, all dichromatic species are diurnal as are most species with warning coloration (Table 2).

Based on phylogenetic logistic regression, potentially conspicuous dichromatic coloration was significantly associated with diel activity ($P < 0.001$; Table S17). Specifically, diurnal species were more likely to evolve dichromatic coloration, as were species from ancestrally diurnal clades (Fig. 4). This pattern was consistent across alternative trees, diel-coding methods (Table S17), and using correlation analyses (Tables S18–S21). Moreover, this association largely holds for all five focal colors independently, with some exceptions (Fig. 4; Table S22).

The association between warning coloration and diel activity was more nuanced, as predicted (Fig. 4). Using conservative coding, we found no association between warning coloration and current diel activity with logistic regression ($z = 0.516$,
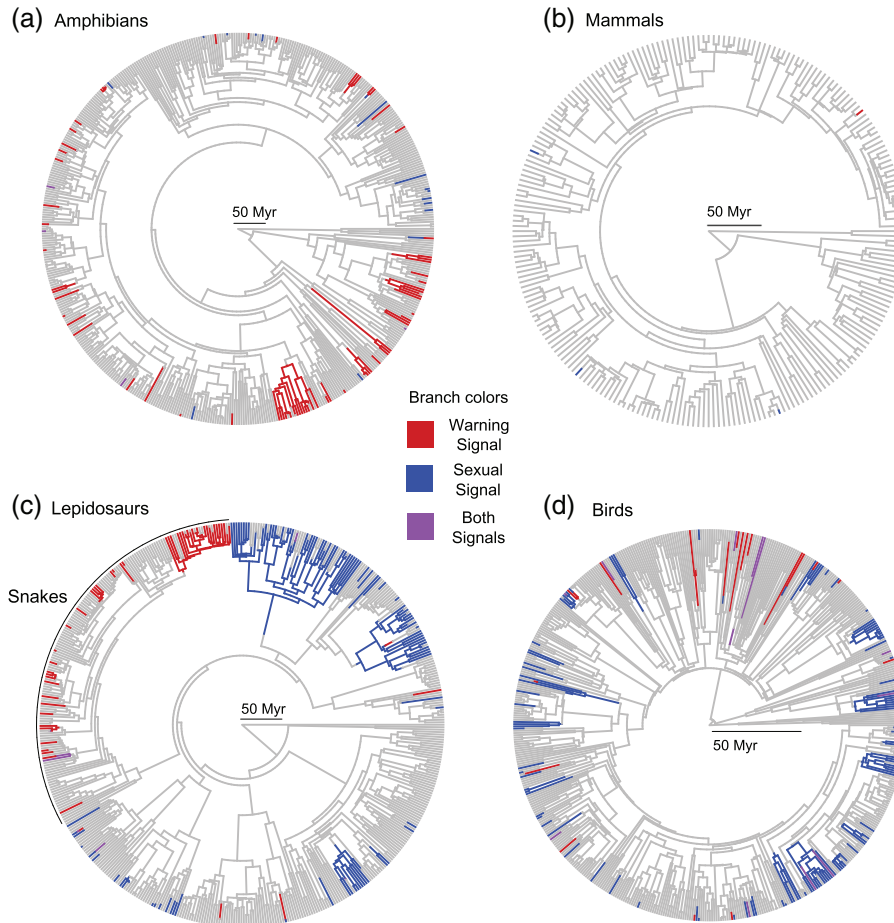
Figure 3. Evolution of warning and sexual signals in the major groups of land vertebrates. Results are shown separately for (a) amphibians, (b) mammals, (c) lepidosaurs, and (d) birds. The entire tree is shown in Figure S1. Gray color on trees indicates that neither warning or sexual signals are present (involving the five focal potentially conspicuous colors). The evolution of traits among species is visualized using stochastic character simulations (assuming the equal rates model), conducted using corHMM with the "makeSimmap" function (Appendix S2). All phylogenies are time calibrated and the scale bar in the middle of each circular phylogeny depicts 50 million years (scales differ between groups). For this figure, species are coded for warning signals based on the liberal coding scheme (see *Methods*). The tree shown is the primary tree used (Tree 1).

$P = 0.606$; Table S17). This pattern was consistent across alternative phylogenies and diel-coding schemes (Table S17). Using liberal coding for warning coloration, there was a significant association between warning coloration and diurnal activity on the primary tree ($z = -2.815$, $P = 0.005$), but not on some alternative trees (Table S17). Correlation analyses were also inconsistent: there was no significant support for models in which warning coloration depended on diel activity, using liberal coding for warning coloration (Tables S23 and S24), but there was using conservative coding (Tables S25 and S26). Overall, current diurnal activity appeared to either have no impact on the evolution of warning coloration or else appeared to promote its evolution.

In contrast, phylogenetic logistic regression consistently showed a significant association between ancestrally nocturnal clades and warning coloration (Fig. 4; Table S17). Correlation analyses also showed that the evolution of warning coloration

was dependent on ancestral diel activity, for both liberal coding (Tables S27 and S28) and conservative coding (Tables S29 and S30). This pattern appeared to be driven by three colors in particular (red, orange, and yellow; Fig. 4; Table S31).

We also investigated the sensitivity of our main results to limited taxon sampling. We randomly sampled 10% of the species in the primary tree in 10 replicates, and repeated the main phylogenetic regression analyses relating diel activity and the evolution of sexual and warning signals (Table S32). We found that sexual dichromatism was associated with diurnal activity across all replicates and coding methods (maximum diurnal, maximum nocturnal, and ancestral), and was significant ($P < 0.05$) in 97% of these analyses (despite the extreme reduction in statistical power). As found for the trees with full sampling, the association between warning coloration and current diel activity was variable among trees and coding methods.
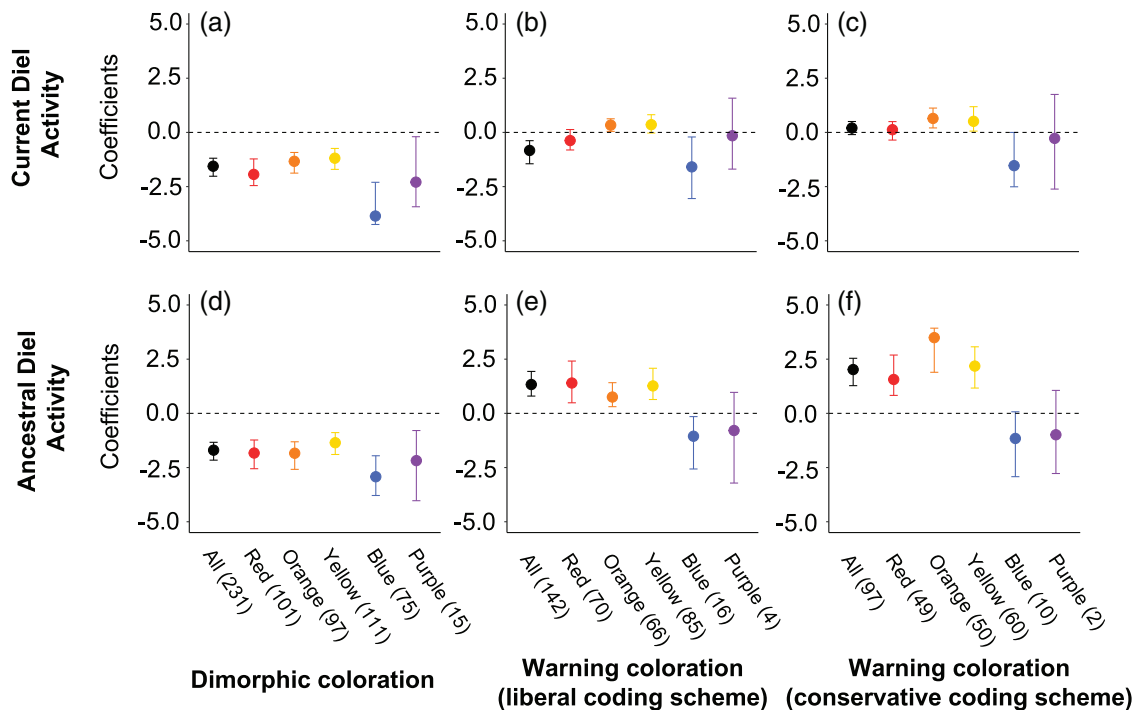
Figure 4. Relationships between diel activity patterns and conspicuous colors used as sexual and warning signals in land vertebrates. The *y*-axis is the estimated coefficient from phylogenetic logistic regression, whereas the *x*-axis is either all five potentially conspicuous colors combined (All) or the specific conspicuous color (including the number of species with that combination of color and function in parentheses). Subfigures a–c show regression coefficients (colored datapoints) when using a species' current diel activity, whereas d–f show results for ancestral diel activity. For each analysis, the color's function (sexual, warning) was the dependent variable and diel activity was the independent variable. Each datapoint includes 95% confidence intervals associated with the estimated coefficient from 100 bootstrap replicates. Intervals that exclude 0 and are below the dashed line generally indicate a significant association between that color and diurnal activity (see Tables S22 and S31). Intervals that exclude 0 and are above the dashed line generally indicate a significant association with nocturnality. Thus, sexually dimorphic coloration is associated with species that are currently (a) and ancestrally (d) diurnal. This association holds for all five colors individually, and all colors combined (all). Warning coloration overall is associated with ancestral nocturnal activity, as are the most common warning colors (red, orange, yellow). Warning coloration is also associated with current diurnal activity, but this is more variable among colors, methods, and trees. Note that a species could have more than one potentially conspicuous color (e.g., red and orange). Results shown here are for the primary tree and using maximum diurnal coding for current diel activity.

However, the evolution of warning coloration was positively associated with ancestral nocturnal activity across nearly all replicates and coding schemes (95%), as with full taxon sampling (although this pattern was not statistically significant in the subsampled trees, as expected given reduced statistical power). Across all these analyses, we found no cases in which the results from the subsampling analyses were both statistically significant and in conflict with those from full sampling.

Discussion

Animals frequently evolve coloration that is potentially conspicuous (Figs. 1, 3). Depending on the species, these colors are typically used as warning signals or sexual signals. But why a given lineage evolves this conspicuous coloration in association with one function or the other has been unresolved and largely unexplored. Here, we tested the hypothesis that current and past diel activity can help explain these patterns. We found that sexual dichromatism is significantly associated with diurnal activity, clearly showing that current day-night activity can explain at least half of this pattern (i.e., evolution of sexual signals). The association between warning signals and current diel activity was more complex: diurnal activity appeared to either promote warning coloration or have no impact on its evolution. However, warning coloration was never significantly associated with (current) nocturnal activity. Yet, there was a significant tendency for warning signals to evolve in clades that were ancestrally nocturnal, such as amphibians and snakes. Overall, we found that the evolution of warning signals and sexual signals in land vertebrates is related to diel-activity patterns, both in the recent and distant past.

These results suggest that ecology (signaling environment) helps drive large-scale patterns of signal evolution and function, across thousands of species and hundreds of millions of years. Diurnal activity likely facilitates the visualization of conspicuous colors, allowing potential mates and rivals to assess subtle differences in coloration that may indicate quality. Conversely, low-light environments (i.e., nocturnal activity) may make discriminating these colors more difficult. Previous studies in nontetrapod species support this idea. For example, experimental manipulations have shown that red coloration in jumping spiders only has a role in courtship when males signal in direct sunlight (Taylor and McGraw 2013). Moreover, in three-spined sticklebacks, water clarity (which affects lighting environment) influences a female's ability to accurately assess male quality from their conspicuous red coloration (Wong et al. 2007). Given that the lighting environment can decrease the effectiveness of visual signals, it may promote the evolution of other types of sexual signals instead (e.g., acoustic; Chen and Wiens 2020). For example, most frogs are nocturnal (Anderson and Wiens 2017) and often use acoustic signals (Pough et al. 2016; Chen and Wiens 2020). Most salamanders and snakes are also nocturnal (Anderson and Wiens 2017), and both often use chemical signals (Pough et al. 2016). Thus, low-light environments may favor the evolution of sexual signals unrelated to conspicuous colors. When conspicuous coloration is not generally used for sexual signaling in a clade (as in amphibians and snakes), it can instead evolve as a warning to potential predators. Furthermore, warning coloration might still be beneficial in nondiurnal species under some circumstances (e.g., if individuals are found by predators when sleeping during the day). In summary, our results suggest that ecology helps explain whether signals are used to communicate with potential mates or with potential predators, and not just the evolution of different sexual signals (Endler and Basolo 1998).

We recognize that many other factors may also help explain these patterns. For example, what if the evolution of warning coloration is instead primarily explained by which clades most frequently produce toxins and which do not? Based on our surveys (Dataset S5), defensive skin toxins are relatively widespread in amphibians (22.8% of 514 amphibian species sampled here), and venoms (used for prey capture and defense) are widespread in advanced (colubroid) snakes (44.4% of all 162 sampled snake species). By contrast, functional toxins appear less widespread in birds (maximum of 7.6%), mammals (1.7%), and lizards (0.3% of 345 sampled species). But because toxins have evolved in all three groups, it is clearly not impossible. Furthermore, the usage of conspicuous coloration as a sexual signal might reduce the likelihood that warning coloration and toxins will evolve. We found relatively few transitions from sexual dichromatism to warning coloration (Figs. 3, S1; Appendix S2). Another possible explanation for these patterns is that only some groups can evolve sexual dichromatism. However, sexual dichromatism does occur in amphibians (Bell and Zamudio 2012; Portik et al. 2019), some snakes (Shine and Madsen 1994), and at least some mammals (e.g., primates; Bradley and Mundy 2008). Therefore, there are not clade-wide constraints against evolving dichromatism in these groups. Variation in mechanisms of color production among groups might also be important. For example, if a conspicuous color is diet and condition dependent, it can potentially function as an honest sexual signal of male quality, as in birds (Hill et al. 2002). In contrast, the production of conspicuous colors by all individuals (regardless of condition, diet, sexual maturity, or other factors) may be crucial for antipredator defense. Yet, the same color may have the same function despite different production mechanisms (e.g., red as a sexual signal produced by diet-derived carotenoids in many birds [Hill et al. 2002] versus endogenously from pteridines in lepidosaurs [Olsson et al. 2013]). Kikuchi et al. (2021) recently reviewed numerous other factors that may influence the evolution of warning signals, including predator and prey abundances and predator sensory ecology. Finally, even if diel activity is a major driver of whether sexual or warning signals evolve most frequently in a given large-scale clade (as we suggest here), these other factors may be important for explaining the specific lineages in which these signals evolved.

We also acknowledge several caveats about our results. First, simply documenting that a species has conspicuous coloration is not necessarily straightforward. Importantly, we did not consider a species to have a sexual signal without evidence of dichromatism, nor a warning signal without evidence of unpalatability (or mimicry). On the other hand, we may have excluded sexual signals that were not dimorphic and warning signals in which relevant defenses are not yet documented. This pattern should not be problematic if these missed sexual signals are predominantly in lizards and birds and the missed warning signals are mostly in amphibians and snakes. Furthermore, in any given species, sexual dichromatism does not guarantee that potentially conspicuous coloration is sexually selected, nor does toxicity guarantee that predators treat conspicuous coloration as a warning signal. Similarly, we likely missed some sexual and warning signals associated with black and white coloration (see *Methods*). Nevertheless, our sampling captured many well-known instances of aposematism, mimicry, and sexual dichromatism across tetrapods, including poison frogs (Maan and Cummings 2012), coral and kingsnakes (Pfennig et al. 2001; Davis Rabosky et al. 2016), and conspicuously colored birds and lizards (Hill et al. 2002; Olsson et al. 2013; Dale et al. 2015). We also acknowledge that warning coloration may by somewhat cryptic under some conditions (e.g., flicker-fusion effect: Titcomb et al. 2014; distance effect: Barnett and Cuthill 2014) and that warning signals need not involve conspicuous coloration (Valkonen et al. 2011). Lastly,

our conclusions might only apply to tetrapods. These patterns should clearly be tested in other groups.

Our results also provide insights into another unresolved question in evolutionary biology: what explains the diversity of sexually selected traits among animal species? For example, why do some lineages have conspicuous colors, whereas others have acoustic signals, and others enlarged horns? One potential explanation (among many) is that different types of signals are associated with different diel-activity patterns (Wiens and Tuschhoff 2020). We show here that the evolution of sexually selected colors is significantly associated with diurnal activity. Conversely, a recent study in tetrapods (Chen and Wiens 2020) used similar data and methods and found that origins of acoustic communication were associated with nocturnal activity (but was retained in some lineages that became diurnal, such as dendrobatid frogs and birds). Taken together, these two sets of results illustrate how different diel-activity patterns contribute to the observed diversity of sexually selected traits among species.

Conclusion

In summary, in this study, we attempt to understand why some species evolve potentially conspicuous colors as warning signals, whereas other species evolve these colors as sexual signals. Using land vertebrates as a model system, we show that similar palettes of conspicuous colors are used for each function. We find that current diurnal activity is associated with the evolution of sexual signals and sometimes with warning signals. The crucial difference is that warning signals tend to evolve in clades that are ancestrally nocturnal, whereas sexual signals tend to evolve in clades that are ancestrally diurnal. Thus, we find that a single ecological factor (diel activity) helps explain the function and evolution of conspicuous coloration across land vertebrates, even among clades with very different physiologies (e.g., endotherms vs. ectotherms) and different mechanisms underlying color production (e.g., exogenous vs. endogenous sources of pigments). These results also suggest that the signaling environment is crucial to understanding the evolution of color, but examining only the current signaling environment of species may be insufficient (even in a phylogenetic context). Our study represents an initial step toward explaining why conspicuous colors evolve as warning signals versus sexual signals in different lineages. We hope these findings will stimulate further empirical and theoretical research on this and related questions.

AUTHOR CONTRIBUTIONS
ZE and JJW designed the study, collected data, and wrote this article. ZE performed analyses.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

DATA ARCHIVING
All data and R code (Datasets S1–S8) are available on Dryad (https://doi.org/10.5061/dryad.7pvmcvdwx).

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendices S1–S3 (single combined file)
Figure S1 (single combined file)
Tables S1–S32 (single combined file)
Datasets S1–S8 (separate files)