Sex-specific morphs: the genetics and evolution of intra-sexual variation

Judith E. Mank [ID]

Abstract | Sex-specific morphs exhibit discrete phenotypes, often including many disparate traits, that are observed in only one sex. These morphs have evolved independently in many different animals and are often associated with alternative mating strategies. The remarkable diversity of sex-specific morphs offers unique opportunities to understand the genetic basis of complex phenotypes, as the distinct nature of many morphs makes it easier to both categorize and compare genomes than for continuous traits. Sex-specific morphs also expand the study of sexual dimorphism beyond traditional bimodal comparisons of male and female averages, as they allow for a more expansive range of sexualization. Although ecological and endocrinological studies of sex-specific morphs have been advancing for some time, genomic and transcriptomic studies of morphs are far more recent. These studies reveal not only many different paths to the evolution of sex-specific morphs but also many commonalities, such as the role of sex-determining genes and hormone signalling in morph development, and the mixing of male and female traits within some morphs.

Alternative mating strategies
Different reproductive behaviours used by male or female animals. Sometimes referred to as alternative reproductive tactics.

Many animal species exhibit multiple sex-specific morphs, that is, discrete phenotypes observed in only one sex (FIG. 1). Sex-specific morphs go beyond polymorphism in any one trait and typically include many different traits, spanning such disparate characteristics as behaviour, physiology, colour and body size[1,2]. Sex-specific morphs represent a form of sexual dimorphism and, therefore, must arise from differences in selection as it acts on females and males. However, sex differences in selection typically result in bimodal phenotypic differences between males and females, and sex-specific morphs represent an expansion beyond this dichotomous view of sexual diversity[3].

Although ecological pressures can differ between the sexes[4], differences in reproductive interests are by far the greatest source of sex differences in selection[5]. It is therefore perhaps not surprising that most sex-specific morphs are associated with alternative mating strategies[2,3], and there are many evocative examples (TABLE 1).

The remarkable diversity of sex-specific morphs offers unique opportunities to understand the genetic basis of complex phenotypes, as the discrete nature of many morphs makes it easier to both categorize and compare genomes and transcriptomes than for continuous traits. They also offer a potentially valuable system to study the way that groups of otherwise unrelated traits become genetically linked, either transcriptionally or physically. Beyond this, sex-specific morphs allow for the study of the forces needed to generate and maintain diversity. Sex-specific morphs can also offer important

insights into how phenotypes can be limited in expression in one sex or the other, a particularly timely model given recent imperatives to better understand sex-specific biology[6–9]. Finally, because sex-specific morphs often display a combination of both male and female traits, they offer a route to expand studies of sexual diversity beyond bimodal male–female comparisons, mirroring in many ways recent societal interest in understanding human sexual diversity.

Ecological and endocrinological studies of sex-specific morphs have been advancing for some time. By contrast, genomic and transcriptomic studies of morphs have emerged far more recently. These studies are revealing many different paths to the evolution of sex-specific morphs and make it possible to study complex selection dynamics at the molecular level.

In this Review, I summarize several fascinating examples of sex-specific morphs, then briefly review theories about their evolutionary origin as well as their development. After discussing recent case studies that have determined the genomic and transcriptomic basis of morph diversity and synthesizing their common findings, I conclude with open questions for the study of these remarkable systems.

Examples of sex-specific morphs
Dimorphic systems. Wild turkeys (*Meleagris gallopavo*) are co-operative breeders with two male morphs. Dominant males display directly to females during the mating season and show more exaggerated
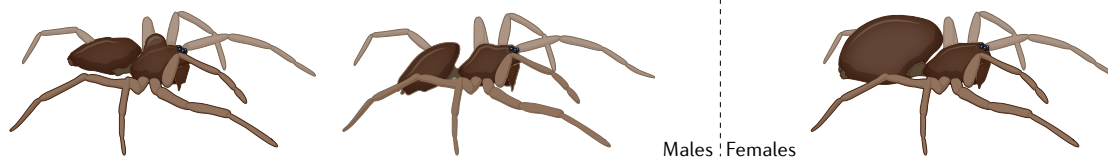
Department of Zoology and Biodiversity Research Centre, University of British Columbia, Vancouver, Canada.

e-mail: mank@zoology.ubc.ca

a Scarab beetle (*Onthophagus taurus*)

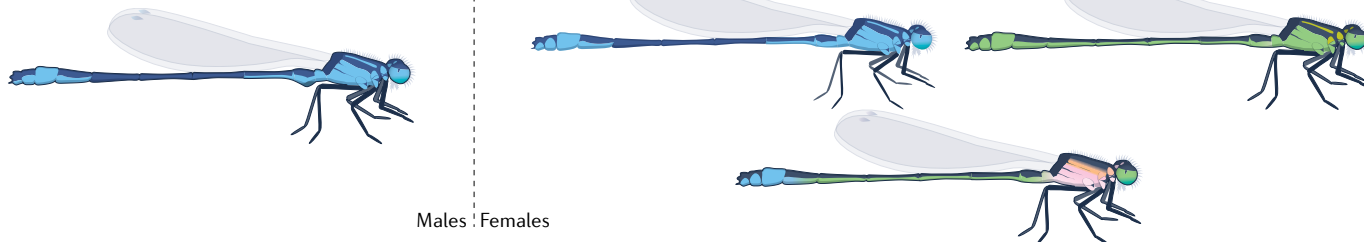Males ┊ Females

b Dwarf spider (*Oedothorax gibbosus*)

Males ┊ Females

c Side-blotched lizard (*Uta stansburiana*)

Males ┊ Females

d Blue-tailed damselfly (*Ishinura elegans*)

Males ┊ Females

e Swallowtail butterfly (*Papilio polytes*)

Males ┊ Females

f Para guppy (*Poecilia parae*)

Males ┊ Females
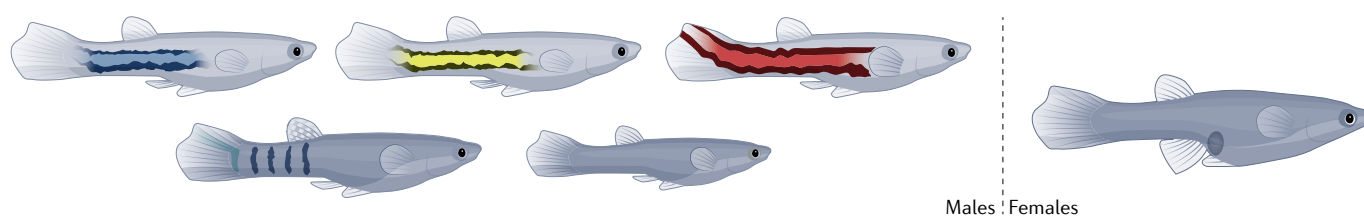
Fig. 1 | **Examples of sex-specific morphs. a** | Scarab beetle (*Onthophagus taurus*). Males from left to right: horned, hornless. **b** | Dwarf spider (*Oedothorax gibbosus*). Males, left to right: hunched, flat. **c** | Side-blotched lizard (*Uta stansburiana*). Males from left to right: orange (large territory), blue (small territory), yellow (sneaker). **d** | Blue-tailed damselfly (*Ishinura elegans*). Females from left to right: androchrome (andromorph), rufenscens-obsoleta, infuscans. **e** | Swallowtail butterfly (*Papilio polytes*). Males are monomorphic within populations, but two morphs exist: alphenor (top) and polytes (bottom). Females from left to right: cyrus (andromorph, alphenor top, polytes bottom), polytes (Batesian mimic of *Pachliopta aristolochiae*), romulus (Batesian mimic of *Pachliopta hector*) and theseus (Batesian mimic of *P. aristolochiae*, black form). **f** | Para guppy (*Poecilia parae*). Males from left to right, top row: blue melanzona, yellow melanzona, red melanzona; bottom row: parae and immaculata (gynomorph).

Sexually selected traits
Traits that help to increase reproductive fitness. Somatic sexually selected traits, such as songs or bright colours, can help to attract mates, or, like antlers, can be used in male–male competition for access to mates. Gonadal sexually selected traits often relate to sperm competition after mating.

Caruncles
Fleshy tuberosities that can be present on head, neck, cheeks or throat of some birds. In the case of turkeys, caruncles include the wattle and the snood, an erectile protuberance on the forehead.

Sneaker male
A male that lacks somatic sexually selected traits and mates primarily via stealth.

Territorial males
Sometimes called bourgeois or alpha males. A male reproductive strategy that is typically associated with somatic sexually selected traits, such as bright colours and mating behaviours, to attract females to a specific defended territory or nesting site.

Helper male
Sometimes called a satellite male in birds and fish. A male morph that lacks sexually selected traits and behaviours, and which assists a territorial male in nest defence and care in exchange for a limited number of fertilization events.

Heritable
A phenotype that is at least partially transmitted genetically from parents to offspring.

Allometry
The scaling relationships between size and shape across different parts of the body.

sexually selected traits, including more vivid head and neck colouration, and more exaggerated caruncles compared with subordinate males[10]. Subordinate brothers cooperate to defend their family's position on the mating grounds against other groups of males and encourage females to mate with their dominant brother[11]. Similar in some ways to subordinate male turkeys, sneaker male morphs exist in many species, such as in the scarab beetle (*Onthophagus taurus*)[12]. Sneaker scarab beetles, like subordinate male turkeys, do not display key sexually selected somatic traits, in this case a horn (FIG. 1a). But, in contrast to subordinate turkey males, which do not breed, sneakers mate by stealth, and although they tend to have small body size relative to territorial males, they often have a very large testes-to-body size ratio so as to maximize fertilization from stolen mating events[3,13].

More complex morph systems. Some species, such as the ruff (*Philomachus pugnax*)[14,15] and the ocellated wrasse (*Symphodus ocellatus*)[16], have three male morphs. Like the dominant male turkey, territorial male morphs defend the nesting site against other males and often invest in somatic and behavioural traits that attract females. A helper male, or satellite male, lacks somatic sexually selected traits but assists the territorial male in territorial defence and/or parental care and, in exchange, obtains a fraction of the fertilization events at the site. Because the sneaker morph invests nothing in territorial defence, parental care or somatic sexually selected traits, it is able to redirect resources into sperm production. The ruff and the ocellated wrasse also make an interesting contrast, as although both species exhibit similar morphs, the underlying genetics differ. Although males of the ocellated wrasse can transition between morph phenotypes over their life cycle[16], the morphs of the ruff are heritable and determined at conception based on genotype[14,15]. There are other variations on three-morph systems, such as in the side-blotched lizard (*Uta stansburiana*), which are also highly heritable[17]. Instead of a satellite morph, this species contains two territorial morphs, one with large territories and one with small, the latter referred to as a mate guarder, as well as a sneaker morph, and each morph is associated with a distinct head colour (FIG. 1c).

Many examples of male dimorphism and trimorphism exist, but there are fewer examples of more complex systems. The tawny dragon (*Ctenophorus decresii*) exhibits four male morphs, which differ in throat colour, aggression and anti-predator behaviour[18,19]. Two species of stag beetle (*Odontolabis sommeri lowei* and *Odontolabis brookeana*) are also tetramorphic, with

alpha, beta, gamma and bolt-cutter males, the latter named because the mandibles resemble a bolt-cutter tool. The stag beetle morphs differ in body size and mandible shape and allometry[20]. Like the scarab beetle horns, stag beetle mandibles are used as weapons in male mating contests, and the different-shaped mandibles of the four morphs represent different competitive strategies.

The largest number of co-occurring clearly discrete morphs may be in the pentamorphic para guppy (*Poecilia parae*), which has a highly aggressive male morph that mates via coercion, a female mimic, or gynomorph, that mates via stealth, and three males with different levels of aggression that entice females through mate preference (FIG. 1f). Each of these morphs is associated with complex differences in size, behaviour, colour and sperm morphology[21–23], and each morph is Y-linked and therefore inherited perfectly from father to son[24,25].

Female-specific morphs. There are female morphs as well, most notably the castes of social insects[26], which represent specialization of labour within the super-organism. Female-limited reproductive morphs are arguably less common than male morphs, and result in some cases from ecological selection[27], and in others from male mating harassment. In many damselflies, such as *Ishinura elegans*[28–30] (FIG. 1d), some females exhibit a male-mimicking andromorph phenotype. Similarly, some swallowtail butterflies in the genus *Papilio* exhibit several female-specific mimetic polymorphisms (FIG. 1e). In this case, some females are Batesian mimics, likely resulting from ecological selection, and some are andromorphs, whereas males are monomorphic within populations and non-mimetic[27,31,32]. In both damselflies and swallowtails, andromorphs may benefit from reduced sexual harassment. However, whereas male morphs, including sneakers, territorial and female mimics, have evolved many times independently across vertebrates and invertebrates, female andromorphs seem to have evolved far less often. The reduced prevalence of female morphs compared with male morphs is likely a function of the directionality of sexual selection. Sex-specific morphs are often associated with mating systems with a high level of sexual selection, resulting from substantial variance in male fitness[13]. Sexual selection is typically more prevalent in males, and this may create more reproductive niches[33] in males than females, which are then filled by various reproductive morphs.

Origin of morph diversity

Sex-specific morphs likely result from disruptive selection, whereby extreme phenotypes have higher reproductive fitness than intermediate ones. What is unusual in the case of sex-specific morphs is that disruptive selection acts on normal continuous phenotypic variation for multiple traits simultaneously[33,34] and in only one sex. For example, in the case of dimorphic species with territorial and sneaker male morphs, the advantage to sneakers comes from being both small and unobtrusive, so as to gain access to females without notice. At the same time, because fertilization success often largely resembles a raffle competition, with each sperm cell representing a raffle ticket[35], sneaker morphs benefit from having

Table 1 | **Examples of sex-specific morphs**

Species	Notes	Heritable?
Dimorphism		
Wild turkey (*Meleagris gallopavo*)	Co-operative breeder with dominant (breeding) and subordinate (non-breeding) males[11]	No, dominance among sibling males is established via fights just before sexual maturation[10]
Scarab beetle (*Onthophagus taurus*) and rhinoceros beetle (*Trypoxylus dichotomus*)	Territorial and sneaker morphs associated with size of horns, which are important in male–male competition[12,60]	No, morph associated with developmental diet[60,68]
Bluehead wrasse (*Thalassoma bifasciatum*)	Terminal (territorial) males are larger and exhibit sexually selected colouration and behaviours. Initial phase males are female mimics	No, sequential hermaphrodite, individuals mature as either females or initial phase males, and both can become terminal phase males later in life under the right social conditions[69]
Peacock blenny (*Salaria pavo*)	Territorial males are larger and have sexually selected somatic traits in contrast to female-mimic males	No, males transition from female-mimic to territorial phenotypes as they grow larger with age[70]
Dwarf spider (*Oedothorax gibbosus*)	Hunched males exhibit cephalic ornaments used in nuptial feeding, while flat males lack these features and mature earlier to gain an initial reproductive advantage[33]	Yes[54]
Trimorphism		
Side-blotched lizard (*Uta stansburiana*)	Males with large territories, small territories and sneaker. Throat colour co-varies with reproductive strategy[17]	Yes[71]
Ruff (*Philomachus pugnax*)	Territorial 'independent' males, satellite helper males and sneaker 'faeder' males[72]	Yes, morphs associated with large autosomal inversions[14,15]
Ocellated wrasse (*Symphodus ocellatus*)	Territorial, satellite and sneaker males[16]	No, males can change their morph throughout the life cycle[16]
Blue-tailed damselfly (*Ishinura elegans*)	Andromorph females mimic males in all aspects except genitalia. Two other female morphs differ in colour[73]	Yes, Mendelian[74]
Tetramorphism		
Tawny dragon lizard (*Ctenophorus decresii*)	Male morphs differ in throat colour and aggression level[19]	At least partially[18]
Stag beetle (*Odontolabis sommeri lowei* and *Odontolabis brookeana*)	Alpha and beta males are large with long mandibles that differ in shape, gamma males are small and likely represent sneaker morphs, and bolt-cutter males have wide mandibles that may give them an advantage in some mating contests[20]	Gamma male morph is likely facultative based on body size at pupation, and other three males may be at least in part heritable[20]
Swallowtail butterfly (*Papilio polytes*)	Three female mimetic and one andromorph. Males are monomorphic within populations and non-mimetic	Yes, based on alleles of the *doublesex* locus[2,27,32]
Pentamorphism		
Para guppy (*Poecilia parae*)	Male morphs include a female mimic, coercive male and three separate male morphs that mate via female choice[21–23]	Yes, Y-linked[24,25]

Female mimic
Also called a gynomorph. A male morph that exhibits female somatic phenotypes.

Andromorph
Also called a male mimic, a female morph that exhibits many male somatic phenotypes.

Batesian mimics
Organisms that mimic the warning colouration of a noxious model, thereby gaining protection from predators.

Disruptive selection
A form of selection in which extreme phenotypes are more fit than intermediate forms.

enlarged testes to produce enormous quantities of sperm, or more tickets in the fertilization raffle, to maximize reproductive success, and therefore their fitness, from limited opportunities[36] (FIG. 2). Being small without high sperm production is not as advantageous, as the male would be unable to quickly flood another male's nest or a female's reproductive tract with gametes to maximize the benefit from rare stolen fertilization events.

For a territorial male, disruptive selection acts in the opposite direction to that for the sneaker male. Being large is often key to defending the territory against other males, and displaying additional traits that attract females increases mating opportunities. Because energy is finite, territorial males cannot invest in all traits. Redirecting energy from somatic growth towards sperm production[2,37] would likely reduce reproductive fitness, as the male would be unable to defend and maintain a territory. Similarly, redirecting energy from female preference traits to spermatogenesis would likely reduce reproductive fitness, as the male would be unable to attract females for mating.

Thus, for both territorial and sneaker males, intermediate forms, or forms with mismatched traits, are less fit. The morph-specific combinations of different extreme phenotypes allow that sex to occupy a greater range of reproductive niches within the fitness landscape. In this framework, the genetic and evolutionary questions about how to maintain the integrity of the suites of characters associated with morphs are similar to questions about local adaptation of multiple traits in the face of gene flow between populations. However, the case of morphs is complicated in that multiple morphs exist within the same, interbreeding, population and the traits are present in only one sex.

How to make a morph
The disruptive selection that acts on multiple traits in morphs leads to shifts in the relationships between those traits. Many traits co-vary — for example, horn or mandible length in beetles co-varies with body size[20,37,38], so larger beetles will also have larger horns or mandibles. Because beetle horns or mandibles are often used

as weapons in male–male competition for mates, the size of these weapons is crucial to different reproductive niches. Therefore, to occupy different reproductive niches, different morphs exhibit different allometric relationships between horn or mandible length and body size[20,38] (FIG. 3).

Body size and horn or mandible size can be thought of as two modules, encoded by different suites of genes, of the body that are developmentally integrated in various ways across morphs to prevent mismatching of traits. Similarly, relative testes size, and therefore sperm count, also varies with body size in species, and the slope of this relationship can differ across male morphs[39], representing another integrated module of morph phenotype. Some phenotypic traits, such as mating behaviour, are less continuous than body size, but they also represent modules that are integrated into the overall morph phenotype.

Morphs represent a range of sexualization. Sexual dimorphism, differences between males and females, also often results from differences in allometric relationships[40,41]. Interestingly, many intra-sexual morphs represent a mixture of male and female allometries. For example, the hornless sneaker beetle males often resemble females for that trait[38,42,43] (FIG. 1a), but are otherwise phenotypically

Relative testes size
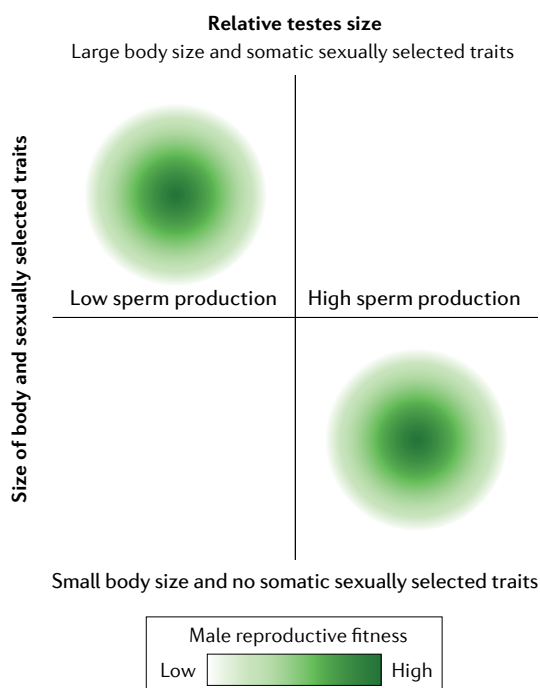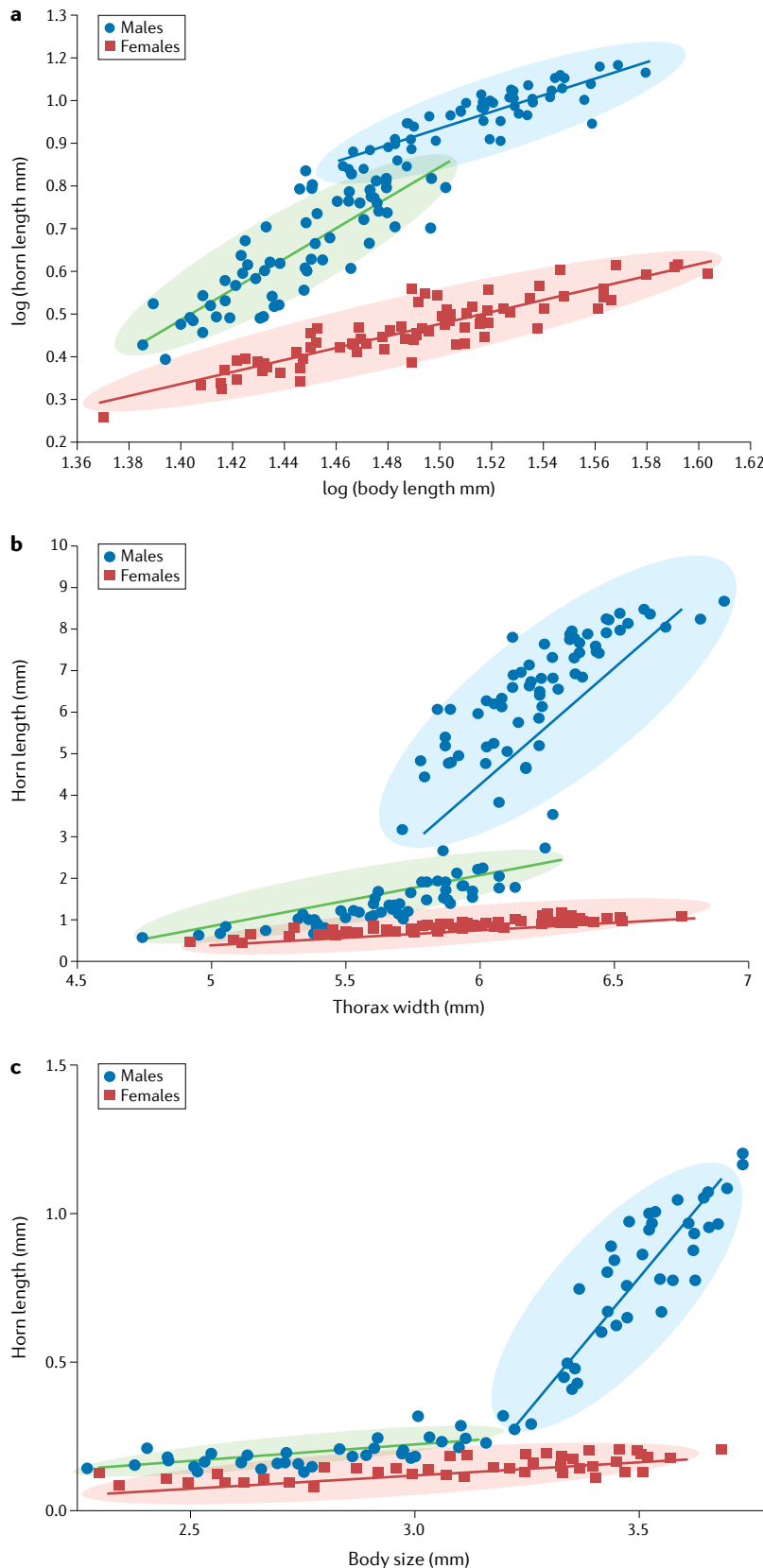Large body size and somatic sexually selected traits

Fig. 2 | **Disruptive selection for extreme male traits results in different reproductive morphs.** Example case of a species with two male morphs, a territorial male and a sneaker. Sneakers benefit from being both small and unobtrusive, and having enlarged testes to produce enormous quantities of sperm to maximize reproductive success. Selection acts in the opposing direction for territorial males, for whom large body size is key to defending the territory against other males, and secondary sexually selected traits attract females. Redirecting investment in either of these traits towards sperm production reduces reproductive fitness for territorial males.

male (FIG. 3). More extreme examples are andromorphs and gynomorphs, which mimic the other sex for many somatic traits but have the gonad of their genetic sex. Therefore, some morphs represent an expansion of the bimodal relationship often observed in males and females and can sometimes be thought of as representing variation in the degree of sexualization, and even different mix-and-match combinations of male and female traits[44].

This variation in sexualization is evident in transcriptome studies of morphs. Many genes differ in expression between males and females, and these sex-biased genes are thought to be related to phenotypic sex differences[45]. Transcriptome studies of intra-sexual morphs reveal a range of sex-biased gene expression. For example, subordinate male turkeys show a pattern of demasculinization in gene expression compared with dominant males, consistent with their phenotype[44]. More simply stated, subordinate males are clearly male, but somewhat less transcriptionally masculine than dominant males.

Mixing and matching male and female traits. Other transcriptome studies reveal greater ranges of sexualization. Female-mimicking male morphs in the bluehead wrasse (*Thalassoma bifasciatum*)[46] and in the peacock blenny (*Salaria pavo*)[47] show greater transcriptional similarity to females in the brain compared with territorial males, as do satellite males in the ocellated wrasse[48]. Female morphs show a similar pattern, with andromorph females in the blue-tailed damselfly transcriptionally more similar to males than to either of the other female morphs[49].

Interestingly, gonad transcriptomes show very different patterns compared with those of the brain[48] among ocellated wrasse morphs, reflecting differences in investment in sperm production[48,50] and illustrating how a morph can comprise mix-and-match combinations of male and female traits. This mix-and-match combination of male and female traits is also evident in *O. taurus* transcriptomes, in which the horn tissue of sneaker (hornless) males is transcriptionally more similar to that of females than that of large-horned (territorial) males. By contrast, the leg tissue of male morphs is more similar to each other than either is to female leg tissue[51].

Heritable morphs
Pedigree analysis of morph phenotypes has been used for some time to determine whether morphs are heritable or not (TABLE 1). Only recently have genomic comparisons across heritable morphs been used to determine the genetic basis of morph phenotypes. Many of these studies have linked morph phenotypes to supergenes, regions of chromosomes, typically inverted, that contain several genes. The inversions prevent recombination and presumably keep alleles at multiple genes underlying each morph linked together to prevent mismatching of traits[52].

Sex determination supergenes. Many sexual dimorphisms are linked to the sex determination pathway, reviewed in[53]. It is therefore perhaps not surprising that genes in the sex determination pathway have been

a | *Oryctes nasicorn.* (plot with log body length vs log horn length)

b | *Onthophagus nigriventris* (plot with Thorax width vs Horn length)

c | *Onthophagus acuminatus* (plot with Body size vs Horn length)

Fig. 3 | **Allometric relationships between and within the sexes for beetle horn and body size.** Horn size scales with body size, but the slope of the relationship differs between large-horned territorial and small-horned sneaker males, and the latter can exhibit a similar allometric relationship to females. **a** | *Oryctes nasicorn.* **b** | *Onthophagus nigriventris.* **c** | *Onthophagus acuminatus.* Females in red, large-horned males in blue, small-horned males in green. Part **a** adapted with permission from REF.[38], OUP. Part **b** adapted from REF.[43], CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/). Part **c** adapted with permission from REF.[75], PNAS.

3 Mb autosomal genomic fragment that contains, among other genes, an extra copy of the key sex-determining gene *doublesex*[54]. For many genes, copy number is related to expression level[55,56], which is also true for *doublesex* in the hunched morph. Hunched males with the *doublesex*-containing fragment have higher total *doublesex* expression than flat males, which lack the extra copy, and elevated *doublesex* expression likely leads to the masculinized hunched morph through the regulation of several downstream genes. Interestingly, *doublesex* is expressed less in females than in either male morph, regardless of whether she carries the hunched male genomic fragment, and this likely represents overarching regulatory control of *doublesex* through sex hormones or other aspects of the sex determination pathway. In the case of dwarf spiders, activation is limited to males, and once activated, expression level is related to copy number.

The pentamorphic para guppy, *P. parae* also represents an interesting case of the interplay between supergenes and sex determination. In this case, each of the five male morphs is associated with a distinct Y chromosome[24,25], ensuring that all the sons from a male share his phenotype. Although the sex-determining gene in this species is not yet known, the presence of a Y chromosome determines maleness. Further work in this system will no doubt reveal more about the interplay between sex determination and the genes that underlie these morphs.

The three male morphs of the ruff, *P. pugnax*, are encoded by large autosomal inversions[14,15,57]. In this case, the inversions associated with both satellite and sneaker (faeder) males are dominant over territorial males, and both inversions are lethal in the homozygous state. Independent males exhibit elevated testosterone levels compared with satellites and faeders[14], consistent with their masculinized phenotype. The gene content of these inversions has recently been annotated[57] but is not yet available, and future work will reveal whether the testosterone differences are due to differences in copy number of sex-determining genes within the inversions.

The andromorph females in *Papilio polytes* are also associated with inversions, but in this case, the inversions are contained within a single gene[27,32]. Supergenes are typically thought to physically link alleles at multiple loci, and instead of linking multiple genes, this supergene is comprised of two inversions, one at either end of the gene. More importantly, the gene that contains the inversions that differentiates andromorphs from mimetic females is *doublesex*, the same gene that apparently controls male morphs in dwarf spiders. However, in contrast

implicated in some morph-associated supergenes, again revealing a link between morphs and sexualization. For example, in the dwarf spider (*Oedothorax gibbosus*; TABLE 1 and FIG. 1b), hunched males are encoded by a

to dwarf spiders, in which *doublesex* expression level is key to male morphs, in swallowtails, different alleles of *doublesex* result in different isoforms, or splice variants, of the gene in andromorph versus mimetic females, which in turn affect expression of many downstream genes.

Balancing selection and the maintenance of morph genetic diversity. Once generated via disruptive selection, morphs that are at least partially heritable are thought to be maintained through balancing selection[17,33], whereby the fitness of each morph is related to the frequency of the others[17,30]. To go back to the two-morph example of territorial and sneaker males, the fitness of territorial males is inversely related to the frequency of sneakers — as more and more sneakers parasitize a territorial male's nest or territory, the proportion of offspring in that nest or territory that he sires decreases. At the same time, sneaker males are reproductive parasites of territorial males, and require the nest or territory provided by territorial males to reproduce. As the frequency of territorial males decreases, so too does the fitness of sneaker males. The resulting frequency of each morph is therefore balanced by the frequency of the other. Put another way, the mean fitness of sneakers relative to territorial males decreases as the frequency of sneakers increases, maintaining a balance in the frequency of alleles underlying each morph type.

The complex dynamics needed to generate and maintain multiple heritable morphs might be expected to result primarily in dimorphic or trimorphic systems. Indeed, the number of tetramorphic examples is relatively small (TABLE 1). However, the para guppy (*P. parae*) exhibits five heritable morphs, each with distinct colour and mating tactics[22,24]. The five Y-linked morphs are maintained via balancing selection that involves a complex interaction of female preference, male–male competition and predation[21–23], suggesting that more morphs are possible when selection dynamics are governed by multiple forces.

Facultative morphs
Many morphs are largely determined on the basis of environmental or social conditions. Although there can be some heritable aspects that influence morph determination, in these cases, technically all individuals have the potential to exhibit any morph phenotype. This represents a form of polyphenism, whereby a single genome can give rise to multiple phenotypes[58]. Morphs can be determined at maturity, often on the basis of developmental nutritional status[59,60], or can be plastic, with individuals transitioning between morphs throughout their lifespan depending on the optimal strategy for their relative size.

Developmental modularity through threshold traits. Some of the best examples of morphs determined at maturity on the basis of developmental nutrition come from beetles. Rhinoceros beetle (*Trypoxylus dichotomus*) males have two morphs associated with the size of the forked horn on their heads. In nutrient-rich conditions, male beetles grow significantly larger horns and adopt a territorial strategy, whereas a nutrient-poor diet causes the development of small horns and a sneaker strategy.

Female beetles, in contrast, lack horns irrespective of nutritional status. In this example, male horn size is mediated by male-specific changes to insulin or insulin-like growth factor activity[60] in response to food intake, demonstrating male-limited interaction between an environmental condition (nutrition) and insulin regulation. In *O. taurus* beetles, the interaction between beetle horn size and nutrition is further mediated by *doublesex*[61], and artificial *doublesex* knockdown mutants exhibit less nutrition-dependent horn growth[59], again showing the important interaction between key sex determination genes and sex-specific morphs.

The interaction between insulin or insulin-like growth factor signalling, nutrition and horn size in male beetles hints at the underlying ecological reason. If males with insufficient nutrition, either from rationed maternal effects or limited nutrition during development, were to invest in horn growth the resulting stunted horn would be insufficient to win competitive battles for access to females. In this situation, the hornless sneaker morph may represent the best strategy to deal with a bad situation. Of course, nutritional status and insulin are continuous traits, and male horn size is bimodal. This represents a threshold trait[42], whereby a morph phenotype is triggered by a critical value, in this case nutrition. Threshold traits represent a form of developmental modularity, in which the threshold mechanism (in this case, insulin signalling), evolves independently of the downstream phenotypes it regulates. Also, multiple phenotypes (in this case horns, body size and mating behaviour), can be integrated into the threshold, or dissociated from it, depending on evolutionary pressures[42,58,62,63].

Sequential morphs. In contrast to the beetle morphs, which are determined by developmental nutrition and, once evident at maturity, are maintained for the life of that male, some facultative sex-specific morphs are sequential, with individuals transitioning across different morphs depending on their relative condition or other factors. This strategy is particularly evident in fish, which exhibit indeterminate growth and for which size is often a key factor in the ability of a male to defend a nest and attract females. In a wide variety of fish species, young males of insufficient size to successfully defend a nest territory experience higher reproductive success as sneakers, satellites or female mimics[13]. Only males that survive long enough to reach a large size relative to others in the population are likely to succeed with a territorial strategy. Thus, in these cases, male morphs represent different positions in the size hierarchy, and males can essentially move through the ranks as they grow.

For all sequential facultative morphs, the pathway that underlies the transition needs to be activated and, just like heritable morphs, this activation is often associated with the sex determination pathway in the form of sex hormones. This process has been well studied in many fish species, and fish differ from many other animal groups in using two forms of androgen to differentiate gonadal from somatic sexual traits. In this case, similar to many other animals, testosterone is associated with male gonadal sex and is observed in

Box 1 | Outstanding questions about sex-specific morphs

- Is the genetic architecture of heritable and facultative morphs fundamentally different? The link between morph phenotypes and supergenes[14,15,25,27,32,54,57], particularly supergenes that contain sex-determining genes[25,27,32,54], suggests that heritable morphs are often encoded by alleles at several linked loci, implying polygenic control. This is somewhat at odds with results from facultative morphs, which suggest a simpler singular hormonal control mechanism that regulates suites of downstream genes[59–61]. How these two models relate to each other, and whether they represent two alternative end points to morph determination or one is an intermediate to the other in an evolutionary progression, remain to be determined.

- The predicted link between disruptive selection and multiple discrete morph phenotypes[2] remains largely theoretical. What is the genetic locus of disruptive selection, and what strength is needed to drive morph evolution in natural systems? Can experimental evolution be used to artificially generate discrete morph phenotypes, and if so, how does this affect development, regulatory networks and genomic organization?

- For heritable morphs, what are the forces of balancing selection needed to maintain diversity? What determines the maximal number of morphs that can be maintained in a population and how is this related to the complexity of genetic architecture?

- Why are morphs more often observed in males than females? Is it generally true that sex-specific morphs are primarily the result of sexual selection providing more reproductive niches for males[33], and are other forces at play as well, such as predation or ecological niche partitioning?

- At what point does continuous variation transition to discrete morphs? Comparative studies in outgroup species would be helpful to determine whether they exhibit evidence of continuous variation for the traits seen in discrete distributions in morphs and how this relates to relative fitness. Recent reports suggest possible intermediate stages of morphs[76,77] and may prove very useful in understanding the initial evolutionary stages of morph diversity.

all types of male morph. However, somatic sexually selected traits are often associated with another sex hormone, 11-ketotestosterone, which typically circulates at the highest levels in territorial males[48,64,65]. The two androgens allow for the uncoupling of somatic sexually selected traits from gonadal sex. Furthermore, arginine vasotocin — a hormone produced by neurosecretory cells in non-mammalian vertebrates, including birds, fishes and amphibians — is associated with female somatic sexual characteristics and spawning behaviour. When expressed in males, arginine vasotocin allows for the female mimicry of somatic traits and behaviours associated with male gynomorphs[13,64,66,67]. Different combinations of androgens and arginine vasotocin therefore underlie the variation in sex-specific morphs, mirroring the phenotypic mixing and matching of male and female traits as well as differing degrees of sexualization.

Concluding remarks

Sex-specific morphs are increasingly useful for studying the evolutionary causes and genetic basis of intra-specific variation. The modularity of the diverse traits associated with many morphs offer a powerful system for studying how the genes that underlie traits can be integrated, through either physical or regulatory linkage. Additionally, the need to understand sex-specific biology has become increasingly clear[6–9], and sex-specific morphs offer important insights into the genetic architecture of phenotypes limited in expression to one sex or the other, which is crucial for understanding sex differences. Finally, because sex-specific morphs often display a range of sexualization and a combination of both male and female traits, they offer a route to expand studies of sexual diversity beyond bimodal male–female comparisons, mirroring in many ways recent societal interest in understanding human sexual diversity.

Although many important questions remain (BOX 1), the recent use of genomic and transcriptomic approaches to the study of sex-specific morphs has revealed several important trends. Transcriptome studies have shown that overall gene expression mirrors the range of sexualization observed in morph phenotypes[44]. This approach has revealed the regulatory modularity that forms the underlying basis for the mix-and-match combinations of male and female traits observed in many morphs[46–51]. For heritable morphs, the link between morphs and supergenes[14,15,25,27,32,54,57], particularly supergenes that contain sex-determining genes[25,27,32,54], suggests that several alleles and linked loci are needed to encode morph phenotypes. However, facultative morphs show that this complex architecture may be simply controlled transcriptionally via sex-specific hormonal signalling[48,60,61,64,65]. Whether these represent two distinct genetic models for morph evolution or they share similar ultimate mechanisms remains an intriguing area for future work.

1. Engqvist, L. & Taborsky, M. The evolution of genetic and conditional alternative reproductive tactics. *Proc. Biol. Sci.* **283**, 20152945 (2016).
2. Taborsky, M., Oliveira, R. F., & Brockmann, H. J. *Alternative Reproductive Tactics: an Integrative Approach* (Cambridge University Press, 2008).
3. Gross, M. R. Alternative reproductive strategies and tactics: diversity within sexes. *Trends Ecol. Evol.* **11**, 92–98 (1996).
4. De Lisle, S. P. Understanding the evolution of ecological sex differences: integrating character displacement and the Darwin–Bateman paradigm. *Evol. Lett.* **3**, 434–447 (2019).
5. Slatkin, M. Ecological causes of sexual dimorphism. *Evolution* **38**, 622–630 (1984).
6. Brady, E., Nielsen, M. W., Andersen, J. P. & Oertelt-Prigione, S. Lack of consideration of sex and gender in COVID-19 clinical studies. *Nat. Commun.* **12**, 4015 (2021).
7. Nature Editorial. Nature journals raise the bar on sex and gender reporting in research. *Nature* **605**, 396 (2022).
8. Rechlin, R. K., Splinter, T. F. L., Hodges, T. E., Albert, A. Y. & Galea, L. A. M. An analysis of neuroscience and

psychiatry papers published from 2009 and 2019 outlines opportunities for increasing discovery of sex differences. *Nat. Commun.* **13**, 2137 (2022).
9. Woitowich, N. C., Beery, A. & Woodruff, T. A 10-year follow-up study of sex inclusion in the biological sciences. *eLife* **9**, e56344 (2020).
10. Watts, C. R. & Stikes, A. W. The social order of turkeys. *Sci. Am.* **224**, 112–119 (1971).
11. Krakauer, A. H. Kin selection and cooperative courtship in wild turkeys. *Nature* **434**, 69–72 (2005).
12. Moczek, A. P. & Emlen, D. J. Male horn dimorphism in the scarab beetle, *Onthophagus taurus*: do alternative reproductive tactics favour alternative phenotypes? *Anim. Behav.* **59**, 459–466 (2000).
13. Mank, J. E. & Avise, J. C. Comparative phylogenetic analysis of male alternative reproductive tactics in ray-finned fishes. *Evolution* **60**, 1311–1316 (2006).
14. Küpper, C. et al. A supergene determines highly divergent male reproductive morphs in the ruff. *Nat. Genet.* **48**, 79–83 (2016).
15. Lamichhaney, S. et al. Structural genomic changes underlie alternative reproductive strategies in the

ruff (*Philomachus pugnax*). *Nat. Genet.* **48**, 84–88 (2016).
16. Alonzo, S. H., Taborsky, M. & Wirtz, P. Male alternative reproductive behaviours in a Mediterranean wrasse, *Symphodus ocellatus*: evidence from otoliths for multiple life-history pathways. *Evolut. Ecol. Res.* **2**, 997–1007 (2000).
17. Sinervo, B. & Lively, C. M. The rock–paper–scissors game and the evolution of alternative male strategies. *Nature* **380**, 240–243 (1996). **One of the first empirical demonstrations of the role of balancing selection in maintaining male morphs**.
18. Rankin, K. J., McLean, C. A., Kemp, D. J. & Stuart-Fox, D. The genetic basis of discrete and quantitative colour variation in the polymorphic lizard, *Ctenophorus decresii*. *BMC Evolut. Biol.* **16**, 179 (2016).
19. Yewers, M. S. C., Pryke, S. & Stuart-Fox, D. Behavioural differences across contexts may indicate morph-specific strategies in the lizard *Ctenophorus decresii*. *Anim. Behav.* **111**, 329–339 (2016).
20. Matsumoto, K. & Knell, R. J. Diverse and complex male polymorphisms in *Odontolabis* stag beetles (Coleoptera: Lucanidae). *Sci. Rep.* **7**, 16733 (2017).

21. Hurtado-Gonzales, J. L., Baldassarre, D. T. & Uy, J. A. C. Interaction between female mating preferences and predation may explain the maintenance of rare males in the pentamorphic fish *Poecilia parae*. *J. Evolut. Biol.* **23**, 1293–1301 (2010).

22. Hurtado-Gonzales, J. L. & Uy, J. A. C. Alternative mating strategies may favour the persistence of a genetically based colour polymorphism in a pentamorphic fish. *Anim. Behav.* **77**, 1187–1194 (2009).

23. Hurtado-Gonzales, J. L. & Uy, J. A. C. Intrasexual competition facilitates the evolution of alternative mating strategies in a colour polymorphic fish. *BMC Evolut. Biol.* **10**, 391 (2010).

24. Lindholm, A. K., Brooks, R. & Breden, F. Extreme polymorphism in a Y-linked sexually selected trait. *Heredity* **92**, 156–162 (2004).

25. Sandkam, B. A. et al. Extreme Y chromosome polymorphism corresponds to five male reproductive morphs of a freshwater fish. *Nat. Ecol. Evol.* **5**, 939 (2021).
   One of the first links between sex chromosomes and male morphs. Identifies the Y chromosome variation that underlies five male morphs in this species.

26. Schwander, T., Lo, N., Beekman, M., Oldroyd, B. P. & Keller, L. Nature versus nurture in social insect caste differentiation. *Trends Ecol. Evol.* **25**, 275–282 (2010).

27. Nishikawa, H. et al. A genetic mechanism for female-limited Batesian mimicry in *Papilio* butterfly. *Nat. Genet.* **47**, 405–409 (2015).

28. Gossum, H. V. G. V., Robb, T. R., Forbes, M. R. F. R. & Rasmussen, L. R. Female-limited polymorphism in a widespread damselfly: morph frequencies, male density, and phenotypic similarity of andromorphs to males. *Can. J. Zool.* **86**, 1131–1138 (2008).

29. Takahashi, M., Takahashi, Y. & Kawata, M. Candidate genes associated with color morphs of female-limited polymorphisms of the damselfly *Ischnura senegalensis*. *Heredity* **122**, 81–92 (2019).

30. Takahashi, Y., Yoshimura, J., Morita, S. & Watanabe, M. Negative frequency dependent selection in female colour polymorphism of a demselfly. *Evolution* **64**, 3620–3628 (2010).

31. Clarke, C. & Sheppard, P. M. The genetics of the mimetic butterfly *Papilio polytes*. *Philos. Trans. R. Soc. Lond. B* **263**, 431–458 (1972).

32. Kunte, K. et al. *doublesex* is a mimicry supergene. *Nature* **507**, 229–232 (2014).
   Linked morph diversity to *doublesex* isoforms. A key paper that illustrates the role of sex-determining genes in morph genetic architecture.

33. Hendrickx, F., Vanthournout, B. & Taborsky, M. Selection for costly sexual traits results in a vacant mating niche and male dimorphism. *Evolution* **69**, 2105–2117 (2015).

34. Morris, M. R., Goedert, D., Abbott, J. K., Robinson, D. M. & Rios-Cardenas, O. in *Advances in the Study of Behavior* Vol. 45 (eds Brockmann, H. J. et al.) 447–478 (Academic Press, 2013).

35. Parker, G. A. Sperm competition games: raffles and roles. *Proc. R. Soc. Lond. Ser. B* **242**, 120–126 (1990).

36. Parker, G. A. Sperm competition games: sneaks and extra-pair copulations. *Proc. R. Soc. Lond. Ser. B* **242**, 127–133 (1990).

37. Simmons, L. W. & Emlen, D. J. Evolutionary trade-off between weapons and testes. *Proc. Natl Acad. Sci. USA* **103**, 16346–16351 (2006).

38. Goczał, J., Rossa, R. & Tofilski, A. Intersexual and intrasexual patterns of horn size and shape variation in the European rhinoceros beetle: quantifying the shape of weapons. *Biol. J. Linn. Soc.* **127**, 34–43 (2019).

39. Alonzo, S. H. et al. Ejaculate allocation and sperm characteristics differ among alternative male types in a species of fish with cooperation and competition among unrelated males. *Cells* **10**, 2612 (2021).

40. Rensch, B. Die Abhängigkeit der relativen Sexualdifferenz von der Körpergrösse. *Bonn. Zool. Beitr.* **1**, 58–69 (1950).

41. Tseng, M. & Rowe, L. Sexual dimorphism and allometry in the giant water strider *Gigantometra gigas*. *Can. J. Zool.* **77**, 923–929 (1999).

42. Emlen, D. J., Hunt, J. & Simmons, L. W. Evolution of sexual dimorphism and male dimorphism in the expression of beetle horns: phylogenetic evidence for modularity, evolutionary lability, and constraint. *Am. Nat.* **166**, S42–S68 (2005).

43. Warren, I. A. et al. Insights into the development and evolution of exaggerated traits using de novo transcriptomes of two species of horned scarab beetles. *PLoS ONE* **9**, e88364 (2014).

44. Pointer, M. A., Harrison, P. W., Wright, A. E. & Mank, J. E. Masculinization of gene expression is associated with exaggeration of male sexual dimorphism. *PLoS Genet.* **9**, e1003697 (2013).
   One of the first morph transcriptome studies to show the range of transcriptome sexualization across morphs.

45. Mank, J. E. The transcriptional architecture of phenotypic dimorphism. *Nat. Ecol. Evol.* **1**, 0006 (2017).

46. Todd, E. V. et al. Female mimicry by sneaker males has a transcriptomic signature in both the brain and the gonad in a sex-changing fish. *Mol. Biol. Evol.* **35**, 225–241 (2018).
   An early morph transcriptome study showing that female-mimic morphs are transcriptionally more similar for some tissues to females than males, illustrating the mix-and-match combination of male and female traits in many morphs.

47. Cardoso, S. D., Gonçalves, D., Goesmann, A., Canário, A. V. M. & Oliveira, R. F. Temporal variation in brain transcriptome is associated with the expression of female mimicry as a sequential male alternative reproductive tactic in fish. *Mol. Ecol.* **27**, 789–803 (2018).

48. Stiver, K. A., Harris, R. M., Townsend, J. P., Hofmann, H. A. & Alonzo, S. H. Neural gene expression profiles and androgen levels underlie alternative reproductive tactics in the ocellated wrasse, *Symphodus ocellatus*. *Ethology* **121**, 152–167 (2015).

49. Chauhan, P., Wellenreuther, M. & Hansson, B. Transcriptome profiling in the damselfly *Ischnura elegans* identifies genes with sex-biased expression. *BMC Genomics* **17**, 985 (2016).

50. Dean, R. et al. Sperm competition shapes gene expression and sequence evolution in the ocellated wrasse. *Mol. Ecol.* **26**, 505–518 (2017).

51. Snell-Rood, E. C. et al. Developmental decoupling of alternative phenotypes: insights from the transcriptomes of horn-polyphenic beetles. *Evolution* **65**, 231–245 (2011).

52. Schwander, T., Libbrecht, R. & Keller, L. Supergenes and complex phenotypes. *Curr. Biol.* **24**, R288–R294 (2014).

53. Mank, J. E. & Rideout, E. J. Developmental mechanisms of sex differences: from cells to organisms. *Development* **148**, dev199750 (2021).

54. Hendrickx, F. et al. A masculinizing supergene underlies an exaggerated male reproductive morph in a spider. *Nat. Ecol. Evol.* **6**, 195–206 (2022).
   This study demonstrates that the supergene that underlies morph diversity contains a duplicate of *doublesex*, a key sex-determining gene.

55. Guo, M., Davis, D. & Birchler, J. A. Dosage effects on gene expression in a maize ploidy series. *Genetics* **142**, 1349–1355 (1996).

56. Malone, J. H. et al. Mediation of *Drosophila* autosomal dosage effects and compensation by network interactions. *Genome Biol.* **13**, r28 (2012).

57. Hill, J. et al. Low mutation load in a supergene underpinning alternative male mating strategies in ruff. Preprint at *bioRxiv* https://doi.org/10.1101/2022.04.27.489720 (2022).

58. West-Eberhard, M. J. *Developmental Plasticity and Evolution* (Oxford Univ. Press, 2003).

59. Casasa, S., Zattara, E. E. & Moczek, A. P. Nutrition-responsive gene expression and the developmental evolution of insect polyphenism. *Nat. Ecol. Evol.* **4**, 970–978 (2020).

60. Emlen, D. J., Warren, I. A., Johns, A., Dworkin, I. & Lavine, L. C. A mechanism of extreme growth and reliable signaling in sexually selected ornaments and weapons. *Science* **337**, 860–864 (2012).

61. Kijimoto, T., Moczek, A. P. & Andrews, J. Diversification of *doublesex* function underlies morph-, sex-, and species-specific development of beetle horns. *Proc. Natl Acad. Sci. USA* **109**, 20526–20531 (2012).

62. West-Eberhard, M. J. Phenotypic plasticity and the origins of diversity. *Annu. Rev. Ecol. Syst.* **20**, 249–278 (1989).

63. West-Eberhard, M. J. Developmental plasticity and the origin of species differences. *Proc. Natl Acad. Sci. USA* **102**, 6543–6549 (2005).

64. Mank, J. E. & Avise, J. C. The evolution of reproductive and genomic diversity in ray-finned fishes: insights from phylogeny and comparative analysis. *J. Fish Biol.* **69**, 1–27 (2006).

65. Nugent, B. M., Stiver, K. A., Alonzo, S. H. & Hofmann, H. A. Neuroendocrine profiles associated with discrete behavioural variation in *Symphodus ocellatus*, a species with male alternative reproductive tactics. *Mol. Ecol.* **25**, 5212–5227 (2016).

66. Carneiro, L. A., Oliveira, R. F., Canario, A. V. M. & Grober, M. S. The effect of arginine vasotocin on courtship behaviour in a blenniid fish with alternative reproductive tactics. *Fish. Physiol. Biochem.* **28**, 241–243 (2003).

67. Foran, C. M. & Bass, A. H. Preoptic AVT immunoreactive neurons of a teleost fish with alternative reproductive tactics. *Gen. Comp. Endocrinol.* **111**, 271–282 (1998).

68. Moczek, A. P. Horn polyphenism in the beetle *Onthophagus taurus*: larval diet quality and plasticity in parental investment determine adult body size and male horn morphology. *Behav. Ecol.* **9**, 636–641 (1998).

69. Munday, P. L., Wilson White, J. & Warner, R. R. A social basis for the development of primary males in a sex-changing fish. *Proc. R. Soc. Lond. Ser. B* **273**, 2845–2851 (2006).

70. Gonçalves, E. J., Almada, V. C., Oliveira, R. F. & Santos, A. J. Female mimicry as a mating tactic in males of the blenniid fish *Salaria Pavo*. *J. Mar. Biol. Assoc. U. K.* **76**, 529–538 (1996).

71. Sinervo, B. & Zamudio, K. R. The evolution of alternative reproductive strategies: fitness differential, heritability, and genetic correlation between the sexes. *J. Heredity* **92**, 198–205 (2001).

72. Lank, D. B., Smith, C. M., Hanotte, O., Burke, T. & Cooke, F. Genetic polymorphism for alternative mating behaviour in lekking male ruff *Philomachus pugnax*. *Nature* **378**, 59–62 (1995).
   One of the first papers to show heritability for alternative male morphs.

73. Van Gossum, H., Stoks, R., Matthysen, E., Valck, F. & De Bruyn, L. U. C. Male choice for female colour morphs in *Ischnura elegans* (Odonata, Coenagrionidae): testing the hypotheses. *Anim. Behav.* **57**, 1229–1232 (1999).

74. Sanchez-Guillen, R. A., Van Gossum, H. & Cordero Rivera, A. Hybridization and the inheritance of female colour polymorphism in two ischnurid damselflies (Odonata: Coenagrionidae). *Biol. J. Linn. Soc.* **85**, 471–481 (2005).

75. Emlen, D. J., Lavine, L. C. & Ewen-Campen, B. On the origin and evolutionary diversification of beetle horns. *Proc. Natl Acad. Sci. USA* **104**, 8661–8668 (2007).

76. Moore, E. C. et al. Polygenic sex determination produces modular sex polymorphism in an African cichlid fish. *Proc. Natl Acad. Sci. USA* **119**, e2118574119 (2022).
   A polygenic sex-determining mechanism may represent an initial stage of male morph evolution.

77. Spottiswoode, C. N. et al. Genetic architecture facilitates then constrains adaptation in a host–parasite coevolutionary arms race. *Proc. Natl Acad. Sci. USA* **119**, e2121752119 (2022).