bioRxiv preprint doi: https://doi.org/10.1101/2023.01.26.525769; this version posted January 27, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Multimodal models of animal sex: breaking binaries leads to a better understanding of ecology and evolution

J. F. McLaughlin*, Kinsey M. Brock*[†], Isabella Gates[‡], Anisha Pethkar[‡], Marcus Piattoni[‡], Alexis Rossi[‡], Sara E. Lipshutz[‡]

The first two authors contributed equally to this work.

*Department of Environmental Science, Policy, and Management, College of Natural Resources, University of California, Berkeley, CA, USA.

[†]Museum of Vertebrate Zoology, University of California, Berkeley, CA, USA

[‡]Department of Biology, Loyola University Chicago, IL, USA

Keywords: sexual selection, polymorphism, biological sex, terminology, reproduction, sexual phenotype

Abstract

'Sex' is often used to describe a suite of phenotypic and genotypic traits of an organism related to reproduction. However, not all of these traits – gamete type, chromosomal inheritance, physiology, morphology, behavior, etc. – are necessarily linked, and the rhetorical collapse of variation into a single term elides much of the complexity inherent in reproductive phenotypes. We argue that consideration of 'sex' as a constructed category operating at multiple biological levels opens up new avenues for inquiry in our study of biological variation. We apply this framework to three case studies that illustrate the diversity of sex variation, from decoupling sexual phenotypes to the evolutionary and ecological consequences of intrasexual polymorphisms. We argue that instead of assuming binary or bimodal sex in these systems, some may be better categorized as multimodal. Finally, we conduct a meta-analysis of terms used to describe diversity in sexual phenotypes in the scientific literature to highlight how a more inclusive and expansive framework for multimodal sex can clarify, rather than cloud, studies of sexual diversity within and across species. We argue that such an expanded understanding of 'sex' better equips us to understand evolutionary processes on their own terms, and that as biologists it is incumbent upon us to push back against misunderstandings of the biology of sexual phenotypes that enact harm on marginalized communities.

Introduction: Sex is not a single trait

In sexually reproducing species, the common assumption is that there are two sexes, strictly classified as female or male (Fisher 1930; Hurst 1996; Jaffe 1996). This assertion is supported by the cellular mechanisms of sexual reproduction, in which multiple (usually two) parental

bioRxiv preprint doi: https://doi.org/10.1101/2023.01.26.525769; this version posted January 27, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

gametes combine genotypes to create an offspring with a novel genetic makeup. In animals, these gametes are of different sizes (e.g., large ova and small sperm), a condition called anisogamy. However, the binary classification of gametic sex breaks down when we consider the broader diversity of gametic phenotypes. For instance, hermaphroditic species possess both gamete types required for reproduction, and do not have separate sexes (Jarne and Auld 2006). Of the estimated 1.2 million animal species, roughly 5-6% are hermaphroditic (Jarne and Auld 2006). Outside of animals, systems of sex determination rely on genetic markers that determine compatibility between equally-sized gametes, a condition known as isogamy (Togashi and Cox 2011) found in fungi (Kothe 1996; Lee et al. 2010; Billiard et al. 2011, 2012), algae (Perrin 2012; Tillmann and Hoppenrath 2013), and amoebozoa (Douglas et al. 2016). Another problem lies in the limitations of the gametic sex definition, summarized by Joan Roughgarden in (2013): “the biggest error in biology today is uncritically assuming that the gamete size binary implies a corresponding binary in body type, behavior, and life history.”

Sex is a reproductive process, and it is also a categorical term. Operationally, the term 'sex' is used to encompass a broad collection of gametic, genetic, hormonal, anatomic, and behavioral traits (Gross 1996; Engqvist and Taborsky 2016; Mank 2022)(Whitfield 2004); (Gross 1996; Engqvist and Taborsky 2016; Mank 2022). 'Sex' is often semantically flattened into a binary model, for which individuals are classified as either 'female' or 'male' (Figure 1A). A more expansive definition of sex is bimodal - with most individuals falling within one of two peaks of a trait distribution (Figure 1B). However, even a bimodal model is an oversimplification, since 'sex' comprises multiple traits, with variable distributions (see Figure 1C and case studies). Individuals may possess different combinations of chromosome type, gamete size, hormone level, morphology, and social roles, which do not always align in female- and male-specific ways or persist across an organism’s lifespan (Karkazis 2019; Griffiths 2021). Reliance on strict binary categories of sex fails to accurately capture the diverse and nuanced nature of sex.

Here we propose a model of sex as multivariate, encompassing multiple independent traits, and multimodal, where any one of these traits may have a distribution outside the binary. Together, the distributions of all traits comprise the sexual phenotype of an individual within a species. We lay out the shortcomings of assuming and analyzing sex as binary across genetic, endocrine, morphological, and behavioral levels of organization, and we propose a multimodal model of animal sex (Figure 1C). We then provide three case studies that illustrate the diversity of sex variation: decoupling sexual phenotypes in 'sex-role reversed' birds, the evolutionary consequences of more than two sexes within a taxon, and the ecological consequences of intrasexual color polymorphism in lizards. We argue that rethinking the default assumption of binary sex can help us better understand the evolutionary worlds these animals operate in. Finally, we offer recommendations for future research and education on sex diversity.

We are certainly not the first, nor the last biologists to interrogate the definition of sex (Hoekstra 1990; Roughgarden 2013; Karkazis 2019). We find it useful to clarify that our focus is primarily on animals. We rely on literature mainly pertaining to organismal biology, spanning the fields of molecular genetics, neuroendocrinology, behavior, ecology, and evolution, and also

bioRxiv preprint doi: https://doi.org/10.1101/2023.01.26.525769; this version posted January 27, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

differentiate between the concepts of sex and gender (Goymann and Brumm 2018). Though the term ‘gender’ has occasionally been used to describe sexual phenotypes (Roughgarden 2013; Kutschera 2016), this term describes multifaceted and abstract self-interpretations of identity, an internal personal experience that expresses itself in the context of an individual’s specific culture, place, and time (Thorne et al. 2019). We validate the important role of gender in the human experience, especially in regard to transgender, nonbinary, and gender nonconforming (TNGC) identities. However, in studies of non-human animals it is key to differentiate between the specifically cultural role of gender and the observed traits of other organisms that we broadly call 'sex' (Goymann and Brumm 2018). Thus, throughout this paper we focus solely on sex, as anything similar to the human experience of gender in other organisms is fundamentally inaccessible to us.

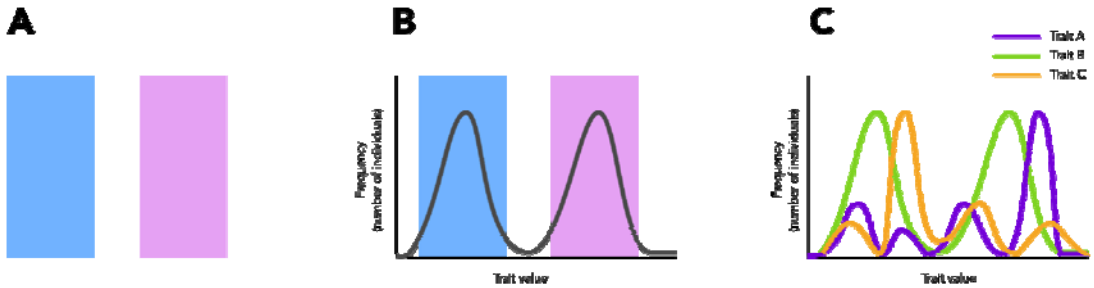

Figure 1: Three models of the distribution of reproductive trait phenotypes, demonstrated by distribution across a hypothetical population: A) A strict binary, for which all individuals are unambiguously grouped into one of two categories. Whereas some traits, like gamete size, operate this way, a binary does not accurately model the distribution of most phenotypic traits. B) A bimodal model, with most individuals falling around two peaks, or means, of a single sex trait with continuous trait variation, which align with the categorization of male or female. Depending on where the category boundaries are drawn, some individuals may fit both or neither. C) A multimodal model of sex as a collection of traits, represented as individual lines, that contribute to the overall sex phenotype. Each trait has its own independent distribution, which may or may not be bimodal, or coincide with other traits. In the example shown, two categories might conceivably be delineated, but doing so flattens trait variation within the system.

Sex at the genetic level

The genetics of sexual determination vary widely (Bachtrog et al. 2014) (Figure 2). In the simple case of highly specialized, non-recombining sex chromosomes working in isolation, biologists classify individuals as homogametic (i.e., have two of the same sex chromosome type) or heterogametic (having two distinct chromosome types), with sexual phenotypes following from those genotypes. However, even these 'simple' systems include intermediate (intersex) phenotypes (Vaiman and Pailhoux 2000). The most widely familiar systems are XX/XY, where the presence of certain sex-determining genes, such as *sry* on the Y chromosome in mammals,

bioRxiv preprint doi: https://doi.org/10.1101/2023.01.26.525769; this version posted January 27, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

activates diverging developmental pathways early in the developmental process (Bachtrog et al. 2014; Stévant et al. 2018) (Figure 2a). ZZ/ZW systems operate similarly, but with the ova-producing individuals being heterogametic (Bachtrog et al. 2014) (Figure 2b). In some taxa, dose-dependent effects of homogametic chromosomes lead to higher expression of genes that start a particular development pathway (Smith et al. 2009). Dose-dependent effects also underlie the haplodiploid systems of many insects, where unfertilized and fertilized ova develop into different social and reproductive classes (Beye et al. 2003; Verhulst et al. 2010) (Figure 2c). Some combination of sex-determining genes and dosage dependence may be at play in species for which the smaller sex chromosome and most of its associated genes have been lost entirely (Sutou et al. 2001; Just et al. 2002; Kuroiwa et al. 2010). In polygenic systems, multiple loci on several chromosomes, both sex-linked and autosomal, interact to determine sexual phenotypes. Polymorphisms in the sex chromosomes can override sex-determining genes (Figure 2D) (Veyrunes et al. 2010; Saunders et al. 2014; Zhao et al. 2017) or lead to multiple reproductive morphs (Sandkam et al. 2021). In several species of cichlid, sex is determined by the interplay of both ZZ/ZW and XX/XY chromosomes, resulting in 4 genotypes but 2 phenotypes (Ser et al. 2010; Moore et al. 2022) (Figure 2E). Not all sexual differentiation is controlled by genetic mechanisms, in part or whole (Bachtrog et al. 2014). Temperature-dependent sex determination is common among reptiles (Figure 2F) (Sarre et al. 2004) and fish (Baroiller et al. 2009). In its simplest form, the temperature of incubation determines which hormonal pathways are triggered in a developing egg (Sarre et al. 2004). In other systems, temperature can override genetic pathways, resulting in animals with the genetic markers usually associated with one sex, but the phenotypic traits of another (Quinn et al. 2009; Holleley et al. 2015; Whiteley et al. 2017; Wiggins et al. 2020). Other environmental variables may likewise interact with genetic sex determination, possibly through epigenetic factors (Capel 2017; Piferrer 2021), but these last mechanisms are only just beginning to be explored. Altogether, evidence from these diverse species reveals that genetic sex is not the universal binary often assumed, and that genetic sex is one variable among the many traits that comprise multimodal sex.

How genetic material is transmitted to the next generation is even more variable, once an assumption of sexual dimorphism is set aside. While we focus here on sexual reproduction, there exist many reproductive strategies that, like 'sex' itself, exist on a continuum between sexual and asexual reproduction, and provide further insight into the evolutionary processes behind reproduction. Parthenogenesis occurs notably in both arthropods (van der Kooi et al. 2017) and squamate reptiles (Fujita and Moritz 2009). While in most species, parthenogenesis is an alternate pathway to otherwise predominantly sexually reproducing taxa (Watts et al. 2006; Lampert 2008), in a small subset, parthenogenesis is the sole form of reproduction (Fujita and Moritz 2009). Whiptail lizards (*Cnemidophorus* and *Aspidoscelis*) have repeatedly evolved via hybridization (Barley et al. 2021), engage in copulatory behavior despite all individuals being capable of laying eggs (Crews and Fitzgerald 1980; Crews et al. 1986), and can even spontaneously produce genetically identical but phenotypically male offspring (Wibbels and Crews 1994; Cabej 2018). Further along the continuum, gynogenesis involves the stimulation of embryonic development in an ovum by sperm without the incorporation of the genetic material of the sperm (Jaylet and Ferrier 1978). This mode of reproduction can be artificially induced in

bioRxiv preprint doi: https://doi.org/10.1101/2023.01.26.525769; this version posted January 27, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

many species, and is frequently used in aquaculture to manage stocks (Manan et al. 2022). Yet another variant of incomplete parthenogenesis (Lampert and Schartl 2010), termed kleptogenesis, is found in unisexual *Ambystoma* salamanders that co-occur with gonochoristic congeners, and involves a triploid ovum being fertilized by a related species with some incorporation of genetic material (Bogart et al. 2007). The vast array of sexual determination and reproductive systems challenge frameworks that impose human cultural binary categories on animals, and show how even at the most fundamental levels, the biological underpinnings of sexual phenotypes are extremely diverse and multimodal.

bioRxiv preprint doi: https://doi.org/10.1101/2023.01.26.525769; this version posted January 27, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

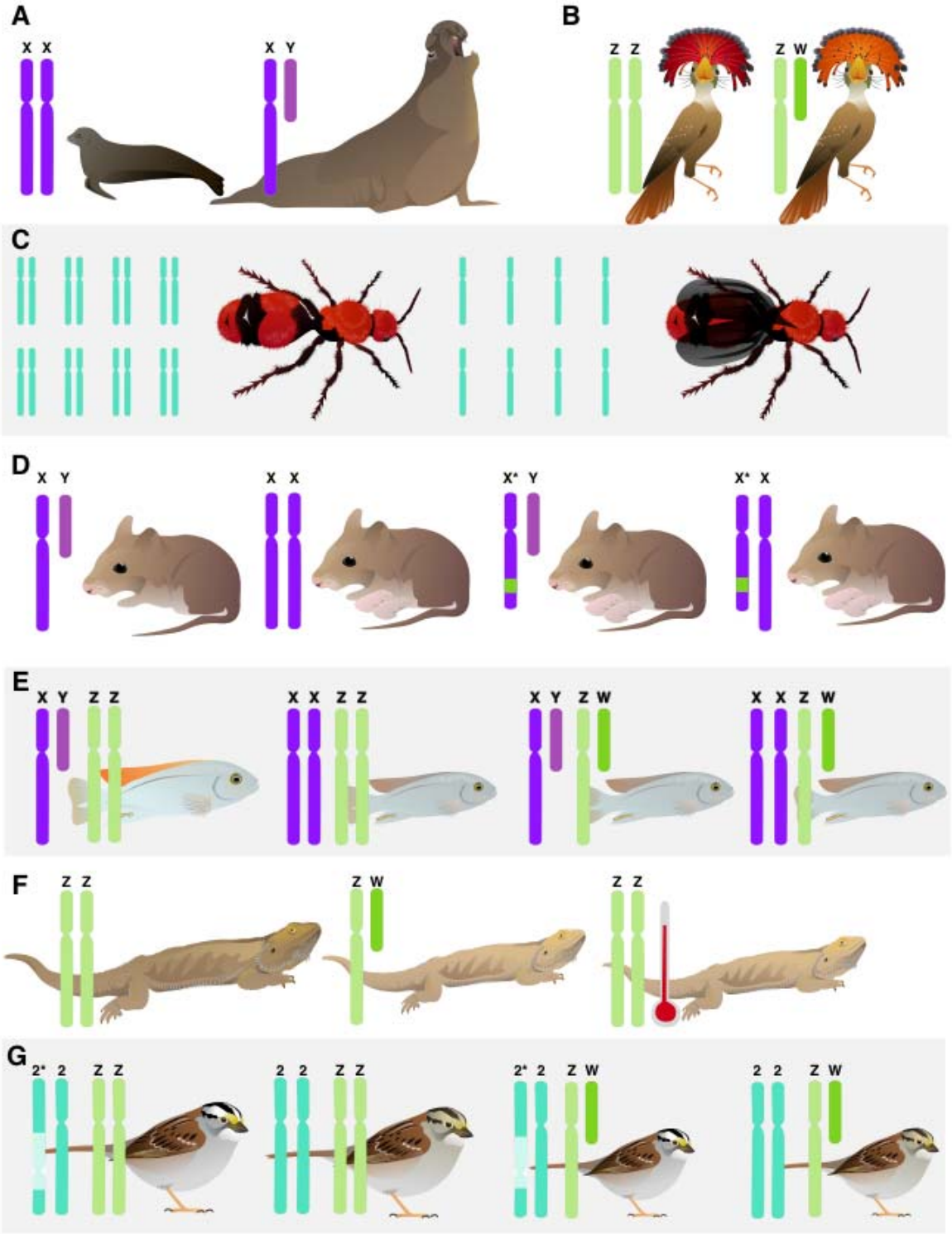

Figure 2: Mechanisms of sex determination vary widely across animals. The most familiar (top row) are the relatively simple (**A**) XX/XY (in purple, here shown with northern elephant seal, *Mirounga angustirostris*, as an example taxon) and (**B**) ZZ/ZW (in green, shown with royal

bioRxiv preprint doi: https://doi.org/10.1101/2023.01.26.525769; this version posted January 27, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

flycatcher, *Onychorhynchus coronatus*). (**C**) Haplodiploid sex determination involves sex being determined by having one or two copies of the genome, and is found predominantly in insects such as the pictured *Dasymutilla occidentalis*. (**D**) A mutation in the X chromosome of some African pygmy mice (*Mus minutoides*) overrides the presence of a Y chromosome, and (**E**) in some cichlids (shown here with *Metriaclima mbenjii*), both the absence of the W chromosome and presence of the Y are required for an individual to develop 'male' characteristics. (**F**) In central bearded dragon (*Pogona vitticeps*), the ZZ genotype is overridden by high temperatures, leading to phenotypically 'female' individuals capable of laying eggs. (**G**)The white-throated sparrow (*Zonotrichia albicollis*) is found in two color morphs linked to a large inversion on chromosome two (in turquoise), which is paired with the Z and W genotype to determine which individuals can successfully reproduce with each other.

Sex at the endocrine level

Hormones influence a number of sexual phenotypes, including 1) morphological traits like gonads, genitals, ornamentation, and body size, 2) reproductive states like estrous cycling and gametogenesis, and 3) behaviors like courtship displays, receptivity to mating, aggression, and parental care (Adkins-Regan 2005). So-called ‘sex’ hormones typically refer to steroid hormones produced in the testes and ovaries, and include estrogens, progestogens, as well as several androgens (testosterone, androstenedione, dihydrotestosterone, and 11-ketotestosterone). However, many of these hormones are also produced outside of the gonads, including in the adrenal cortex, fat, and brain (Schmidt et al. 2008). Furthermore, steroid hormones are often metabolized into different forms before they bind to nuclear receptors and regulate the transcription of hormonally responsive genes (Aranda and Pascual 2001). For instance, in male zebra finches (*Taeniopygia guttata*), testosterone is converted to 17β-estradiol via the enzyme aromatase in the brain, which then regulates directed singing (Walters et al. 1991). A common misconception is that androgens regulate ‘masculine’ phenotypes and estrogens regulate ‘feminine’ phenotypes, which has led to the widely used terms ‘masculinization’ and ‘feminization’. We argue that this terminology is circular and confusing. Steroid hormones are not sex-specific - both females and males possess the capacity to produce, metabolize, and respond to these hormones (Staub and De Beer 1997).

Whereas it is often the case that average peak hormone levels may be higher or lower in one sex, this depends on various contexts, including when animals are developing (organizational effects) and when they are breeding (activational effects) (Phoenix et al. 1959), as well as in response to social stimuli (Wingfield et al. 1990; Goymann et al. 2019). For example, testosterone in temperate avian species peaks seasonally during territorial establishment and mating, but decreases to basal, non-detectable levels during the non-breeding season, when gonads regress ((Wingfield et al. 2019). Within an individual, testosterone levels vary substantially throughout the day (Panico et al. 1990; Brambilla et al. 2009; Greives et al. 2021) Though testosterone levels in circulation are typically higher in males than in females, for many species(Goymann and Wingfield 2014; Van Leeuwen and Bladh 2016), there is no testosterone threshold for ‘maleness.’ In the sex-changing bluebanded goby (*Lythrypnus dall*i), 11-ketotestosterone and testosterone do not differ between females and males ((Lorenzi et al. 2008). Furthermore, many hormone signals circulate in the bloodstream at small concentrations, to target neural and peripheral tissues where they activate a cascade of

bioRxiv preprint doi: https://doi.org/10.1101/2023.01.26.525769; this version posted January 27, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

events that amplify their effects. For instance, a study examining the ventromedial telencephalon in dark-eyed juncos (*Junco hyemalis*) found that individual variation in the expression of genes encoding the androgen receptor, estrogen receptor, and aromatase correlated with aggression in both sexes (Rosvall et al. 2012) Given the daily, seasonal, and cross-species variation in hormone levels, hormone levels are not a binary indicator of sex.

Sex at the morphological level

Organismal biologists often make binary sex assignments to individuals based on their primary sexual characteristics, despite the diversity of genital morphology within and among taxa. For example, female spotted hyenas (*Crocuta crocuta*) mate, give birth, and urinate through a urogenital canal at the tip of a hypertrophied clitoris, or pseudo-penis (Glickman et al. 2006), which they use to signal social status (Hofer and East 1995). In *Neotrogla* barkflies, females have a highly elaborate penis (gynosome) that retrieves sperm from the male's vagina (Yoshizawa et al. 2014). In color polymorphic lizards, male color morphs have distinct hemipene morphology associated with their alternative mating strategies (Gilman et al. 2019). Phallus polymorphism is common in hermaphroditic gastropods, in which some individuals have a functional penis (euphally), some have a non-functional penis (hemiphally), and some have no penis (aphally) (Leonard et al. 2007). In birds, only 3% of species even have phalluses (external genitalia) (Brennan 2022). As with phallic diversity, animal morphologies involved in birthing and brooding exhibit extraordinary intra- and intersexual variation. Male pregnancy has evolved several times in syngnathid fishes (seahorses and pipefish) where gestation in males requires morphological and physiological changes in paternal tissues to incubate developing embryos in a specialized brooding pouch, analogous to structures in viviparous females (Carcupino et al. 2002). In birds, for which biparental care is common (Clutton-Brock 2019), both sexes develop brood patches to incubate eggs. In mammals (humans included), genital structures are not formed de novo in males and females, but develop from a shared anatomical basis that can differentiate into forms other than the two most commonly observed pathways, leading to intermediate phenotypes that are unfortunately pathologized (Quigley et al. 1995; Dreger 2006; Grimstad et al. 2021) Thus, the presence, shape, and size of genitalia, such as the vaginal opening or phallus, do not always fall into neat categories of sex.

Secondary sexual characteristics include ornamentation, weaponry, and many other sexual morphologies that are not directly involved in reproduction. Although these traits are expected to be sexually dimorphic, there are many exceptions. For example, intraspecific polymorphisms present a classic example of more than two sexual morphologies (Mank 2022). The three morphs of male ruffs (*Philomachus pugnax*) exhibit extremely divergent size and plumage ornamentation, which correspond to alternative mating strategies and chromosomal genotypes (Küpper et al. 2016). In many odonates there are three female morphs, consisting of two ‘gynomorphs’ that vary in color, and one ‘andromorph’ that closely resembles males in color, sexual ornamentation, and behavior, presumably to avoid mating harassment (Gallesi et al. 2015). These so-called ‘andromorphs’ exhibit morphological and behavioral variation distinct from males and ‘gymnomorph’ females (Paulson 1998; Fincke et al. 2005; Gallesi et al. 2015), resulting in an overall phenotype that does not conform to a binary distribution and likely arises

bioRxiv preprint doi: https://doi.org/10.1101/2023.01.26.525769; this version posted January 27, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

due to a variety of factors beyond simple male mimicry (Fincke et al. 2005). For both primary and secondary sexual morphologies, there are many cases in which the distribution of these traits is multimodal, rather than binary.

Sex at the social level

Scientific notions of how animals 'should' behave originate with Darwin in the Victorian era (Darwin 1871). The Darwin-Bateman paradigm posits that anisogamy drives the direction of sexual selection to be stronger in males, resulting in 'traditional' sex roles for male-male mate competition and female mate choice (Dewsbury 2005). Trivers (1972) added to this paradigm with the hypothesis that gametic investment drives postzygotic parental investment, which should be higher in females because of larger gamete size. Sex role theories have dominated sexual selection research, but not without criticism (Gowaty et al. 2012; Ah-King 2013; Tang-Martínez 2016), as others have argued for the importance of ecology, sex ratio, and life history traits on the evolution of sex-specific behaviors (Kokko and Jennions 2008; Mokos et al. 2021; Kappeler et al. 2022). Outside of mammals, many examples in fish, frogs, and birds contradict the notion that the sex with the larger gamete suffers higher mating costs and invests more resources into the next generation (Gross 2005; Furness and Capellini 2019).

Within species, alternative reproductive strategies are a classic example of multimodal variation in behavior (Gross 1996; Sinervo and Lively 1996; Stiver et al. 2015), and can arise via different mechanisms of genotype, condition dependence, and social environment. For example, in the bluegill sunfish (*Lepomis macrochirus*), there are three different reproductive strategies found in males, one of which overlaps with courtship soliciting behaviors in females (Dominey 1980). The diversity of sexual behaviors also includes the common occurrence of same-sex sexual behavior (SSB) in animals (Monk et al. 2019). SSBs have been observed across a large number of animals (Bailey and Zuk 2009), but were frequently unreported by early naturalists due to societal biases (Russell et al. 2012).

Compared to the other traits we review, sex studied at the social level seems to more readily acknowledge variation outside of binary categories, perhaps because plasticity is a fundamental aspect of behavioral research. Furthermore, several recent studies in animal behavior and evolutionary biology have acknowledged that the positionality of the scientists themselves influences their research (Ahnesjö et al. 2020; Tang-Martínez 2020; Ah-King 2022; Pollo and Kasumovic 2022), making more space for scientists to reflect on how their identities shape their scientific practices.

Case study 1: 'Sex-role reversal' and the decoupling of sexual phenotypes

Now that we have surveyed multiple, independent levels of sex, how can we integrate them in a multimodal framework? 'Sex-role reversed' birds like black coucals (*Centropus grillii*), spotted sandpipers (*Actitis macularius*), barred buttonquails (*Turnix suscitator*), and northern jacanas (*Jacana spinosa)* present fascinating cases of multimodal sex (Figure 3). 'Sex-role reversed' species are so-named because they defy 'traditional' expectations of social sex roles - male

bioRxiv preprint doi: https://doi.org/10.1101/2023.01.26.525769; this version posted January 27, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

competition and female parental care. However, sex role terminology imposes binary thinking, limiting our understanding of continuous variation in phenotypes (Ah-King and Ahnesjö 2013). At the social level, females face stronger sexual selection to compete for multiple mates (i.e. social polyandry), and males conduct the majority of parental care (Emlen and Oring 1977). As in most birds, they have ZW/ZZ sex determination and females are heterogametic. At the endocrine level, females have testosterone levels similar to females in other mating systems, a pattern that applies broadly across independent origins (Eens and Pinxten 2000; Lipshutz and Rosvall 2020a). There is some evidence that sexually selected traits like weaponry, plumage ornamentation, and aggression are associated with hormonal levels in females, but not males (Goymann et al. 2008; Muck and Goymann 2011; Lipshutz and Rosvall 2020b). At the morphological level, sexual size dimorphism is biased towards females, who have more exaggerated ornamentation, weaponry, and body size (Emlen and Wrege 2004; Blizard and Pruett-Jones 2017). Larger, more ornamented individuals gain access to breeding territories, whereas floater individuals do not reproduce. During the breeding season, the gonadal size of territorial males fluctuates between stages of courtship and parental care, whereas territorial females remain in a continuous state of fertility (Lipshutz and Rosvall 2020b). Altogether, the genetic, endocrine, morphological, and social traits of 'sex-role reversed' systems clearly defy a binary framework. Rather than considering these systems as rare exceptions, we can use them as a model to understand the multimodality of sexual phenotypes.

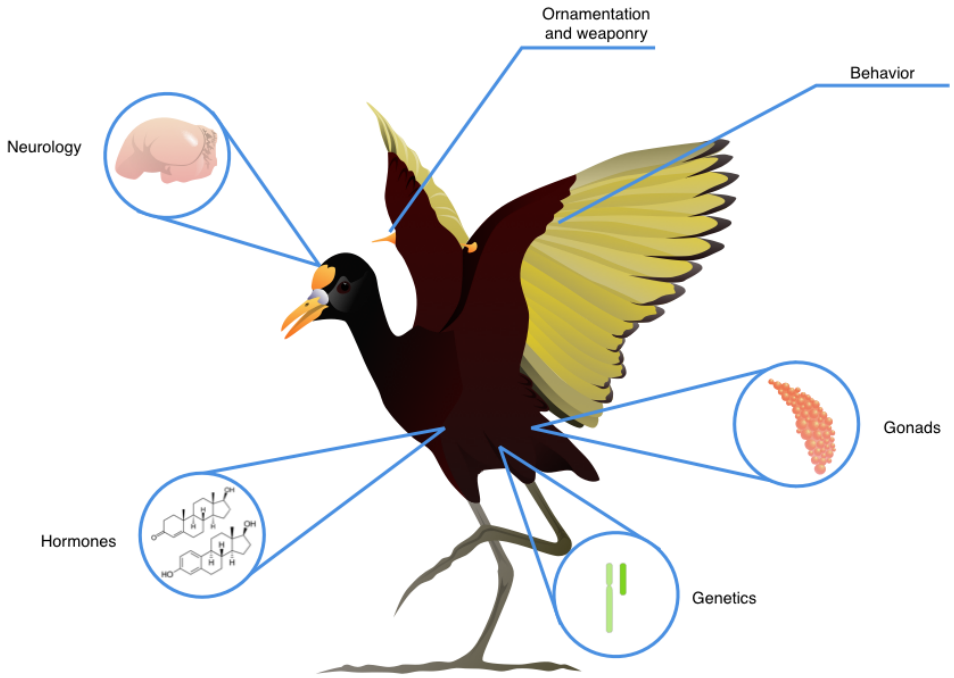

Figure 3: Multimodal sex in the northern jacana. 'Sex' includes phenotypic variation at multiple levels, from genetic makeup, anatomy and morphology, hormone levels, and social roles and behaviors.

Case study 2: The evolutionary consequences of more than two sexes

bioRxiv preprint doi: https://doi.org/10.1101/2023.01.26.525769; this version posted January 27, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Here we pose a systems-level question: how does the number of operative sexes influence the evolution of a species? Several species have been described as having more than two sexes. In white-throated sparrows (*Zonotrichia albicollis*), the more aggressive white-stripe morph has a large inversion on chromosome 2 ('supergene'), which tan-stripe individuals lack (Tuttle et al. 2016; Falls and Kopachena 2020; Maney et al. 2020) (Figure 2G). This species mates disassortative by color morph, with tan stripe morphs predominantly mating with white stripe morphs (Hedrick et al. 2018). White-throated sparrows are sometimes referred to as 'the bird with four sexes', because population persistence as a whole requires that all four morphs be present (Campagna 2016; Maney et al. 2020). A similar system occurs in hybrid *Pogonomyrmex* ants, where each colony requires three sexes to operate, and four to persist beyond a single generation (Parker 2004).

There are significant evolutionary consequences to having more than two operative sexes. A key parameter in population genetics is effective population size (N_e) (Charlesworth 2009), which is fundamentally impacted by the number of sexes in a given population (Caballero 1994). Assuming an equal sex ratio, the absolute maximum of N_e will be equal to $\frac{N}{S_O}$, where N is the census population size and S_o is the operative number of sexes. As N_e influences the strength of selection, genetic drift, and genetic diversity of a population (Charlesworth 2009), the operative number of sexes substantially impacts the evolutionary trajectory of a lineage. The number of sexes in a system also shapes genome evolution (Charlesworth and Mank 2010), mating kinetics (Power 1976; Iwasa and Sasaki 1987), and sexual selection (Emlen and Oring 1977), and thus a framework of sex that accounts for diversity in sexual phenotypes allows us to more accurately model natural processes at the system level (Mokos et al. 2021).

We can gain novel insights on the evolution of other taxa by applying this systems-level framework. The previously mentioned ruff also has a large chromosomal inversion that functions as a supergene (Küpper et al. 2016; Lamichhaney et al. 2016). This genetic polymorphism is linked to both phenotypic and behavioral differences in reproductive strategies (Lank et al. 1995; Jukema and Piersma 2006). It is typical to frame such systems as having *subtypes* of two sexes– in this case, three males and one female. But if we are willing to consider the white-throated sparrows and *Pogonomyrmex* systems as having more than two sexes, why not apply a similar framework to the ruff and other polymorphic systems (Galeotti et al. 2003; Roulin 2004)? Our binary framing of sex in these systems may actively interfere with understanding their evolutionary trajectories.

Case study 3: Ecological consequences of multimodal sex in color polymorphic lizards

Not all members of the same sex look and behave the same way, and this intrasexual variation can have profound evolutionary and ecological consequences. Color polymorphism, the evolution of two or more genetically-determined color morphs within a single population (Ford 1945), is a classic example of multimodal sexual diversity (Mank 2022). Intrasexual color morphs have evolved in every major animal group across the tree of life, and are particularly common in lizards (Stuart-Fox et al. 2021; Brock, McTavish, et al. 2022). In lizards, intrasexual color morphs occur in both females and males, and are often associated with alternative sexual

bioRxiv preprint doi: https://doi.org/10.1101/2023.01.26.525769; this version posted January 27, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

phenotypes comprising discontinuous variation in genital morphology (Gilman et al. 2019), clutch size (Sinervo et al. 2000), mate preference (Pérez i de Lanuza et al. 2013), mating strategies (Gross 1982; Sinervo and Lively 1996), and endocrine profiles (Huyghe et al. 2009; Brock et al. 2020). These distinct sexual phenotypes give rise to ecological specialization among morphs, including thermal preference (Thompson et al. 2022), microhabitat selection (BeVier et al. 2022), diet (Lattanzio and Miles 2016), and behavior (Brock and Madden 2022; Brock, Chelini, et al. 2022). Although each morph utilizes a specialized set of resources, in aggregate this contributes to a greater niche breadth in polymorphic populations, relative to monomorphic populations (Forsman et al. 2008); Figure 4). As an ecological consequence, the overall niche breadth of polymorphic populations is hypothesized to confer greater capacity for range expansion, less susceptibility to environmental change, and a larger buffer to extinction risk (Forsman et al. 2008). Evolutionary consequences of multimodal morphs include punctuated patterns of evolution, greater mobility on the adaptive landscape, and increased diversification rates (West-Eberhard 1986; Corl et al. 2010; Hugall and Stuart-Fox 2012; Brock, McTavish, et al. 2022). New species could form from different morphs if they become reproductively isolated or fixed within a population (West-Eberhard 1986; Gray and McKinnon 2007); (Seehausen et al. 2008; Corl et al. 2010; McLean and Stuart-Fox 2014; Brock, Indiana Madden, et al. 2022). In addition to facilitating speciation, character release associated with morph fixation could produce punctuated accelerations of morphological change (Eldredge 1976; West-Eberhard 1986; Corl et al. 2010). Thus, the ecological differences that emerge from intrasexual polymorphism are a crucial component of biodiversity within a species, which has far-reaching consequences for population persistence in a rapidly changing world. Collapsing intrasexual polymorphisms into a female-male binary erases extensive multivariate phenotypic variation (Figure 4), including its ecological and evolutionary consequences.

bioRxiv preprint doi: https://doi.org/10.1101/2023.01.26.525769; this version posted January 27, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

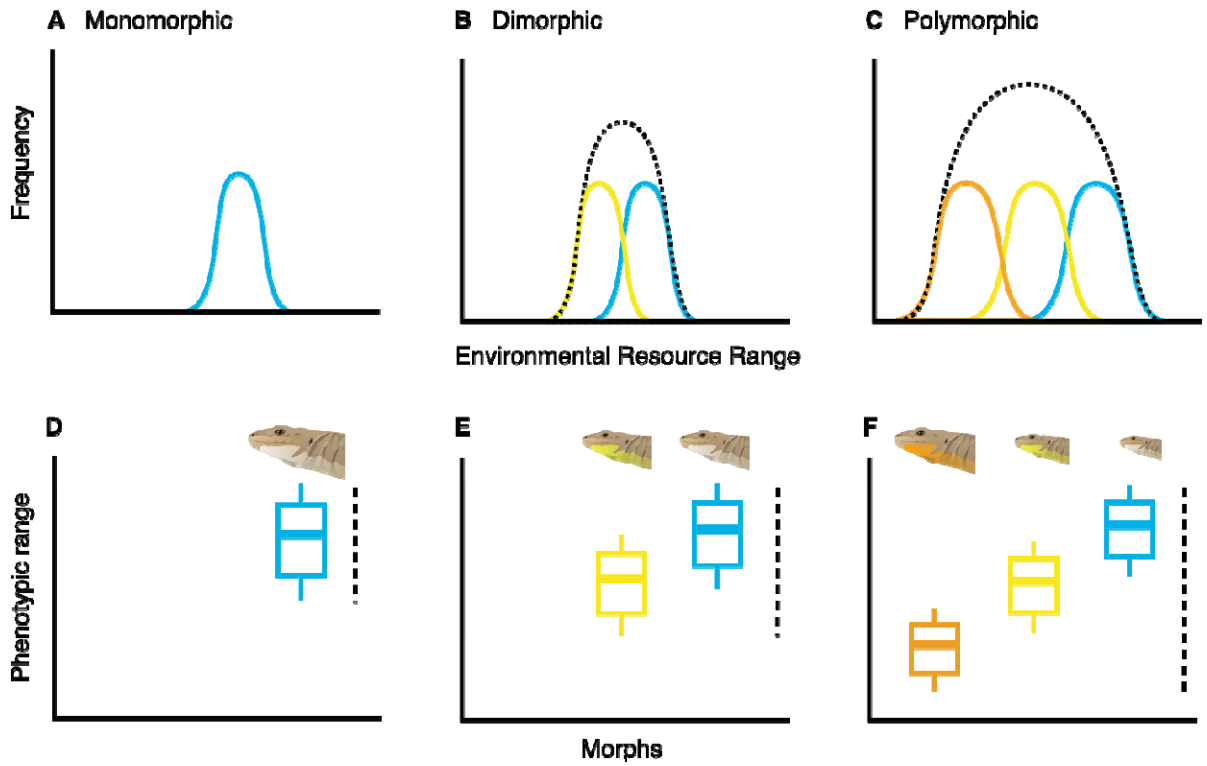

Figure 4: Illustration of alternative patterns of population-level environmental niche breadth (dotted line) distributions for A) monomorphic, B) dimorphic, and C) polymorphic populations of wall lizards (*Podarcis*). Different color morphs (solid color lines) have divergent environmental resource use that, in the aggregate, result in an overall greater niche breadth in an A) monomorphic population compared to B) dimorphic and C) polymorphic populations.

Literature survey: How does terminology for sexual diversity change over time?

The language used in published research forms the foundation of how we as scientists think about, discuss, and investigate the natural world. To track how terminology usage has changed over time, we conducted a systematic review on Web of Science of search terms associated with genetic, phenotypic, behavioral, and physiological levels of sex. Our search terms included several cases such as “andromorph/gynomorph” (60 papers) and “female-like male/male-like female”(124), and “she-male” (5), which illustrate framing sexual polymorphisms relative to one sex or another, or both. We also included “female hormone/male hormone” (40) and “feminize/masculinize” (539), which attribute physiological mechanisms as inherently sex-specific. Additionally, we surveyed the conflation of sex and gender (1047). More details are available in the Supplementary Materials.

bioRxiv preprint doi: https://doi.org/10.1101/2023.01.26.525769; this version posted January 27, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

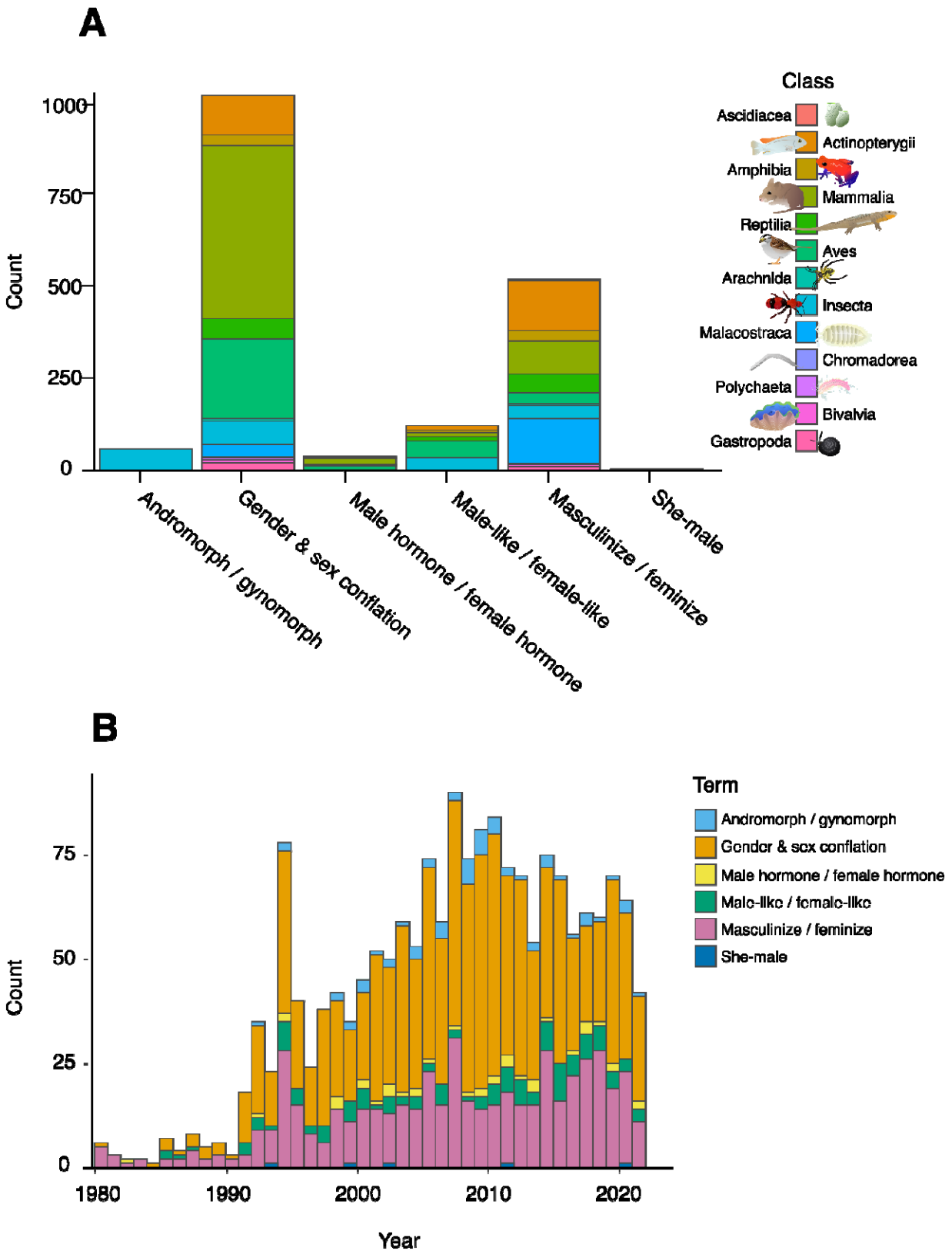

Figure 5. Literature survey for terms (a) by taxonomy and (b) over time.

bioRxiv preprint doi: https://doi.org/10.1101/2023.01.26.525769; this version posted January 27, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

We initially identified 2,222 papers (Figure S1, Supplementary Materials), 1653 of which were exclusive to non-human animals, could be assigned a taxonomic class, and were available to Loyola University Chicago online. We found that the conflation of sex and gender increased in frequency in the literature in the past three decades (Figure 5), consistent with findings from another study (Goymann and Brumm 2018). This was most prevalent in studies of mammals, followed by birds. The usage of “feminize” and “masculinize” was most prevalent in bony fish and crustaceans, and has also increased over time. Other terms were consistently used across time, primarily female-like/male-like, female hormone/male hormone, and andromorph/gynomorph (Figure 5). Andromorph/gynomorph are almost exclusively used in insects, particularly damselflies, while “male-like” female and “female-like” males are used more generally across taxa. Finally, application of “she-male” was confined only to reptiles, and garter snakes (*Thamnophis sp.*) in particular.

The interplay of science and society

Multimodal models of sex reveal overlapping but not necessarily coincident phenotypes at every biological level, from the molecular to the macroevolutionary. In zoology, we impose a binary categorization of sex as an emergent property of many traits. Whereas some of these traits do typically have a bimodal distribution (some chromosomes, gametes), others demonstrate largely continuous or multimodal variation (hormone levels [(Wingfield et al. 1990), behavior [(Dominey 1980)], morphology [Mank 2022]), suggesting that most individual animals can best be studied from the framework of many axes of behavioral and physiological phenotypes– some categorical, but most continuous. Even the basic inclusion of sex as a variable is missing from many studies, particularly in fields related to human health (Woitowich et al. 2020; Garcia-Sifuentes and Maney 2021). However, uncritically applying a simple binary without considering the mechanisms shaping sex-specific effects can confound inferences (Casto et al. 2022) and completely erases the biological realities of TGNC and intersex people (Cheung et al. 2021; Phiri-Ramongane and Khine 2022).

Embracing complex models of sex has led to greater understanding of fungi and plants (Lee et al. 2010; Billiard et al. 2011, 2012). Here, we have argued that applying such a lens to animals may lead to similarly novel insights. Some readers may consider our examples of sexual diversity as rare anomalies – exceptions to the evolutionary rule, which do not require a novel framework to explain. We acknowledge some variations in sexual phenotypes may be relatively less common in animals, but that does not minimize their importance in our understanding of evolution. When we regard diversity as a deviation from the norm, we ignore its successful role in generating novel solutions to evolutionary challenges. Our frameworks of sex need to accommodate and embrace this diversity, lest we fall prey to a deterministic model of evolution, where the ideal endpoint is always binary.

As biologists, we seek to understand the vast diversity of life in all its wonderful strangeness - yet the lens we use fundamentally shapes that which we can observe. The historical legacies of sexism, racism, queerphobia, and ableism have deeply influenced the

bioRxiv preprint doi: https://doi.org/10.1101/2023.01.26.525769; this version posted January 27, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

frameworks we use to study nature (Branch et al. 2022; Kamath et al. 2022). Challenging these foundations is difficult but vital to both increasing inclusion in biology (Hales 2020; Casper et al. 2022) and dismantling assumptions that interfere with our ability to observe the natural world on its own terms (Monk et al. 2019; Ahnesjö et al. 2020; Kamath et al. 2022; Packer and Lambert 2022). There is pressure for scientists to avoid making the politics of our work explicit, especially those of us who do not directly study social issues. However, especially in the United States, legislation targeting TGNC people is increasingly undergirded with simplistic binary language purportedly rooted in biology (such as OH HB454 §3129.02 2021, WV HB3293 §18-2-25d.b1 2021, MO SB22 §191.1720 2022, and TX HB672 88R §71.004.1A 2022). We believe it is imperative that all biologists challenge the misuse and abuse of this language (Miyagi et al. 2021) and confront how our scientific models impact society (Bazzul and Sykes 2011). Our science can be weaponized to discriminate against marginalized groups if we are not explicitly mindful of our responsibility to dispel misconceptions and recognize diversity. At the same time, we scientists are best situated to communicate how nature is a rich tapestry of diversity that affirms, rather than invalidates, human experience.

Acknowledgements

Thanks to Kelsey Lewis and Sam Sharpe for the opportunity to contribute to this symposium. Aqdas Aftab, Wolfgang Goymann, and Kim Rosvall for fruitful conversations discussing sex and gender diversity. Claire Curry and Elliott Smith provided vital assistance in designing the systematic review and mapping analysis. Anusha Bishop, Anne Chambers, Jay Falk, Ambika Kamath, Daniel Olivera, and Erin Westeen provided feedback on an early version of the manuscript. Thank you to the Trans Formations Project, whose database of legislation was very helpful in understanding the use of the term ‘biological sex' in recent legislation.

Conflict of Interest

This research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

References

Adkins-Regan E. 2005. Hormones and animal social behavior. Monographs in Behavior and Ecology. Princeton, NJ, USA: Princeton University Press.

Ah-King M. 2013. On anisogamy and the evolution of “sex roles.” Trends Ecol Evol 28:1-2.

Ah-King M. 2022. The Female Turn. Singapore: Springer Nature.

Ah-King M, Ahnesjö I. 2013. The “Sex Role” Concept: An Overview and Evaluation. Evol Biol 40:461–70.

Ahnesjö I, Brealey JC, Günter KP, Martinossi-Allibert I, Morinay J, Siljestam M, Stångberg J, Vasconcelos P. 2020. Considering Gender-Biased Assumptions in Evolutionary Biology. Evol Biol 47:1–5.

Aranda A, Pascual A. 2001. Nuclear hormone receptors and gene expression. Physiol Rev

bioRxiv preprint doi: https://doi.org/10.1101/2023.01.26.525769; this version posted January 27, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

81:1269–1304.
Bachtrog D, Mank JE, Peichel CL, Kirkpatrick M, Otto SP, Ashman T-L, Hahn MW, Kitano J, Mayrose I, Ming R, Perrin N, Ross L, Valenzuela N, Vamosi JC, Tree of Sex Consortium. 2014. Sex determination: why so many ways of doing it? PLoS Biol 12:e1001899.
Bailey NW, Zuk M. 2009. Same-sex sexual behavior and evolution. Trends Ecol Evol 24:439–46.
Barley AJ, Reeder TW, Nieto-Montes de Oca A, Cole CJ, Thomson RC. 2021. A New Diploid Parthenogenetic Whiptail Lizard from Sonora, Mexico, Is the “Missing Link” in the Evolutionary Transition to Polyploidy. Am Nat 198:295–309.
Baroiller JF, D’Cotta H, Saillant E. 2009. Environmental effects on fish sex determination and differentiation. Sex Dev 3:118–35.
Bazzul J, Sykes H. 2011. The secret identity of a biology textbook: straight and naturally sexed. Cult Stud Sci Educ 6:265–86.
BeVier GT, Ayton C, Brock KM. 2022. It ain’t easy being orange: lizard colour morphs occupying highly vegetated microhabitats suffer greater ectoparasitism. Amphib-reptil 43:287-97.
Beye M, Hasselmann M, Fondrk MK, Page RE, Omholt SW. 2003. The gene csd is the primary signal for sexual development in the honeybee and encodes an SR-type protein. Cell 114:419–29.
Billiard S, López-Villavicencio M, Devier B, Hood ME, Fairhead C, Giraud T. 2011. Having sex, yes, but with whom? Inferences from fungi on the evolution of anisogamy and mating types. Biol Rev Camb Philos Soc 86:421–42.
Billiard S, López-Villavicencio M, Hood ME, Giraud T. 2012. Sex, outcrossing and mating types: unsolved questions in fungi and beyond. J Evol Biol 25:1020–38.
Blizard M, Pruett-Jones S. 2017. Plumage pattern dimorphism in a shorebird exhibiting sex-role reversal (*Actitis macularius*). Auk 134:363–76.
Bogart JP, Bi K, Fu J, Noble DWA, Niedzwiecki J. 2007. Unisexual salamanders (genus *Ambystoma*) present a new reproductive mode for eukaryotes. Genome 50:119–36.
Brambilla DJ, Matsumoto AM, Araujo AB, McKinlay JB. 2009. The effect of diurnal variation on clinical measurement of serum testosterone and other sex hormone levels in men. J Clin Endocrinol Metab 94:907–13.
Branch HA, Klingler AN, Byers KJRP, Panofsky A, Peers D. 2022. Discussions of the “Not So Fit”: How Ableism Limits Diverse Thought and Investigative Potential in Evolutionary Biology. Am Nat 200:101–13.
Brennan PLR. 2022. Bird genitalia. Curr Biol 32:R1061–62.
Brock KM, Baeckens S, Donihue CM, Martín J, Pafilis P, Edwards DL. 2020. Trait differences among discrete morphs of a color polymorphic lizard, *Podarcis erhardii*. PeerJ 8:e10284.
Brock KM, Chelini MC, Ayton C, Madden IE, Ramos C. 2022. Colour morph predicts social behaviour and contest outcomes in a polymorphic lizard (*Podarcis erhardii*). Anim Behav 191:91-103.
Brock KM, Indiana Madden, Rosso A, Ramos C, Degen R, Stadler SR, Ayton C, Fernandez MEL, Servin JR. 2022. Patterns of colour morph diversity across populations of Aegean Wall Lizard, *Podarcis erhardii* (Bedriaga, 1882). HNO 15:361–64.
Brock KM, Madden IE. 2022. Morph-specific differences in escape behavior in a color polymorphic lizard. Behav Ecol Sociobiol 76:104.
Brock KM, McTavish EJ, Edwards DL. 2022. Color Polymorphism is a Driver of Diversification in the Lizard Family Lacertidae. Syst Biol 71:24–39.
Caballero A. 1994. Developments in the prediction of effective population size. Heredity 73 (Pt 6):657–79.
Cabej NR. 2018. Epigenetic Principles of Evolution. Elsevier Science.
Campagna L. 2016. Supergenes: The Genomic Architecture of a Bird with Four Sexes. Curr Biol 26:R102-24.

bioRxiv preprint doi: https://doi.org/10.1101/2023.01.26.525769; this version posted January 27, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Capel B. 2017. Vertebrate sex determination: evolutionary plasticity of a fundamental switch. Nat Rev Genet 18:675–89.

Carcupino M, Baldacci A, Mazzini M, Franzoi P. 2002. Functional significance of the male brood pouch in the reproductive strategies of pipefishes and seahorses: a morphological and ultrastructural comparative study on three anatomically different pouches. J Fish Biol 61:1465–80.

Casper AMA, Rebolledo N, Lane AK, Jude L, Eddy SL. 2022. “It’s completely erasure”: A Qualitative Exploration of Experiences of Transgender, Nonbinary, Gender Nonconforming, and Questioning Students in Biology Courses. LSE 21:ar69.

Casto KV, Leininger E, Tan T. 2022. Teaching About Sex and Gender in Neuroscience: More than meets the “XY.” J Undergrad Neurosci Educ 17:A1-3.

Charlesworth B. 2009. Fundamental concepts in genetics: effective population size and patterns of molecular evolution and variation. Nat Rev Genet 10:195–205.

Charlesworth D, Mank JE. 2010. The birds and the bees and the flowers and the trees: lessons from genetic mapping of sex determination in plants and animals. Genetics 186:9–31.

Cheung AS, Lim HY, Cook T, Zwickl S, Ginger A, Chiang C, Zajac JD. 2021. Approach to interpreting common laboratory pathology tests in transgender individuals. J Clin Endocrinol Metab 106:893–901.

Clutton-Brock TH. 2019. The Evolution of Parental Care. Princeton, NJ, USA: Princeton University Press.

Corl A, Davis AR, Kuchta SR, Comendant T, Sinervo B. 2010. Alternative mating strategies and the evolution of sexual size dimorphism in the side-blotched lizard, *Uta stansburiana*: a population-level comparative analysis. Evolution 64:79–96.

Crews D, Fitzgerald KT. 1980. “Sexual” behavior in parthenogenetic lizards (*Cnemidophorus*). Proc Natl Acad Sci U S A 77:499–502.

Crews D, Grassman M, Lindzey J. 1986. Behavioral facilitation of reproduction in sexual and unisexual whiptail lizards. Proc Natl Acad Sci 83:9547–50.

Darwin C. 1871. The descent of man: and selection in relation to sex. London: J. Murray.

Dewsbury DA. 2005. The darwin-bateman paradigm in historical context. Integr Comp Biol 45:831–37.

Dominey WJ. 1980. Female mimicry in male bluegill sunfish—a genetic polymorphism? Nature 284:546–48.

Douglas TE, Strassmann JE, Queller DC. 2016. Sex ratio and gamete size across eastern North America in *Dictyostelium discoideum*, a social amoeba with three sexes. J Evol Biol 29:1298–1306.

Dreger AD. 2006. Intersex and human rights: The Long View. In: International Library of Ethics, Law and the New Medicine. Dordrecht: Kluwer Academic Publishers. p. 73–86.

Eens M, Pinxten R. 2000. Sex-role reversal in vertebrates: behavioural and endocrinological accounts. Behav Processes 51:135–47.

Eldredge N. 1976. Differential Evolutionary Rates. Paleobiology 2:174–77.

Emlen ST, Oring LW. 1977. Ecology, sexual selection, and the evolution of mating systems. Science 197:215–23.

Emlen ST, Wrege PH. 2004. Size Dimorphism, Intrasexual Competition, and Sexual Selection in Wattled Jacana (*Jacana jacana*), A Sex-Role-Reversed Shorebird in Panama. Auk 121:391–403.

Engqvist L, Taborsky M. 2016. The evolution of genetic and conditional alternative reproductive tactics. Proc Biol Sci 283:20152945.

Falls JB, Kopachena JG. 2020. White-throated Sparrow (*Zonotrichia albicollis*). Birds of the World.

Fincke OM, Jödicke R, Paulson DR, Schultz TD. 2005. The evolution and frequency of female color morphs in Holarctic Odonata: why are male-like females typically the minority? Int J

bioRxiv preprint doi: https://doi.org/10.1101/2023.01.26.525769; this version posted January 27, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Odonatol 8:183–212.
Fisher RA. 1930. The Genetical Theory of Natural Selection Oxford, UK: Oxford University Press.
Ford EB. 1945. Polymorphism. Biol Rev Camb Philos Soc 20:73–88.
Forsman A, Ahnesjö J, Caesar S, Karlsson M. 2008. A model of ecological and evolutionary consequences of color polymorphism. Ecology 89:34–40.
Fujita MK, Moritz C. 2009. Origin and evolution of parthenogenetic genomes in lizards: current state and future directions. Cytogenet Genome Res 127:261–72.
Furness AI, Capellini I. 2019. The evolution of parental care diversity in amphibians. Nat Commun 10:4709.
Galeotti P, Rubolini D, Dunn PO, Fasola M. 2003. Colour polymorphism in birds: causes and functions. J Evol Biol 16:635–46.
Gallesi MM, Sacchi R, Hardersen S. 2015. Does wing shape of andromorph females of *Calopteryx splendens* (Harris, 1780) resemble that of males? Int J Odonatol 18:305–15.
Garcia-Sifuentes Y, Maney DL. 2021. Reporting and misreporting of sex differences in the biological sciences. Elife 10.
Gilman CA, Corl A, Sinervo B, Irschick DJ. 2019. Genital morphology associated with mating strategy in the polymorphic lizard, *Uta stansburiana*. J Morphol 280:184–92.
Glickman SE, Cunha GR, Drea CM, Conley AJ, Place NJ. 2006. Mammalian sexual differentiation: lessons from the spotted hyena. Trends Endocrinol Metab 17:349–56.
Gowaty PA, Kim Y-K, Anderson WW. 2012. No evidence of sexual selection in a repetition of Bateman's classic study of *Drosophila melanogaster*. Proceedings of the National Academy of Sciences 109:11740–45.
Goymann W, Brumm H. 2018. Let's Talk About Sex - Not Gender. Bioessays 40:e1800030.
Goymann W, Moore IT, Oliveira RF. 2019. Challenge Hypothesis 2.0: a fresh look at an established idea. Bioscience 69:432–42.
Goymann W, Wingfield JC. 2014. Male-to-female testosterone ratios, dimorphism, and life history—what does it really tell us? Behav Ecol 25:685–99.
Goymann W, Wittenzellner A, Schwabl I, Makomba M. 2008. Progesterone modulates aggression in sex-role reversed female African black coucals. Proc Biol Sci 275:1053–60.
Gray SM, McKinnon JS. 2007. Linking color polymorphism maintenance and speciation. Trends Ecol Evol 22:71–79.
Greives T, Eshleman M, Galante H, Elderbrock E, Deimel C, Hau M. 2021. Early nighttime testosterone peaks are correlated with GnRH-induced testosterone in a diurnal songbird. Gen Comp Endocrinol 312:113861.
Griffiths PE. 2021. What are biological sexes? PhilSci Archive.
Grimstad F, Boskey ER, Taghinia A, Estrada CR, Ganor O. 2021. The role of androgens in clitorophallus development and possible applications to transgender patients. Andrology 9:1719–28.
Gross MR. 1982. Sneakers, satellites and parentals: Polymorphic mating strategies in North American sunfishes. Z Tierpsychol 60:1–26.
Gross MR. 1996. Alternative reproductive strategies and tactics: diversity within sexes. Trends Ecol Evol 11:92–98.
Gross MR. 2005. The evolution of parental care. Q Rev Biol 80:37–45.
Hales KG. 2020. Signaling inclusivity in undergraduate biology courses through deliberate framing of genetics topics relevant to gender identity, disability, and race. CBE Life Sci Educ 19:es2.
Hedrick PW, Tuttle EM, Gonser RA. 2018. Negative-assortative mating in the white-throated sparrow. J Hered 109:223–31.
Hoekstra RF. 1990. The evolution of male-female dimorphism: Older than sex? J Genet 69:11–15.

bioRxiv preprint doi: https://doi.org/10.1101/2023.01.26.525769; this version posted January 27, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Hofer H, East ML. 1995. Virilized sexual genitalia as adaptations of female spotted hyaenas. Rev Suisse Zool 102.

Holleley CE, O'Meally D, Sarre SD, Marshall Graves JA, Ezaz T, Matsubara K, Azad B, Zhang X, Georges A. 2015. Sex reversal triggers the rapid transition from genetic to temperature-dependent sex. Nature 523:79–82.

Hugall AF, Stuart-Fox D. 2012. Accelerated speciation in colour-polymorphic birds. Nature 485:631–34.

Hurst LD. 1996. Why are there only two sexes? Proceedings of the Royal Society of London Series B: Biological Sciences 263:415–22.

Huyghe K, Husak JF, Herrel A, Tadić Z, Moore IT, Van Damme R, Vanhooydonck B. 2009. Relationships between hormones, physiological performance and immunocompetence in a color-polymorphic lizard species, *Podarcis melisellensis*. Horm Behav 55:488–94.

Iwasa Y, Sasaki A. 1987. Evolution of the number of sexes. Evolution 41:49–65.

Jaffe K. 1996. The dynamics of the evolution of sex: Why the sexes are, in fact, always two? Interciencia 21:259-67.

Jarne P, Auld JR. 2006. Animals mix it up too: The distribution of self-fertilization among hermaphroditic animals. Evolution 60:1816–24.

Jaylet A, Ferrier V. 1978. Experimental gynogenesis in the newt species *Pleurodeles waltlii* and *P. poireti*. Chromosoma 69:65–80.

Jukema J, Piersma T. 2006. Permanent female mimics in a lekking shorebird. Biol Lett 2:161–64.

Just W, Baumstark A, Hameister H, Schreiner B, Reisert I, Hakhverdyan M, Vogel W. 2002. The sex determination in *Ellobius lutescens* remains bizarre. Cytogenet Genome Res 96:146–53.

Kamath A, Velocci B, Wesner A, Chen N, Formica V, Subramaniam B, Rebolleda-Gómez M. 2022. Nature, data, and power: how hegemonies shaped this special section. Am Nat 200:81–88.

Kappeler PM, Benhaiem S, Fichtel C, Fromhage L, Höner OP, Jennions MD, Kaiser S, Krüger O, Schneider JM, Tuni C, van Schaik J, Goymann W. 2022. Sex roles and sex ratios in animals. Biol Rev Camb Philos Soc, early view.

Karkazis K. 2019. The misuses of "biological sex." Lancet 394:1898–99.

Kokko H, Jennions MD. 2008. Parental investment, sexual selection and sex ratios. J Evol Biol 21:919–48.

Kothe E. 1996. Tetrapolar fungal mating types: sexes by the thousands. FEMS Microbiol Rev 18:65–87.

Küpper C, Stocks M, Risse JE, Dos Remedios N, Farrell LL, McRae SB, Morgan TC, Karlionova N, Pinchuk P, Verkuil YI, Kitaysky AS, Wingfield JC, Piersma T, Zeng K, Slate J, Blaxter M, Lank DB, Burke T. 2016. A supergene determines highly divergent male reproductive morphs in the ruff. Nat Genet 48:79–83.

Kuroiwa A, Ishiguchi Y, Yamada F, Shintaro A, Matsuda Y. 2010. The process of a Y-loss event in an XO/XO mammal, the Ryukyu spiny rat. Chromosoma 119:519–26.

Kutschera U. 2016. Sex versus gender in sea urchins and leeches two centuries after Lamarck 1816. J Marine Sci Res Dev 6:5.

Lamichhaney S, Fan G, Widemo F, Gunnarsson U, Thalmann DS, Hoeppner MP, Kerje S, Gustafson U, Shi C, Zhang H, Chen W, Liang X, Huang L, Wang J, Liang E, Wu Q, Lee SM-Y, Xu X, Höglund J, Liu X, Andersson L. 2016. Structural genomic changes underlie alternative reproductive strategies in the ruff (*Philomachus pugnax*). Nat Genet 48:84–88.

Lampert KP. 2008. Facultative parthenogenesis in vertebrates: reproductive error or chance? Sex Dev 2:290–301.

Lampert KP, Schartl M. 2010. A little bit is better than nothing: the incomplete parthenogenesis of salamanders, frogs and fish. BMC Biol 8:78.

bioRxiv preprint doi: https://doi.org/10.1101/2023.01.26.525769; this version posted January 27, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Lank DB, Smith CM, Hanotte O, Burke T, Cooke F. 1995. Genetic polymorphism for alternative mating behaviour in lekking male ruff *Philomachus pugnax*. Nature 378:59–62.

Lattanzio MS, Miles DB. 2016. Trophic niche divergence among colour morphs that exhibit alternative mating tactics. R Soc Open Sci 3:150531.

Lee SC, Ni M, Li W, Shertz C, Heitman J. 2010. The evolution of sex: A perspective from the fungal kingdom. Microbiol Mol Biol Rev 74:298–340.

Leonard JL, Westfall JA, Pearse JS. 2007. Phally polymorphism and reproductive biology in *Ariolimax (Ariolimax) buttoni* (Pilsbry and Vanatta, 1896) (Stylommatophora: Arionidae)*. Malb 23:121–35.

Lipshutz SE, Rosvall KA. 2020a. Neuroendocrinology of Sex-Role Reversal. Integr Comp Biol 60:692–702.

Lipshutz SE, Rosvall KA. 2020b. Testosterone secretion varies in a sex- and stage-specific manner: Insights on the regulation of competitive traits from a sex-role reversed species. Gen Comp Endocrinol 292:113444.

Lorenzi V, Earley RL, Rodgers EW, Pepper DR, Grober MS. 2008. Diurnal patterns and sex differences in cortisol, 11-ketotestosterone, testosterone, and 17beta-estradiol in the bluebanded goby (*Lythrypnus dalli*). Gen Comp Endocrinol 155:438–46.

Manan H, Noor Hidayati AB, Lyana NA, Amin-Safwan A, Ma H, Kasan NA, Ikhwanuddin M. 2022. A review of gynogenesis manipulation in aquatic animals. Aquaculture and Fisheries 7:1–6.

Maney DL, Merritt JR, Prichard MR, Horton BM, Yi SV. 2020. Inside the supergene of the bird with four sexes. Horm Behav 126:104850.

Mank JE. 2022. Sex-specific morphs: the genetics and evolution of intra-sexual variation. Nat Rev Genet 1–9.

McLean CA, Stuart-Fox D. 2014. Geographic variation in animal colour polymorphisms and its role in speciation. Biol Rev Camb Philos Soc 89:860–73.

Miyagi M, Guthman EM, Sun SED-K. 2021. Transgender rights rely on inclusive language. Science 374:1568–69.

Mokos J, Scheuring I, Liker A, Freckleton RP, Székely T. 2021. Degree of anisogamy is unrelated to the intensity of sexual selection. Sci Rep 11:19424.

Monk JD, Giglio E, Kamath A, Lambert MR, McDonough CE. 2019. An alternative hypothesis for the evolution of same-sex sexual behaviour in animals. Nat Ecol Evol 3:1622–31.

Moore EC, Cicotto PJ, Peterson EN, Lamm MS, Albertson RC, Roberts RB. 2022. Polygenic sex determination produces modular sex polymorphism in an African cichlid fish. Proceedings of the National Academy of Science 119:e2118574119.

Muck C, Goymann W. 2011. Throat patch size and darkness covaries with testosterone in females of a sex-role reversed species. Behav Ecol 22:1312–19.

Packer M, Lambert MR. 2022. What's gender got to do with it? Dismantling the human hierarchies in evolutionary biology and environmental toxicology for scientific and social Progress. Am Nat 200:114–28.

Panico S, Pisani P, Muti P, Recchione C, Cavalleri A, Totis A, Berrino F. 1990. Diurnal variation of testosterone and estradiol: a source of bias in comparative studies on breast cancer. J Endocrinol Invest 13:423–26.

Parker JD. 2004. A major evolutionary transition to more than two sexes? Trends Ecol Evol 19:83–86.

Paulson DR. 1998. Possible morphological and behavioral male mimicry in a libellulid dragonfly, *Erythrodiplax umbrata* (L.)(Anisoptera: Libellulidae). Odonatologica 27:249–52.

Pérez i de Lanuza G, Font E, Carazo P. 2013. Color-assortative mating in a color-polymorphic lacertid lizard. Behav Ecol 24:273–79.

Perrin N. 2012. What uses are mating types? The "developmental switch" model. Evolution 66:947–56.

bioRxiv preprint doi: https://doi.org/10.1101/2023.01.26.525769; this version posted January 27, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Phiri-Ramongane B, Khine AA. 2022. Role of clinical laboratories in reporting results of transgender individuals on hormonal therapy. Journal of Endocrinology, Metabolism and Diabetes of South Africa 27:8–13.

Phoenix CH, Goy RW, Gerall AA, Young WC. 1959. Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. Endocrinology 65:369–82.

Piferrer F. 2021. Epigenetic mechanisms in sex determination and in the evolutionary transitions between sexual systems. Philos Trans R Soc Lond B Biol Sci 376:20200110.

Pollo P, Kasumovic MM. 2022. Let's talk about sex roles: what affects perceptions of sex differences in animal behaviour? Anim Behav 183:1–12.

Power HW. 1976. On forces of selection in the evolution of mating types. Am Nat 110:937–44.

Quigley CA, De Bellis A, Marschke KB, el-Awady MK, Wilson EM, French FS. 1995. Androgen receptor defects: historical, clinical, and molecular perspectives. Endocr Rev 16:271–321.

Quinn AE, Radder RS, Sarre SD, Georges A, Ezaz T, Shine R. 2009. Isolation and development of a molecular sex marker for *Bassiana duperreyi*, a lizard with XX/XY sex chromosomes and temperature-induced sex reversal. Mol Genet Genomics 281:665–72.

Rosvall KA, Bergeon Burns CM, Barske J, Goodson JL, Schlinger BA, Sengelaub DR, Ketterson ED. 2012. Neural sensitivity to sex steroids predicts individual differences in aggression: implications for behavioural evolution. Proc Biol Sci 279:3547–55.

Roughgarden J. 2013. Evolution's Rainbow: Diversity, Gender, and Sexuality in Nature and People Univ of California Press.

Roulin A. 2004. The evolution, maintenance and adaptive function of genetic colour polymorphism in birds. Biol Rev Camb Philos Soc 79:815–48.

Russell DGD, Sladen WJL, Ainley DG. 2012. Dr. George Murray Levick (1876–1956): unpublished notes on the sexual habits of the Adélie penguin. Polar Rec 48:387–93.

Sandkam BA, Almeida P, Darolti I, Furman BLS, van der Bijl W, Morris J, Bourne GR, Breden F, Mank JE. 2021. Extreme Y chromosome polymorphism corresponds to five male reproductive morphs of a freshwater fish. Nat Ecol Evol 5:939–48.

Sarre SD, Georges A, Quinn A. 2004. The ends of a continuum: genetic and temperature-dependent sex determination in reptiles. Bioessays 26:639–45.

Saunders PA, Perez J, Rahmoun M, Ronce O, Crochet P-A, Veyrunes F. 2014. XY females do better than the XX in the African pygmy mouse, *Mus minutoides*. Evolution 68:2119–27.

Schmidt KL, Pradhan DS, Shah AH, Charlier TD, Chin EH, Soma KK. 2008. Neurosteroids, immunosteroids, and the Balkanization of endocrinology. Gen Comp Endocrinol 157:266–74.

Seehausen O, Terai Y, Magalhaes IS, Carleton KL, Mrosso HDJ, Miyagi R, van der Sluijs I, Schneider MV, Maan ME, Tachida H, Imai H, Okada N. 2008. Speciation through sensory drive in cichlid fish. Nature 455:620–26.

Ser JR, Roberts RB, Kocher TD. 2010. Multiple interacting loci control sex determination in lake Malawi cichlid fish. Evolution 64:486–501.

Sinervo B, Lively CM. 1996. The rock–paper–scissors game and the evolution of alternative male strategies. Nature 380:240–43.

Sinervo B, Svensson E, Comendant T. 2000. Density cycles and an offspring quantity and quality game driven by natural selection. Nature 406:985–88.

Smith CA, Roeszler KN, Ohnesorg T, Cummins DM, Farlie PG, Doran TJ, Sinclair AH. 2009. The avian Z-linked gene DMRT1 is required for male sex determination in the chicken. Nature 461:267–71.

Staub NL, De Beer M. 1997. The role of androgens in female vertebrates. Gen Comp Endocrinol 108:1–24.

Stévant I, Papaioannou MD, Nef S. 2018. A brief history of sex determination. Mol Cell Endocrinol 468:3–10.

bioRxiv preprint doi: https://doi.org/10.1101/2023.01.26.525769; this version posted January 27, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

Stiver KA, Harris RM, Townsend JP, Hofmann HA, Alonzo SH. 2015. Neural gene expression profiles and androgen levels underlie alternative reproductive tactics in the ocellated wrasse, *Symphodus ocellatus*. Ethology 121:152–67.

Stuart-Fox D, Aulsebrook A, Rankin KJ, Dong CM, McLean CA. 2021. Convergence and divergence in lizard colour polymorphisms. Biol Rev Camb Philos Soc 96:289–309.

Sutou S, Mitsui Y, Tsuchiya K. 2001. Sex determination without the Y chromosome in two Japanese rodents *Tokudaia osimensis osimensis* and *Tokudaia osimensis spp*. Mamm Genome 12:17–21.

Tang-Martínez Z. 2016. Rethinking Bateman's Principles: challenging persistent myths of sexually reluctant females and promiscuous males. J Sex Res 53:532–59.

Tang-Martínez Z. 2020. The history and impact of women in animal behaviour and the ABS: a North American perspective. Anim Behav 164:251–60.

Thompson A, Kapsanaki V, Liwanag HEM, Pafilis P, Wang IJ, Brock KM. 2022. Some like it hotter: differential thermal preferences among lizard color morphs. bioRxiv.

Thorne N, Yip AK-T, Bouman WP, Marshall E, Arcelus J. 2019. The terminology of identities between, outside and beyond the gender binary - A systematic review. Int J Transgend 20:138–54.

Tillmann U, Hoppenrath M. 2013. Life cycle of the pseudocolonial dinoflagellate *Polykrikos kofoidii* (Gymnodiniales, Dinoflagellata). J Phycol 49:298–317.

Togashi T, Cox PA. 2011. The Evolution of Anisogamy: A Fundamental Phenomenon Underlying Sexual Selection. Cambridge University Press.

Trivers RL. 1972. Parental investment and sexual selection In: Campbell B, editor. Sexual selection and the descent of man, 1871–1971. Chicago: Aldine.

Tuttle EM, Bergland AO, Korody ML, Brewer MS, Newhouse DJ, Minx P, Stager M, Betuel A, Cheviron ZA, Warren WC, Gonser RA, Balakrishnan CN. 2016. Divergence and Functional Degradation of a Sex Chromosome-like Supergene. Curr Biol 26:344–50.

Vaiman D, Pailhoux E. 2000. Mammalian sex reversal and intersexuality: deciphering the sex-determination cascade. Trends Genet 16:488–94.

van der Kooi CJ, Matthey-Doret C, Schwander T. 2017. Evolution and comparative ecology of parthenogenesis in haplodiploid arthropods. Evol Lett 1:304–16.

Van Leeuwen AM, Bladh ML. 2016. Textbook of Laboratory and Diagnostic Testing: Practical Application of Nursing Process at the Bedside. F.A. Davis.

Verhulst EC, Beukeboom LW, van de Zande L. 2010. Maternal control of haplodiploid sex determination in the wasp *Nasonia*. Science 328:620–23.

Veyrunes F, Chevret P, Catalan J, Castiglia R, Watson J, Dobigny G, Robinson TJ, Britton-Davidian J. 2010. A novel sex determination system in a close relative of the house mouse. Proc Biol Sci 277:1049–56.

Walters MJ, Collado D, Harding CF. 1991. Oestrogenic modulation of singing in male zebra finches: differential effects on directed and undirected songs. Anim Behav 42:445–52.

Watts PC, Buley KR, Sanderson S, Boardman W, Ciofi C, Gibson R. 2006. Parthenogenesis in Komodo dragons. Nature 444:1021–22.

West-Eberhard MJ. 1986. Alternative adaptations, speciation, and phylogeny (a review). Proc Natl Acad Sci U S A 83:1388–92.

Whiteley SL, Holleley CE, Ruscoe WA, Castelli M, Whitehead DL, Lei J, Georges A, Weisbecker V. 2017. Sex determination mode does not affect body or genital development of the central bearded dragon (*Pogona vitticeps*). Evodevo 8:25.

Whitfield J. 2004. Everything you always wanted to know about sexes. PLoS Biol 2:e183.

Wibbels T, Crews D. 1994. Putative aromatase inhibitor induces male sex determination in a female unisexual lizard and in a turtle with temperature-dependent sex determination. J Endocrinol 141:295–99.

Wiggins JM, Santoyo-Brito E, Scales JB, Fox SF. 2020. Gene dose indicates presence of sex

bioRxiv preprint doi: https://doi.org/10.1101/2023.01.26.525769; this version posted January 27, 2023. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY-NC-ND 4.0 International license.

chromosomes in collared lizards (*Crotaphytus collaris*), a species with temperature-influenced sex determination. Herp 76:27–30.

Wingfield JC, Goymann W, Jalabert C, Soma KK. 2019. Concepts derived from the Challenge Hypothesis. Horm Behav 115:104550.

Wingfield JC, Hegner RE, Dufty AM, Ball GF. 1990. The “Challenge Hypothesis”: theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. Am Nat 136:829–46.

Woitowich NC, Beery A, Woodruff T. 2020. A 10-year follow-up study of sex inclusion in the biological sciences. Elife 9.

Yoshizawa K, Ferreira RL, Kamimura Y, Lienhard C. 2014. Female penis, male vagina, and their correlated evolution in a cave insect. Curr Biol 24:1006–10.

Zhao L, Quinn A, Ng ET, Veyrunes F, Koopman P. 2017. Reduced activity of SRY and its target enhancer Sox9-TESCO in a mouse species with X*Y sex reversal. Sci Rep 7:41378.