

Review



Cite this article: Stöck M *et al.* 2021 A brief review of vertebrate sex evolution with a pledge for integrative research: towards 'sexomics'. *Phil. Trans. R. Soc. B* **376**: 20200426.
<https://doi.org/10.1098/rstb.2020.0426>

Accepted: 8 March 2021

One contribution of 12 to a theme issue 'Challenging the paradigm in sex chromosome evolution: empirical and theoretical insights with a focus on vertebrates (Part I)'.

Subject Areas:

developmental biology, genetics, evolution, genomics

Keywords:

evolution, genomics, reproduction, vertebrates, sex chromosomes, sex determination

Authors for correspondence:

Matthias Stöck
e-mail: matthias.stoeck@igb-berlin.de
Lukáš Kratochvíl
e-mail: lukas.kratochvil@natur.cuni.cz

*Co-senior authors.

Electronic supplementary material is available online at <https://doi.org/10.6084/m9.figshare.c.5438942>.

A brief review of vertebrate sex evolution with a pledge for integrative research: towards 'sexomics'

Matthias Stöck^{1,2}, Lukáš Kratochvíl³, Heiner Kuhl¹, Michail Rovatsos², Ben J. Evans⁴, Alexander Suh^{5,6}, Nicole Valenzuela⁷, Frédéric Veyrunes⁸, Qi Zhou^{9,10}, Tony Gamble¹¹, Blanche Capel¹², Manfred Scharl^{13,14,*} and Yann Guiguen^{15,*}

¹Leibniz-Institute of Freshwater Ecology and Inland Fisheries—IGB (Forschungsverbund Berlin), Müggelseedamm 301, 12587 Berlin, Germany

²Amphibian Research Center, Hiroshima University, Higashi-Hiroshima 739-8526, Japan

³Department of Ecology, Faculty of Science, Charles University, Viničná 7, 12844 Prague, Czech Republic

⁴Department of Biology, McMaster University, Life Sciences Building Room 328, 1280 Main Street West, Hamilton, Ontario, Canada L8S 4K1

⁵School of Biological Sciences, University of East Anglia, Norwich Research Park, Norwich NR4 7TU, UK

⁶Department of Organismal Biology—Systematic Biology, Evolutionary Biology Centre, Science for Life Laboratory, Uppsala University, Norbyvägen 18D, 75236 Uppsala, Sweden

⁷Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, IA 50011, USA

⁸Institut des Sciences de l'Évolution de Montpellier, ISEM UMR 5554 (CNRS/Université de Montpellier/IRD/EPHE), Montpellier, France

⁹MOE Laboratory of Biosystems Homeostasis and Protection and Zhejiang Provincial Key Laboratory for Cancer Molecular Cell Biology, Life Sciences Institute, Zhejiang University, Hangzhou, Zhejiang 310058, People's Republic of China

¹⁰Department of Neuroscience and Developmental Biology, University of Vienna, A-1090 Vienna, Austria

¹¹Department of Biological Sciences, Marquette University, Milwaukee, WI 53201, USA

¹²Department of Cell Biology, Duke University Medical Center, Durham, NC 27710, USA

¹³Developmental Biochemistry, Biocenter, University of Würzburg, 97074 Würzburg, Germany

¹⁴The Xiphophorus Genetic Stock Center, Department of Chemistry and Biochemistry, Texas State University, San Marcos, TX 78666, USA

¹⁵INRAE, LPGP, 35000, Rennes, France

ID MSt, 0000-0003-4888-8371; LK, 0000-0002-3515-729X; HK, 0000-0002-3515-729X; MR, 0000-0002-8429-5680; BJE, 0000-0002-9512-8845; AS, 0000-0002-8979-9992; NV, 0000-0003-1148-631X; FV, 0000-0002-1706-9915; QZ, 0000-0002-7419-2047; TG, 0000-0002-0204-8003; BC, 0000-0002-6587-0969; MSc, 0000-0001-9882-5948; YG, 0000-0001-5464-6219

Triggers and biological processes controlling male or female gonadal differentiation vary in vertebrates, with sex determination (SD) governed by environmental factors or simple to complex genetic mechanisms that evolved repeatedly and independently in various groups. Here, we review sex evolution across major clades of vertebrates with information on SD, sexual development and reproductive modes. We offer an up-to-date review of divergence times, species diversity, genomic resources, genome size, occurrence and nature of polyploids, SD systems, sex chromosomes, SD genes, dosage compensation and sex-biased gene expression. Advances in sequencing technologies now enable us to study the evolution of SD at broader evolutionary scales, and we now hope to pursue a *sexomics* integrative research initiative across vertebrates. The vertebrate *sexome* comprises interdisciplinary and integrated information on sexual differentiation, development and reproduction at all biological levels, from genomes, transcriptomes and proteomes, to the organs involved in sexual and sex-specific processes, including gonads, secondary sex organs and those with transcriptional sex-bias. The *sexome* also includes ontogenetic and behavioural aspects of sexual differentiation, including

malfunction and impairment of SD, sexual differentiation and fertility. Starting from data generated by high-throughput approaches, we encourage others to contribute expertise to building understanding of the *sexomes* of many key vertebrate species.

This article is part of the theme issue 'Challenging the paradigm in sex chromosome evolution: empirical and theoretical insights with a focus on vertebrates (Part I)'.

1. Introduction

(a) Towards an integrative understanding of vertebrate sexual differentiation, development and sex determination

In gonochoristic (for this and other terms see Glossary) vertebrates, the genetic and cellular biological processes determining whether an undifferentiated gonad develops towards male or female exhibit great diversity [1,2]. Sex determination (SD) in vertebrates ranges from environmental SD (ESD) to simple or complex genetic systems (genotypic SD (GSD)) that have evolved repeatedly and independently [3–6]. Great plasticity of the developmental processes determining gonads and their initiation during embryogenesis contrasts with the evolutionary conservation of pathways that regulate development of most other tissues and organs [3,7]. In poikilothermic vertebrates, much of the epigenetics and genetics of SD, sex differentiation and sexual development remains poorly understood, and knowledge in homeotherms is mostly restricted to a few models such as humans, mice and chickens [7]. For fishes and amphibians, a diversity of master SD genes defining sex chromosomes was early postulated [8], with some downstream components of the SD networks appearing conserved. Fascinatingly, recent work has illustrated that the molecular control and regulation of SD factors and gonadal differentiation can substantially differ even among closely related groups with indistinguishable gonadal development at the morphological, histological and cellular levels [3,7,9,10].

An interesting heterogeneity exists in the evolution of SD in that some clades exhibit very ancient conservation of sex chromosomes (e.g. birds, therian mammals and many reptile lineages, figure 1), whereas others show frequent evolutionary turnovers with variation even between related clades or even species, such as in many amphibians and fishes, and some reptilian lineages [11]. Highly diverse sex chromosomes may derive from frequent turnovers of SD genes [12,13], suggesting that new SD systems may evolve *de novo* and independently. Deep homology of some sex chromosome systems across disparate taxa suggest that gene content may predispose certain linkage groups to become sex chromosomes [4,14–16], however, so far with relatively weak support in amniotes [17]. Numerous theoretical concepts and models about transitions among SD systems, degeneration and turnover of sex chromosomes [18–23] often remain to be empirically tested in vertebrates. To understand the diversity of SD and sexual development, a deeper and broader knowledge in multiple species from major phylogenetic lineages is necessary. This may have far-reaching consequences also for other fields, owing to likely coevolution of SD, reproductive modes and

life history, which are up to now poorly studied, especially in poikilothermic vertebrates [24–26], although these aspects are very relevant for theoretical and empirical studies of sex ratio ecology and evolution [27].

Here we present an overview of the current knowledge about SD and the genomic resources available for each vertebrate group, as an overture towards a more comprehensive understanding of vertebrate sex evolution. We review the available whole-genome information in all major clades across the vertebrate tree of life, in relation to knowledge about SD, sexual development and reproductive modes, and available genomic resources. We provide an up-to-date overview on divergence times, species numbers, available genomes, genome size, occurrence and nature of polyploids, SD systems, sex chromosomes, SD genes, dosage compensation and sex-biased gene expression.

Despite the fast-developing sequencing technologies allowing genome assemblies of many vertebrates, we consider high-quality genomes only as a starting point that should be complemented by and synthesized with additional information types in order to comprehensively understand sex evolution. We then pledge for an integrative *sexomics* research initiative, which uses high-throughput approaches (e.g. RADSex, PoolSex, RNASex, epigenomics) that would integrate the growing numbers of vertebrate species with an available genome assembly to better understand the evolution of genetic SD and differentiation in vertebrates. This *sexomics* approach could be a starting point for a more in-depth characterization of the 'complete' *sexome* of representative species that would require physiological, cell-biological, behavioural information and beyond to better understand sexual reproduction across lineages.

2. Overview of current knowledge about sex evolution across the vertebrate phylogeny

(a) Vertebrate sister groups: CEPHALOCHORDATA (LANCELETS) and TUNICATA (TUNICATES)

Extant fish-like lancelets (also called amphioxii; [28,29] are considered the sister group to tunicates and vertebrates (e.g. [30]). Lancelets are gonochorists, but little is known about their SD. Recent genetic evidence suggests a female-heterogametic (ZZ/ZW) GSD system [31]. The karyotype of lancelets is considered to resemble that of ancestral vertebrates [32]. According to traditional models, the early vertebrate ancestors experienced two successive rounds of whole-genome duplications (assigned as 1R, 2R) between approximately 500 and 450 Ma [33,34]. However, Simakov *et al.* [29] suggested three duplication events—the first before the diversification of extant chordates, the second in the ancestor of lampreys, and the third in the ancestor of jawed vertebrates.

Tunicates, the putative sister group of vertebrates, possess a wide array of reproductive systems. Sedentary ascidians are mostly sequential hermaphrodites, but some produce sperms and eggs simultaneously with incompatible cell-surface proteins, preventing self-fertilization [35]. Colonial species reproduce asexually by budding. Appendicularian tunicates are mostly sequential hermaphrodites [36] but the pelagic tunicate *Oikopleura dioica* has an XX/XY genetic sex-determining system with possible dosage compensation [37]. Pyrosomes are hermaphroditic as well, reproducing both

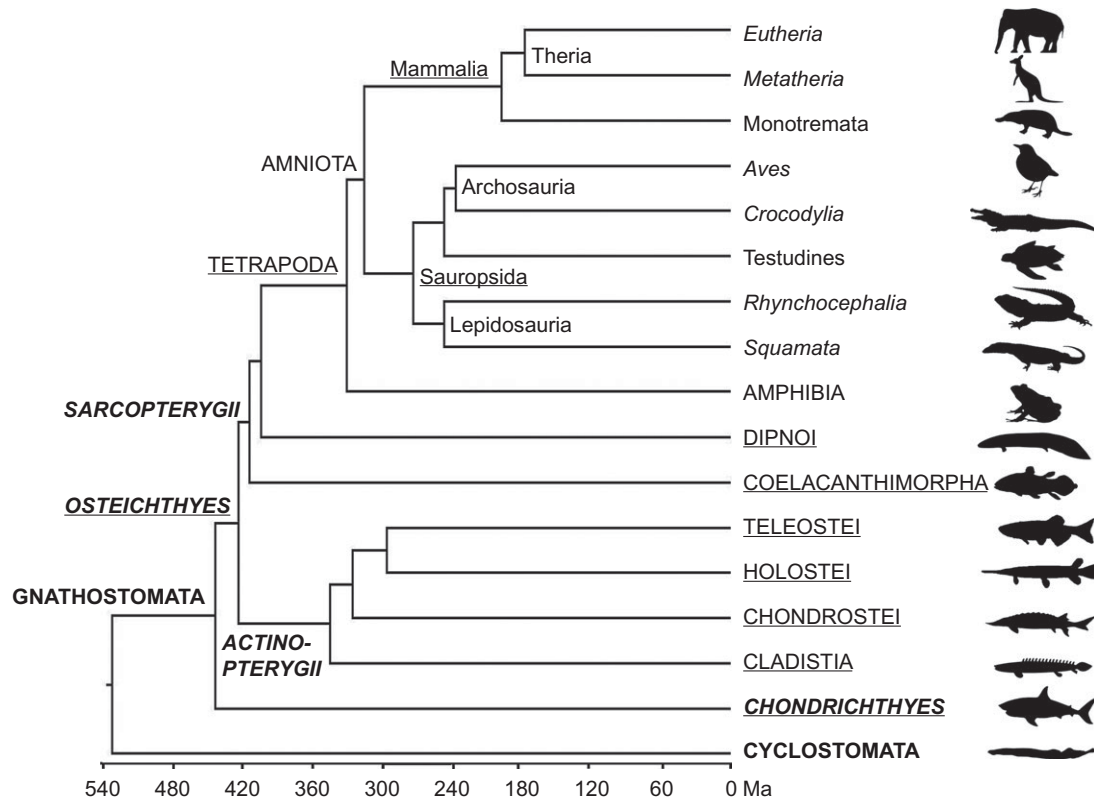


Figure 1. Phylogenetic tree of major clades of vertebrates. Divergence times in millions of years ago (Ma) according to sources provided in the text; typesetting indicates cladistic hierarchies as also used in the text and in table 1.

asexually and sexually with internal fertilization. Thaliaceans (salps) have complex life cycles, obligatorily alternating between sexual and asexual reproduction, allowing rapid population growth while preserving genetic variability [38]. The oozoid develops from a zygote produced by budding, resulting in a chain of individuals that contains an ovary and a testis. The eggs are fertilized internally and the embryo is brooded by the 'mother'. The life cycle of doliolids is the most complex, again including asexual reproduction with a sequential hermaphroditic phase (for overview: [39]).

With respect to the chordate ancestor, extant lancelets and tunicates may have a derived sexual development, life cycles and SD systems, which evolved during the hundreds of millions of years of divergence from the vertebrate lineage. Nevertheless, as the closest living vertebrate outgroups, they might provide important insights into the deep evolutionary history of sex-related traits and SD genes in vertebrates.

(b) VERTEBRATA (VERTEBRATES)

CYCLOSTOMATA (JAWLESS FISHES)

The branch of jawless vertebrates with its approximately 120 living species branched off approximately 540 Ma from the lineage leading to all other vertebrates during the Cambrian (figure 1). Agnatha comprises the extant clades of hagfishes (Myxini) and lampreys (Petromyzontiformes). Four lamprey genomes are available [40–42], and an assembly from hagfish (Myxinidae) is available. Hagfish genome size (c-value: 2.4–4.5 Gb; [43]) exceeds that of lampreys (1.4–2.4 Gb). Hagfishes and lampreys are oviparous (table 1). SD and sexual development of hagfishes is poorly understood. Some species appear as protogynous hermaphrodites [44], but no functional

simultaneous hermaphrodites have been documented [45,55], other species are gonochoristic [56]. It is possible that SD in lampreys is epigenetic/environmental [57]. However, the critical sex differentiation period is unexplored, and the evidence for ESD in lampreys remains equivocal since GSD as a possible alternative has been proposed recently [58]. The sea lamprey genome contains several hundred genes that are eliminated from somatic cells during early development [41]. Other lampreys and hagfish likewise undergo genome elimination [46], but it remains unknown whether genome elimination plays a role in sexual development.

GNATHOSTOMATA (JAWED VERTEBRATES)

CHONDRICHTHYES (CARTILAGINOUS FISHES)

With approximately 1200 extant species, cartilaginous fishes comprise the sister group to all other living jawed vertebrates, with elasmobranchs (sharks, rays and relatives) and holocephalans (chimaeras) sharing an Ordovician common ancestor with Osteichthyes approximately 450 Ma [59]. The genomes of only a few species have been characterized, hindered by large genome sizes (2.6–16.6 Gb; [43]). Currently, six sharks [60], two skates [61] and two chimaera genomes [62] have been assembled (recent overview: table 1). The modes of reproduction are very diverse, including yolk-sac viviparity, histotrophy (nutrition of an embryo by uterine secretions), oophagy and placental reproduction [45,63]. Several studies report cases of occasional (facultative) parthenogenetic reproduction giving rise to all-female offspring [64]. Intersexes (often reported as hermaphrodites) were reported in more than 30 elasmobranch species. They frequently showed improper development or maturation rendering one or both sexes nonfunctional [47]. Nevertheless,

Table 1. Overview on available genome assemblies of vertebrates [42], as December 2020) contrasted with generally known information on sexual phenotype, sex determination mode (SD mode) in gonochorists, system of genotypic sex determination (GSD), reproductive mode, reproduction and master sex determination genes (in GSD species). (Total numbers of species and families were derived from the NCBI taxonomy database and may contain higher than expected numbers owing to the presence of extinct species/families. Herein: x, trait is present; NRSF, trait 'not reported so far' despite the availability of at least some data on this topic; no data, to our knowledge, this topic has not been studied/examined; ?, questionable/equivocal evidence.)

vertebrate genome assemblies at NCBI as of December 2020:				SD mode in gonochorists		system of GSD			reproductive mode				reproduction		gonogenesis				master SD gene		comments	
				sexual phenotype																		
				gonochorism	hermaphroditism	ESD	GSD	male heterogamety	female heterogamety	multitocus sex det.	oviparous	viviparous	bisexual	parthenogenesis, facultative	parthenogenesis, obligate	gonogenesis	hybridogenesis					
				x	?	no data	no data	no data	no data	no data	x	x	x	no data	NRSF	no data	no data	no data				
CYCLOSTOMATA	2 (of 3)	5 (of 546)																			hermaphroditism in hagfish questionable [44,45]; genome elimination in both lampreys and hagfishes [46]	

no functional hermaphroditism was described in this group. SD in Chondrichthyes appears to be largely genotypic with cytogenetic data suggesting XX/XY sex chromosomes in the few studied species of sharks [65] and rays [66], or possibly other forms of male heterogamety in freshwater stingrays (*Potamotrygon*; [67,68]). A ZZ/ZW system was tentatively reported in the stingray *Hypanus americana* [65]. We can conclude that there is currently more information about the evolution of the male genitalia, the claspers [69], than on genetic or possible environmental triggers of SD.

OSTEICHTHYES (BONY FISHES)

ACTINOPTERYGII (RAY-FINNED FISHES)

With some more than 31 000 species, the ray-finned fishes are a very diverse vertebrate class, largely comprising extant Teleostei and few non-teleosts: Cladistia (bichirs), Chondrostei (sturgeons and paddlefish, and Holostei (bowfins and gars). The ancestor of Teleostei underwent another round of whole-genome duplication (traditionally called '3R WGD', or 'teleost-specific WGD'; [33,70]).

CLADISTIA (BICHIRS)

These ray-finned fishes diverged more than 350 Ma in the Devonian from other actinopterygians [71]. Cladistia comprise 13 *Polypterus* and a single *Erpetoichthys* species, with large genomes (4.6 Gb to possibly 7.00 Gb) in *Polypterus*, and a similar genome size (4.4 Gb) in *Erpetoichthys* [43]. A BAC-library of the Senegal bichir, *Polypterus senegalus*, was prepared [72]. For the reedfish, *Erpetoichthys calabaricus*, a chromosome-scale genome assembly is available (table 1). Bichirs are egg-laying and share holoblastic embryonic cleavage with sturgeons. Heteromorphic sex chromosomes have not been found in bichirs [73–75]. Given their phylogenetic position, information on SD and development might provide important insights into the ancestral condition of Actinopterygii.

CHONDROSTEI (STURGEONS and PADDLEFISH)

Sturgeons and paddlefish comprise 27 living species [76] that diverged 330 Ma [77] from the ancestor of the Teleostei and Holostei [33,70]. After their divergence from the other ray-finned fish lineages, sturgeons and paddlefish experienced several polyploidization events, yielding extant species karyotypes from basal approximately 120 up to as many as about 380 chromosomes [78] (and even more in single individuals), and moderate to large genome sizes from 1.4 to 4.4 Gb [43]. Several projects are underway to assemble high-quality sturgeon genomes [77], and a paddlefish genome has been recently published [79]. In contrast to other polyploid fishes, sturgeon genomes maintain a high proportion of ohnologues, i.e. they exhibit a slow deduplication process and loss of several homeologous chromosomes (segmental rediploidization), posing major challenges for genome assembly [77,80]. Chondrostei have exclusively oviparous reproduction [76] and share holoblastic cleavage with most amphibians but not teleosts [81]. Sturgeons do not possess cytologically differentiated sex chromosomes [77,82]. The sex ratio of offspring from experimental gynogenesis yielded contradictory results suggesting either male (XX/XY; [48]) or female heterogamety (ZZ/ZW; [49]) in a paddlefish (*Polyodon spathula*) and a female-heterogametic (ZZ/ZW) SD system in sturgeon [83], yet a sex-linked marker was not found for decades [77]. Using

chromosome-scale assemblies and pool-sequencing, an approximately 16 kb female-specific sequence from sterlet (*Acipenser ruthenus*) was detected by Kuhl *et al.* [84]. A polymerase chain reaction-genotyping test, yielding female-specific products in six sturgeon species, spanning the entire phylogeny with the most divergent extant lineages (*Acipenser sturio*, *Acipenser oxyrinchus* versus *Acipenser ruthenus*, *Huso huso*), stemming from an ancient tetraploidization. Similar results were obtained in two octoploid species (*Acipenser gueldenstaedtii*, *Acipenser baerii*). Phylogenetic conservation during 180 Myr of sturgeon evolution and across at least one polyploidization event revealed the oldest known vertebrate system with undifferentiated sex chromosomes, based presumably on a ZZ/ZW-mode of sex determination [84].

HOLOSTEI (BOWFINS and GARS)

A single species of extant bowfin (*Amia calva*) [85] from North America and closely related gars (*Lepisosteiformes*), occurring in North and Central America plus the Caribbean, with seven living species [86] represent the sister taxon of teleosts. These two lineages diverged in the Early Permian (approx. 300 Ma; [71]), before the teleost-specific WGD. Eased by reasonable genome sizes in bowfin (1.0–1.3 Gb) and gars (1.0–1.3 Gb), a gar [87], and most recently the bowfin genome [88] have been assembled. Gars and bowfins are oviparous [89,90] and show holoblastic embryonic cleavage. No information on the SD in Holostei is available and no sex-specific genome regions have been identified so far [88].

TELEOSTEI (TELEOSTS)

The rise of teleosts, which comprise approximately 31 000 species [91]) and thus make up over 99% of all ray-finned fishes (Actinopterygii), was accompanied by the teleost-specific WGD (traditionally assigned as '3R WGD') in their common ancestor approximately 300 Ma [34,71]. Some lineages, e.g. salmonids and carps, independently experienced yet additional WGD events. Teleosts evolved meroblastic embryonic cleavage [92]. To date several hundreds of teleost genomes have been assembled (table 1). Owing to advanced deduplication and diploidization of genomes and relatively small to large genome sizes (0.4–5.3 Gb; with most genomes less than 2.0 Gb; [43], whole-genome sequencing (WGS) of teleosts shows great progress among vertebrates. Teleosts feature the largest diversity of reproductive modes [93]. All-female sperm-dependent parthenogenetic (gynogenetic) or hybridogenetic species of hybrid origin [94,95], and even sequential (protandrous, protogynous or serial, i.e. bidirectional) hermaphroditism [96], in some cases involving socially controlled sex change [97,98] and simultaneous hermaphroditism exist [99,100], the latter including the only self-fertilizing vertebrate [101]. Sexual development of teleosts is also very plastic [102], and sex reversal can be easily induced by hormonal and sometimes by environmental triggers or treatments [103], rendering them susceptible to endocrine-disruptive pollution [104]. Data in zebrafish and medaka indicate that germ cell number can drive SD [105,106].

Teleosts show the widest variety of sex-determining mechanisms among vertebrates [107]. This includes gonochorism with ESD and GSD (as well as its environmental modulation), GSD ranging from homomorphic to heteromorphic female (ZZ/ZW) or male heterogametic (XX/XY) systems, plus polygenic SD [108,109] or multiple sex chromosomes [110], with different systems evolved in closely related

species. Pure temperature-dependent sex determination (TSD, e.g. [111]) appears to occur in teleosts relatively rarely [107]. In teleosts, the largest number of master SD genes in vertebrates has been characterized [112]. Teleost master SD genes evolved from well-known members of the sexual development regulatory network (the ‘usual suspects’, [3]), stemming in some cases from transcription factors (*Dmrt1*, *Sox3*), *Tgf-beta* signalling pathway members (*Amh*, *Amhr2*, *Gsdf*, *Gdf6*), or exceptionally from an immune gene in salmonids (*Irf9*; [113]), triggering male gonadogenesis through an unknown mechanism. The non-recombining region of young teleost sex chromosomes may be remarkably small, e.g. 300 kb in Atlantic herring (*Clupea harengus*; [114]), some sex chromosomes may even freely recombine, and rarely, the X and Y may differ by just a single nucleotide polymorphism, as reported in the Japanese pufferfish, *Takifugu rubripes* [115]. In teleosts, the research on rewiring of SD- and sex differentiation gene networks is the most advanced [3]. Compared to the huge teleost biodiversity, the discovery of novel SD genes and systems can be expected from WGS of additional species. Many teleosts, among them sequential or simultaneous hermaphrodites and recent polyploids with specific reproductive modes, such as gynogenesis or hybridogenesis [116], still lack the characterization of their genomes, SD systems and SD genes as well as interactions of allospecific sex chromosomes in taxa of hybrid origin.

SARCOPTERYGII (LOBE-FINNED FISHES)

Coelacanth and lungfishes are the only living sarcopterygian fishes [117] that all trace back to a divergence in Silurian times, i.e. more than 420 Ma [71]; all other extant sarcopterygians comprise tetrapods.

COELACANTHIMORPHA (COELACANTHS)

There are two coelacanth species from southeastern Africa and Sulawesi [118]. Coelacanth is ovoviviparous [119,120]. A coelacanth 2.86 Gb genome of *Latimeria chalumnae* has been assembled [121]. Over 50 genes involved in sex differentiation and gametogenesis were sequenced in *L. chalumnae* and *Latimeria menadoensis*, but no master SD genes have been characterized [122,123]. This situation may not change, given the secretive deep-sea lifestyle of these species and their conservation status (CITES).

DIPNOI (LUNGFISHES)

The six living known species of lungfish occur in Africa, South America and Australia. As their closest living relatives, lungfishes are in a uniquely informative phylogenetic position to infer the ancestral condition of tetrapods [124]. Lungfishes are oviparous [125] and show a pattern similar to holoblastic cleavage [92,125]. While coelacanth has moderate vertebrate genome sizes (2.6 Gb; [121]), lungfish genomes range among the largest in vertebrates (49–60 Gb; [43]). Despite their huge size, the assemblies without information about SD systems from the Australian lungfish (*Neoceratodus forsteri*) and the African lungfish (*Protopterus annectens*) have recently been obtained [126,127]. In *P. annectens*, more than 50 genes related to sex differentiation and gametogenesis have been characterized [123]. Master SD genes have not been identified in lungfishes. The availability of captive breeding in some lungfish species might ease elucidation of SD.

TETRAPODA (TETRAPODS)

AMPHIBIA (AMPHIBIANS)

Soon after their Devonian divergence (335 Ma; [128]) from Amniota, the amphibian lineage to Gymnophiona (caecilians) branched off from that of Anura (frogs and toads) and Urodela (= Caudata: newts and salamanders), while the latter two clades (Anura, Caudata) diverged in the Early Permian (300 Ma, [129]). Many amphibian families are deeply (100–150 Ma) diverged [130,131], with recent evidence that 88% of anurans (Hylaidea, Microhylidae, Natatanura) underwent a rapid Cretaceous–Palaeogene boundary diversification [132]. Gymnophiona also exhibit deep divergences, raising expectations for major genomic evolutionary differences [133]. Cleavage in most frog and salamander embryos is radially symmetrical and holoblastic. The limited knowledge on caecilians, however, suggests meroblastic cleavage in this group [134].

Although there are more species of amphibians (over 8260; [135]) than mammals (6485; [136,137]), to date only 19 amphibian genomes of various quality have been assembled, including 15 out of 7291 Anura, 1 out of 760 of Urodela (Caudata) and 3 out of 213 Gymnophiona [42,135]. Even fewer assemblies have reached chromosome-scale quality. The so far slow progress in amphibian genomics is mostly caused by large genome sizes, reaching from 3.9 to 9.8 Gb in Gymnophiona, 1.9–13.1 Gb in Anura, and huge 16.6–78.2 Gb in Urodela [138], and by the large proportions of repetitive sequences. The ongoing dawn of amphibian genomics will be much enlightened by long-read and three-dimensional technologies [139], with many amphibian families still awaiting their first WGS.

Anurans evolved a great diversity of reproductive modes, with terrestrial eggs and exotrophic aquatic larvae, preceding the frequent and repeated evolutionary rise of direct development (terrestrial eggs, no tadpoles), while non-feeding (endotrophic) larvae never led to direct developers [140]. Newts and salamanders exhibit aquatic larvae (rarely involving exceptional or even obligate neoteny, i.e. larval reproduction), as well as terrestrial eggs, and ovo-viviparity with birth of larvae or fully metamorphosed offspring, rarely boosting development by intrauterine cannibalism [141]. Gymnophiona are oviparous or viviparous [142,143], including rare direct developers [144].

While true parthenogenesis most likely did not evolve in amphibians (table 1), hybridogenetic systems, including male- or female-biased and probably GSD-governed population systems occur in anurans [145] as well as kleptogenesis (previously called ‘gynogenesis’; [51]) in salamanders [50], where all-female hybrids of five ploidy levels acquire full or partial genomes from allospecific males and ‘purge’ genomes from deleterious alleles. Recent auto- and allo- (i.e. hybrid origin) polyploids, presenting in amphibians the highest frequency of all vertebrates, are known from several families of anurans and salamanders [146] but are so far unknown in Gymnophiona. Occasional reports on natural sex change in adult anurans (e.g. [147] in *Hyperolius viridiflavus*) require further examination.

About 96% of the amphibians exhibit undifferentiated sex chromosomes [148,149]. All studied amphibians show GSD and either male (XY/XX) or female (ZZ/ZW) heterogamety [150–152], in addition a putative case of a female W0/00 male SD system [153] and several cases of multiple sex chromosomes

[154] have been reported, which form a ring during meiosis in the smoky jungle frog, *Leptodactylus pentadactylus* [155]. While the vast majority of amphibians exhibit homomorphic XX/XY or ZZ/ZW sex chromosome systems, there are several prominent examples of cytogenetically differentiated sex chromosomes [156,157], and for the African bullfrog, *Pyxicephalus adspersus*, a draft genome is pre-published [158], from which potential upregulation of the heterogametic W-chromosome and/or repression in the homogametic Z might inform about dosage compensation. In the cytologically indistinguishable sex chromosomes of the western clawed frog, *Xenopus tropicalis*, male-biased expression of sex-linked transcripts is suspected to be owing to degeneration of the non-recombining portion of the W-chromosome, coupled with incomplete or absent dosage compensation [159]. Cases of sex chromosome-autosome translocations have been shown by cytogenetics [160]. A balanced lethal system in newts (*Triturus*) may have evolved from a vestigial sex chromosome pair [161,162]. Sex chromosomes of most newts and salamanders are homomorphic [157,163], and the observation of balanced sex ratios from clutches is interpreted as indication for GSD but has remained without genetic evidence [164]. Whole-genome approaches in multiple individuals identified the homomorphic sex chromosome of axolotl (*Ambystoma mexicanum*), and a putative approximately 300 kb SD region on the W-chromosome [164,165]. Genomic approaches recently also suggested sex-linked loci in ancient clades of giant salamanders (family Cryptobranchidae; [166,167]). Transcriptomic approaches try to circumvent limitations of huge urodelean genome sizes to address sexual developmental aspects [168,169]. Evidence for heteromorphic sex chromosomes exists for at least one species of Gymnophiona [170].

Homomorphic sex chromosomes in amphibians may be caused by high turnover rates [171], where autosomes evolve into new sex chromosomes [8], as documented in ranid frogs [15] and pipid frogs [10]. Another hypothesis to explain homomorphy is occasional X-Y recombination ('fountain-of-youth'-model; [9]), assuming recombination arrest in males to be controlled by maleness (i.e. by the sexual phenotype rather than the sex chromosomal genotype). Thus, Y chromosomes may recombine, for example, in sex-reversed XY-females, preventing long-term Y degeneration, supported by data from tree frogs [172], true frogs [173] and Palaearctic green toads [174]. Generally, sex reversal in early developmental stages owing to environmental cues is possible, making semi-aquatic amphibians, like fishes, vulnerable to pollution of aquatic ecosystems with endocrine-disruptive compounds [104,175].

Early studies on SD involved experimental sex reversal [176,177], cytogenetics and crossing experiments [148,149]. In-depth molecular studies on amphibian SD stem mostly from clawed frogs (*Xenopus*), where LG7 is sex-linked in diploid *X. tropicalis* [178,179] and coexisting X, Y and W-chromosomes are suggested [154,159] but no master SD gene is known [180]. The only well-characterized anuran master SD gene is a *Dmrt1*-paralogue, the W-linked *Dm-w* of *Xenopus laevis* [151,181], present in some closely related *Xenopus* species but not in the entire pipid radiation [10,182]. *Dm-w* arose after (and perhaps in response to) tetraploidization [182–184] and may initially not have governed sexual differentiation. *Dmrt1* itself is considered a candidate master SD gene in some hylid frogs [185,186], bufonid toads [174] and common frogs (*Rana temporaria*; [173]), and is also sex-linked in several other

ranids [15]. The male versus female-determining molecular mechanisms suggest that parallel amino acid substitutions contributed to the establishment of *Dmrt1Y* (medaka fish) and *Dm-w* (*Xenopus*) as SD genes [187]. A well-studied ranid frog system is that of the Japanese wrinkled frog, *Glandirana rugosa*, with five genetic lineages: the west Japan, east Japan and XY-groups possess XX/XY systems; the ZW- and Neo-ZW groups ZZ/ZW SD systems [188]. In all lineages, the genes *androgen receptor* (*Ar*), *splicing factor 1* (*Sf-1*) and *Sry-box transcription factor 3* (*Sox3*) are located on the Z and W or X and Y chromosomes [189,190]. In most amphibians, the characterization of diploid and polyploid SD systems, evolution by hybridization and introgression and generally the characterization of SD systems, sex chromosomes and their evolution remain unknown from a genomic perspective.

AMNIOTA (AMNIOTES)

Sauropsida (sauropsids, reptiles and birds)

Lepidosauria (lepidosaurs)

Rhynchocephalia/*Sphenodontia* (*tuatara*)

The only extant species in the reptilian order Rhynchocephalia (*Sphenodontia*), diverged approximately 250 Ma from their sister taxon Squamata (lizards and snakes), is the tuatara (*Sphenodon punctatus*), endemic to New Zealand. The 5 Gb tuatara genome has been recently reported [191], including a list of sex developmental genes [192]. The oviparous tuataras exhibit a unique form of TSD, with females produced below, and males above 22°C [193]. Tuataras possess no sex chromosomes with neither population genomic resources nor global CG-methylation patterns revealing sex specificity [191,193]. Orthologues of genes acting antagonistically in masculinizing (e.g. *Sf1*, *Sox9*) or feminizing (e.g. *Rspo1*, *Wnt4*) networks promoting testicular or ovarian development, have been identified, as were genes implicated in TSD (e.g. *Cirbp*; [7]). This example shows that WGS alone can be insufficient to understand SD, particularly TSD. However, a high-quality genome is an important resource for the evaluation of embryonic transcriptomes or proteomes, which are critical data sources for characterization of genes related to sexual development.

Squamata (*squamates*, *lizards and snakes*)

Squamates, comprising currently more than 11 000 species [194], diverged approximately 250 Ma from Rhynchocephalia [195], while lepidosaurs diverged 277 Ma from archosaurs and turtles [191]. To date, more than 35 genomes have been assembled (table 1). Genome studies are eased by moderate genome sizes, ranging from 1.3 to 3.7 Gb [196]. Squamate reptiles are oviparous or viviparous; 'ovo-viviparity' may be difficult to distinguish from viviparity [197]. They mostly exhibit gonochorism, and very rarely true parthenogenesis (females give birth to genetically identical—'clonal'—daughters; [94,198]). In several clades of lizards and in a blind snake, diploid or triploid all-female obligate parthenogenetic complexes, mostly of hybrid origin, are known [199,200] or arose in the laboratory [201]. Interestingly, natural polyploid reptiles appear only fertile as triploids [202]. Squamates exhibit GSD with male (XX/XY) or female (ZZ/ZW) heterogamety, having undifferentiated or often differentiated, heteromorphic sex chromosomes, or ESD, mostly in the form of TSD [6,18,203–205]. ESD seems relatively rare, currently estimated to occur in roughly 5% of non-avian reptile species [206]. Multiple neo-sex chromosomes evolved via sex

chromosome-autosome fusions more frequently in iguanas with male heterogamety than in snakes with female heterogamety [207], which agrees with similar apparent patterns in other vertebrates [110,207,208]. Neither simultaneous nor sequential hermaphrodite species are known in reptiles [209]. Facultative parthenogenesis is well documented in many snake and lizard lineages, with all-female progeny under male heterogamety but all-male progeny under female heterogamety with degenerated W-chromosomes [210]. Facultative parthenogenesis yielding genetically variable offspring of both sexes was discovered in a xantusiid lizard [211]. Five squamate clades (iguanas, lacertid lizards, varanids, skinks and caenophidian snakes) covering approximately 60% of extant squamates show evolutionary conserved sex chromosomes [206,212–216], while other lineages, particularly Acrodonta (agamid lizards and chameleons), boas and pythons, and geckos exhibit more variable SD [18,205,217–219]. In two snake families and the Komodo dragon (*Varanus komodoensis*) with female heterogamety, substantial W-chromosome degeneration and the absence of global Z-chromosome dosage compensation has been shown, dosage balance is largely lacking in Z-specific genes in these species [215,220,221]. By contrast, X-linked genes are twofold upregulated in males and thus fully dosage-compensated in *Anolis carolinensis* [222,223], a species with a 160 Myr-old sex chromosome system [212]. However, a lack of dosage balance under male heterogamety was found in *Basiliscus vittatus* and *Lialis burtonis* [224–226]. Rates of evolution in Z-linked genes were demonstrated to be increased, relative to their autosomal homologues in snakes, supporting the fast-Z effect [220]. Nevertheless, many questions remain regarding SD, dosage compensation and evolutionary rates of sex-linked loci, including the reasons for differences in the variability of SD among squamate lineages.

Testudines (turtles)

Despite their derived anatomy, turtles, containing 361 extant species [194], are related to the bird-crocodylian (Archosaurian) lineage, from which they split between the Upper Permian and Triassic, approximately 270–250 Ma [227], or earlier, in the Carboniferous, 320 Ma [228]. Twenty-two species have draft genomes assembled [42], specifically 18 Cryptodira and four Pleurodira (table 1). Turtles exhibit highly homologous and similarly sized genomes as crocodiles and some birds [229], ranging from 2 to 2.9 Gb [43]. Turtles are exclusively oviparous [197]. They comprise ESD (TSD) or GSD species, the latter with either ZZ/ZW or XX/XY systems [204,230–233]. While ESD is possibly ancestral to turtles and has been found in most studied species, GSD evolved independently at least five times and stayed notably stable in trionychids (ZZ/ZW) and probably also in chelids for many millions of years [233–235], although in chelids their XX/XY sex chromosomes display considerable morphological evolution, including a Y-to-autosome fusion [236]. No global dosage compensation was found in the female-heterogametic trionychid *Apalone ferox* [237], yet, dosage compensation varying by tissue, age, and temperature is suggested in *Apalone spinifer* [238]. Preliminary analyses of few sex-linked genes hint to fast-Z and slow-X effects in turtles [239,240]. Despite efforts to elucidate the molecular basis of GSD in turtles by searching for reptilian homologues of genes [232] involved in sexual development of mammals [241] and birds [242], no master SD gene has been identified yet [204]. However, *Sfl* (a testis development gene)

is translocated to the ZW-chromosomes in *Apalone* and remains a candidate [243]. Natural polyploids are found in *Platemys platycephala*, specifically triploids, diploid–triploid mosaic and triploid–tetraploid mosaicism [54]. Transcriptomic analyses in turtles with ESD targeted the network of gonadal development [244–246], including its epigenetic regulation [246,247]. In early embryos of *Trachemys scripta*, the histone H3 lysine 27 (H3K27) demethylase *Kdm6b* has temperature-dependent sexually dimorphic expression. Knockdown of *Kdm6b* at 26°C (all-male offspring) triggers male-to-female sex reversal in more than 80% of embryos. *Kdm6b* directly promotes transcription of *Dmrt1* by eliminating the trimethylation of H3K27 near its promoter. Additionally, overexpression of *Dmrt1* was sufficient to rescue the sex reversal induced by disruption of *Kdm6b* [248]. Recent research revealed that temperature-mediated influx of calcium at 31°C drives phosphorylation of *Stat3*, which represses transcription of *Kdm6b* [249]. Still, many research questions on the genomics and molecular mechanisms of SD remain unanswered.

Archosauria (archosaurs)

Crocodylia (crocodiles)

Crocodiles, containing only 24 extant species [194] diverged from birds more than 240 Ma [250,251], whereas forms, morphologically similar to the living crocodylians (Alligatoridae, Crocodylidae, Gavialidae), first appear in the fossil record 80–90 Ma [252]. With moderately large genome sizes (2.3–2.9 Gb; [251]), four genomes (*Alligator mississippiensis*, *Alligator sinensis*, *Crocodylus porosus*, *Gavialis gangeticus*) have been sequenced [251,253]. All crocodiles are oviparous [254]. Crocodiles have no sex chromosomes [255], and sexual differentiation is determined during development by a temperature-sensing mechanism with a poorly understood molecular basis. Earlier gene expression studies [256,257] have more recently been extended using gonadal RNAseq and revealed 41 differentially expressed/spliced genes at a male-producing temperature, including *Wnt1*, *Kdm6b*, *C/EBP* [258] and *Jumonji* chromatin modifiers [259]. In the Chinese alligator, orthologues of male-determining genes show an increasing or steady expression during gonadogenesis under the male-inducing but a decreasing expression pattern under the female-inducing temperature [260].

Aves (birds)

Birds contain more than 10 000 extant species [261]. They shared the last common ancestor with the sister taxon of crocodiles earlier than 240 Ma [251,252]. Eased by high synteny [262] and compact genome sizes (0.9–2.1 Gb; [43]), over 502 [42] of bird genome assemblies have been published [263] and more are in preparation (table 1). Birds share homologous female-heterogametic sex chromosomes, i.e. a ZZ/ZW system [264]. No candidate for a female (W-specific) SD gene has been identified [265,266] and current knowledge strongly suggests that SD in birds is based on copy-number (i.e. dosage) variation of the Z-linked master SD gene *Dmrt1* with a key role in testis development, which is missing on the W [267]. The gene *Dmrt1* resides in the oldest evolutionary stratum of the Z-chromosome [268], shared by palaeognath and neognath birds [269–271]. A recent study using a CRISPR-Cas9 based mono-allelic targeting approach with sterile surrogate chicken hosts supports this hypothesis [272]. Such a chromosomally male (ZZ) chicken with a single

functional copy of *Dmrt1* developed ovaries with typical female markers and exhibited follicular development. Interestingly, these animals were indistinguishable in external appearance from wild-type adult males, supporting that the development of male secondary sexual characters is driven by cell-autonomous sex identity and independent of gonadal hormones [272,273]. The rarity of Z0 and ZZW individuals in birds may suggest that these genotypes are often lethal or infertile [274], and that a locus on the W might control dosage compensation of some Z-linked genes [275,276]. Lethality of polyploid bird embryos may be owing to a general disruption of development. Mortality was high among ZZZ individuals, which developed as males [277]. In a study of 4182 chicken embryos, haploids (1.4%), triploids (0.8%, 9 ZZZ, 7 ZZW, 15 ZWW) and tetraploids (0.1%, 1 ZZZZ, 1 ZZWW) were found, none of which survived to hatching ([278]; discussed in: [279]). ZZ-eggs can be sex-reversed to female by oestrogen-exposure during the critical period of gonad formation [7]. Gynandromorphs with male versus female bilateral morphology can arise from double fertilization of a binucleate egg and this bilaterally distinctive chromosomal constitution of cells governs perception of the hormone environment [7]. Facultative parthenogenesis in birds mostly leads to early embryonic mortality, but hatchlings or even adults (all males) were reported in turkey and chicken [52,53]. Multiple neo-sex chromosomes have been found only extremely rarely in birds [280]; however, extended Z and W chromosomes, formed by addition of autosomal material to both Z and W chromosomes, evolved within songbirds in the Sylvioidea superfamily [281–283] and in *Eopsaltria australis* [284].

Genomics of avian sex chromosomes is well studied and revealed great interspecies diversity of pseudoautosomal regions (PAR) and Z/W differentiation, from relatively modest degradation in some palaeognath species to extreme degradation in most modern birds [285,286]. The PAR is short in many neognaths, and even without genes in chicken [287]. Similar to the surviving genes on the mammalian Y chromosomes, the retained genes on the bird W chromosomes are enriched for housekeeping or putative dosage-sensitive genes with stronger selective constraints than the lost ones, and are conserved between distantly related lineages of birds [287,288]. Shared or lineage-specific recombination suppression produced ‘evolutionary strata’, i.e. punctuated sequence divergence owing to stepwise suppression of recombination between Z and W [268]. These strata evolved by a complex process of W- and Z-linked inversions, the latter comprising 25 in total across avian lineages [270].

All studied birds exhibit incomplete ZZ/ZW dosage compensation [289], which seems gene-specific and partial [290]. Moderation of expression levels partially balances out the otherwise twofold difference [291,292], presumably because not all genes are equally sensitive to dosage differences. For many genes, this twofold expression difference does not appear to be associated with severe fitness costs. In addition, other bird genes have evolved sex-biased expression [285,293]. Likewise, in palaeognath birds, sex chromosome genomics recently revealed incomplete dosage compensation, confirmed large (more than 100 Myr-old) PARs, where genes in some species, however, evolve faster than autosomal ones [294]. Like other sex chromosomes, those of birds accumulate transposable elements in the non-recombining regions of the W [295]. On the W, Peona *et al.* [296] revealed enrichment of

endogenous retroviruses, which can be expressed and may retrotranspose, inducing genome-wide female-biased mutation rates. Furthermore, probably all songbirds have a germline-restricted chromosome (GRC) and thus undergo a form of partial genome elimination [40,41]. First cytogenetically described in zebra finch, *Taeniopygia guttata* [297], GRC is absent in somatic cells but present in one copy in male germline cells (but eliminated during spermatogenesis) and two copies in female germline cells (reviewed in [298,299]). Recent genomic, transcriptomic and comparative cytogenetic work suggests that the GRC is enriched in genes [300–302]. The zebra finch GRC contains more than 115 paralogues to single-copy genes on 18 autosomes and the Z is enriched in genes involved in female gonadal development. These genes are transcribed in testes and ovaries [301]. Although the exact function of GRC is currently unclear, the GRC resembles an XX/X0 system, albeit one limited to the germline on top of a ZZ/ZW system in germline and soma. Another level of complexity for understanding the songbird *sexome* arises from the proposed maternal inheritance of the GRC (but see [303]), implying that it is co-inherited with the W and the mitochondrial genome.

Mammalia (mammals)

Monotremata (monotremes)

With five extant species [137], this order includes the sole representatives of the subclass Prototheria, which diverged 200 Ma from viviparous mammals (Theria; [304]), represented by Ornithorhynchidae with a single species (platypus) and the 50 Ma diverged Tachyglossidae (echidnas) with four species. Platypus and echidna genomes are among the smallest in mammals (2.7–2.8 Gb; [43]). Monotremes display a fascinating mixture of derived mammalian and primitive amniote morphological and physiological features shared with sauropsids (reptiles including birds), and have a unique reproductive system that combines egg-laying with lactation. Likewise, the platypus genome exhibits a combination of derived and plesiomorphic characters [305]. The echidna genome has just become available [306]. The monotreme karyotypes have been controversial for almost half a century (cf. [307]) but turned out to contain multiple sex chromosomes, which probably arose from sequential rearrangements between ancient sex chromosomes and several autosomes. During gametogenesis, meiotic chains form that comprise 10 sex chromosomes (five Xs and five Ys) in male platypus and 9 (five Xs and four Ys) in male echidnas [307,308]. This monotreme sex chromosome system evolved independently of the sex chromosomes of viviparous mammals approximately 175 Ma [304,309]. The mammalian master SD gene, *Sry*, is absent from the genome, while the putative avian SD gene, *Dmrt1*, is located on the chromosome X₅, in two copies in females and one in males, i.e. the opposite situation from birds [310]. The most promising master SD candidate is *Amh* (*AmhY*), which is known to have a fundamental role in SD of fishes, and is carried by the Y₅ chromosome that corresponds to the oldest of the evolutionary strata of the monotreme sex chromosomes [304,311]. The recent improvement of a male platypus genome revealed seven strata, distributed across the five Xs, which sequentially suppressed recombination with their homologous Ys, five of which are shared with echidna [306]. This work also provided insights into the origin and evolution of the 10

platypus sex chromosomes. Sequence homology was found between the chromosome Y₅, where *Amh*Y is located, and the chromosome X₁, suggesting that the 10 platypus sex chromosomes ancestrally formed a ring, rather than a chain. In contrast to autosomes, there are extensive interchromosomal contacts between the extant platypus sex chromosome pairs. Unusually frequent interchromosomal contacts were also found between the autosomal regions in humans homologous to the platypus sex chromosomes, suggesting that reciprocal translocations leading to the evolution of the multiple platypus sex chromosomes were facilitated by spatial proximity of these chromosomes that pre-existed in the mammalian ancestor. Monotreme dosage compensation of X-linked genes occurs on a gene-by-gene basis [312], rather than through chromosome-wide silencing, as in eutherians and marsupials [313,314].

Theria (viviparous mammals)

Metatheria (marsupials)

Marsupials diverged approximately 180 Ma from Eutheria (placentals) [304] and contain 385 extant species [136,137], inhabiting Australasia and the Americas. Marsupials exhibit moderate genome sizes of approximately 3.9 Gb [43,315]. To date, eight genomes have been sequenced [42] and genomic evolution has recently been reviewed [316]. Marsupials differ from eutherian mammals in many features of reproduction and development, e.g. extraembryonic tissues have undergone remarkable modifications to accommodate reduced egg size and quantity of yolk/deutoplasm versus increasing emphasis on viviparity and placentation [317]. While all marsupials show male heterogamety (XX/XY), the X of marsupials vary substantially in size, morphology and banding patterns, even between species with an ancestral-like $2n = 14$ karyotype [318]. The marsupial X shares complete homology with two-thirds of the eutherian X, the remaining third is autosomal in marsupials and corresponds to an early addition on the eutherian lineage. The marsupial X, therefore, represents the ancestral therian X [285]. Translocations or fusions between autosomes and sex chromosomes have been observed in several marsupials [207,319]. While marsupials usually inactivate the paternal X chromosome in the female soma by a marsupial-specific non-coding RNA (RSX: RNA on silent X; [320]), dosage compensation often remains incomplete, contrasting to random but tightly controlled eutherian X inactivation [321]. Marsupial dosage compensation is associated with specific epigenetic modifications [322]. Cytogenetics in some bandicoots (family Peramelidae) revealed somatic elimination of one X in females and the Y in males at different ontogenetic stages, resulting in sex chromosome mosaics in various tissues [323]. The marsupial Y is much smaller than the eutherian Y; marsupial X and Y do not share a PAR, and thus cannot form a synaptonemal complex or recombine during the first meiotic division, but a special structure, the dense plate, maintains sex chromosome association to ensure proper segregation [319,324,325]. Marsupial Y chromosomes share the master male SD gene, *Sry*, with placental Y chromosomes [316,326].

Eutheria (placentals)

Placental mammals diverged 180 Ma from marsupials [304] and with 6992 species comprise the vast majority of living mammals [136,137]. Genome size varies between approximately 2.7 Gb in Laurasiatheria, approximately 3.3 Gb in Supraprimates/Euarchontoglires, approximately 4.4 Gb in

Xenarthra and approximately 5.3 Gb in Afrotheria [315], with the largest mammalian genome (approx. 7.7 Gb) being that of a rodent from South America, *Tympanoctomys barrerae* [327]. Placental genome assemblies are available from 411 species [42] (table 1). Presumably owing to sex-specific methylation [328] and/or other aspects of development [279], no polyploid mammals are viable and reports on natural polyploids have been disproved [327,329]. Eutherian sex chromosomes evolved from a pair of autosomes in the therian lineage around 180 Ma, they are nowadays highly differentiated in both size and gene content owing to the arrest of recombination causing the degeneration of the Y [285,330]. The eutherian X chromosome carries more than 1000 genes, whereas the Y contains only a few protein-coding genes [304]. The degree of heteromorphism and PAR of eutherian sex chromosomes can differ dramatically, e.g. humans exhibit two PARs with the larger of about 2.5 Mb, whereas the house mouse PAR is only 0.5 Mb, and other species have even lost their PAR [331,332]. Lineages with multiple neo-sex chromosomes (X₁X₂X₁X₂/X₁X₂Y or XX/XY₁Y₂) have independently evolved by fusion with an autosome at least 20 times [207]. In some rare cases, a translocation of an autosome to both sex chromosomes has restored a large segment of homology between X and Y, creating a neo-PAR, as found in the African pygmy mouse (*Mus minutoides*), where it appears to show signs of early stages of sex chromosome differentiation [333]. Eutherians randomly inactivate one of two Xs in female somatic cells by a non-coding RNA (*Xist*: X-inactive specific transcript; [334]). Active and inactive X chromosomes localize to different subnuclear positions with distinct chromosomal architectures and epigenetic signatures, reflecting their activity state [335]. The eutherian Y exhibits strata that stopped recombining at well-dated time points [304] and carries the master SD gene (*Sry*). This testis development initiating transcription factor is homologous to the X-linked *Sox3* [7,336]. The gene regulatory network of male and female SD- and developmental pathways are best-studied in laboratory house mice [7]. While for 30 years *Sry* has been thought to comprise a single exon, a cryptic second exon, essential for male SD in mice has just been identified [337]. Although eutherian XX/XY sex determination is extremely conserved, a few rodent species evolved unusual, derived sex chromosome systems [338]. For example, spiny rats, *Tokudaia osimensis*, and mole voles, *Ellobius lutescens*, have lost their Y chromosomes including *Sry* [339,340], and the gene *etv* is hypothesized to activate *Sox9* [7]. On the other hand, fertile females with a Y chromosome are known in some rodents (e.g. *Akodon azarae*). The situation is probably best explored in the African pygmy mouse (*Mus minutoides*), with a sex reversal mutation on a mutant X (called X*) and only XY individuals presenting phenotypic males, while genotypic XX, XX* and X*Y mice are females [341]. Genotypic XX females in moles (*Talpa occidentalis*) develop ovotestes instead of ovaries and exhibit a masculinized phenotype (musculature, external genitalia, aggressiveness). The testicular part of the ovotestes lacks fertile germ cells but contains typical male androgen-producing cells. Recently, it was uncovered that the increased androgen synthesis in female moles is caused by a tandem triplication of a region containing *Cyp17A1*, a gene controlling androgen synthesis, and an intrachromosomal inversion involving the pro-testicular growth factor gene *Fgf9*, heterochronically expressed in the ovotestes [342]. Adult mammals cannot

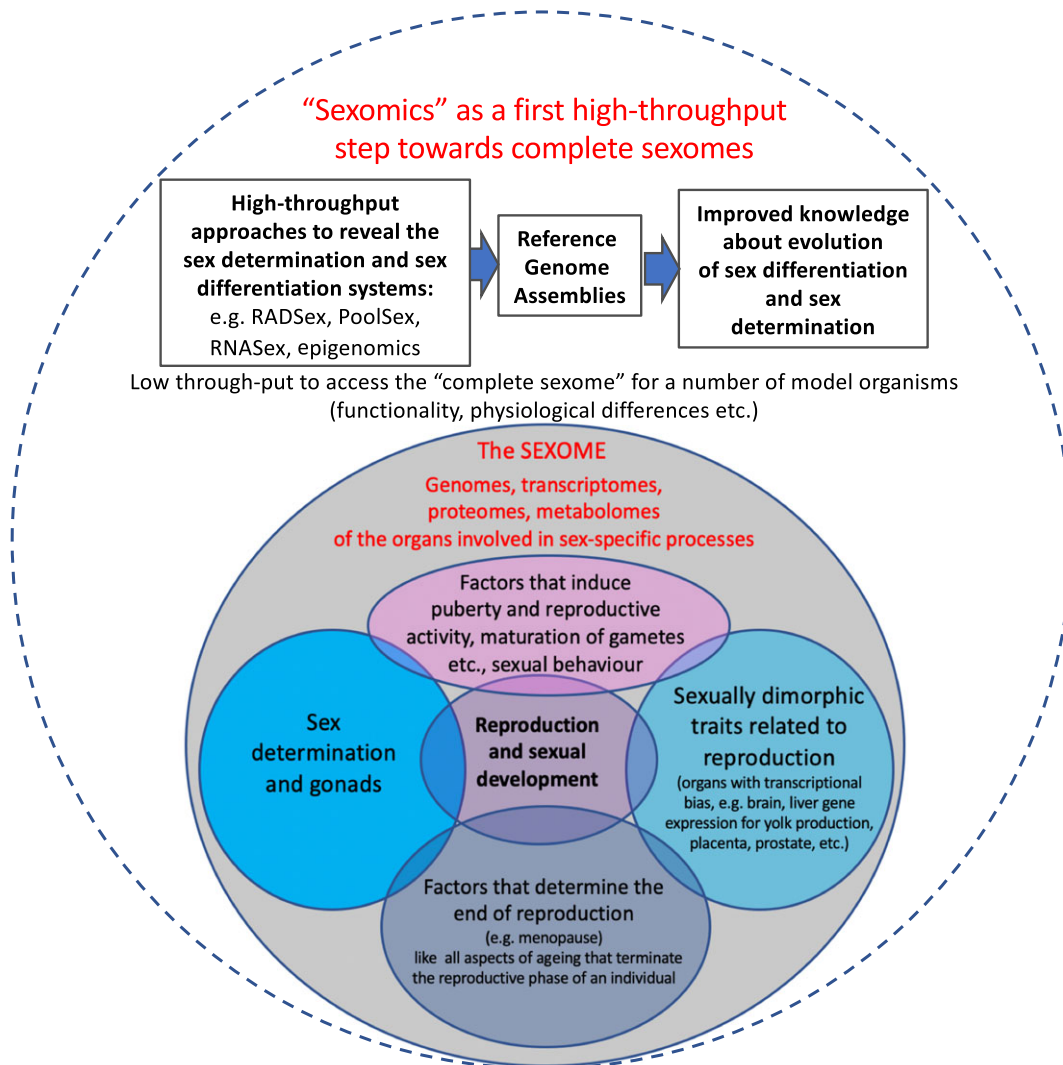


Figure 2. Scheme on *sexomics* as a first high-throughput step to improve our understanding of the complete *sexome* of vertebrates.

perform sex reversal but genetic perturbations can destabilize the commitment to Sertoli and granulosa cell fate in adult life [7], showing that adult mammalian testes or ovaries require repression of the alternative state [343,344]. Gene expression in eutherians across 12 tissues (human, macaque, mouse, rat, dog) revealed hundreds of genes with conserved sex-biased expression but showed that it has arisen recently and is thus not shared between most mammals [345]. XX-genotypes have been experimentally shown to increase lifespan in mice [346].

3. Beyond whole vertebrate genomes: a pledge for '*sexomics*'

There are several ongoing initiatives to sequence many of the 71 000 vertebrate genomes [347–350]. In context to future research on vertebrate SD and differentiation, we hereby suggest that future sequencing efforts target species with missing information on their SD system, the sex chromosomes or special developmental and/or reproductive modes of interest. As an overview, we have prepared table 1, a summary of the electronic supplementary material, table S1, which summarizes currently (December 2020) available whole-genome information in the context of knowledge on sex evolution from [42]. This is where we are now and we think that sequencing technology and bioinformatics will

make it increasingly easier to obtain high-quality genomes from non-model species.

An obvious priority for the *sexome* (figure 2) to be examined by *sexomics* is the sequencing and assembly of sex chromosomes in taxa possessing them. Assembling the sex-limited sex chromosome, the Y or W, has been historically difficult owing to the accumulation of repetitive elements and palindromic sequences on the Y and W [287,351]. Many early genome assembly projects chose to sequence the homogametic sex (XX or ZZ individuals) to avoid problems with assembling sex chromosomes and prevent mis-assembly [352,353]. The advent of long-read sequencing, e.g. PacBio and Oxford Nanopore, has made assembly of the hemizygous sex chromosome (Y or W) feasible, and many genome assembly consortia are now using the heterogametic sex as the reference assembly [354,355]. However, *sexomics* is more than just including sex chromosomes in genome assemblies.

While genome sequencing *per se* will undoubtedly present a driving force towards our understanding of vertebrate sex, we wish to point out that genome sequencing is only a starting point to comprehensively understand SD and sex evolution. For integrative research from here and far beyond, we propose to introduce the terms *sexome* (and *sexomics*; figure 2). As the *sexome*, we consider the information about an individual regarding its sexual differentiation, development and reproduction on all levels of biological organization. This includes the genomic and epigenetic information, the transcriptomes

of the organs involved in these sex-specific processes. These organs comprise the gonads, secondary sex organs and characters, organs with a transcriptional sex-bias (e.g. brain, liver gene expression for yolk production, placenta, prostate), the respective proteomes as well as information about environmental factors that induce puberty and reproductive activity, maturation of gametes, etc., sexual behaviour, and finally the factors that determine fertility and the end of or transitions in reproduction (e.g. menopause). It should also include information on malfunction and impairment (e.g. teratology and endocrine disruption).

While we will not be able to cover this universe, we first focus on the analyses of genomes, transcriptomes and proteomes and how they influence the whole picture. We also would like to encourage others (neurobiologists, ethologists, ecologists) to contribute their expertise to complete the *sexome* (figure 2) of as many species as possible.

Like other ‘-omics’ terms, *sexomics* describes a special feature of an organism, and the *sexomics* idea is a term to gather all relevant ‘-omics’ approaches, applicable in high-throughput mode. We argue that the *sexome* in the first place is a comprehensive description, which comprises all aspects of sexual development and is an archive of data that characterizes a complex phenotype, specific to the reproductive mode of an organism (e.g. female, male, hermaphrodite). Information about the *sexome* feeds into the classical disciplines (see above) and should be considered at the level of ‘comparative *sexomics*’ as a tool for improving the approaches to a better understanding of molecular and phenotypic evolution, population dynamics, ecology and more. We are convinced that only such comparative approaches across the phylogeny as well as information on intraspecific and intra-population variation, and its regulation will lead to substantial scientific progress. We are sure that this holds particularly true for the *sexome*.

Elucidating the evolution of sex chromosomes and SD in non-model vertebrates primarily addresses fundamental research questions [2,11], including turnovers of SD systems [356], speciation [357], hybridization [358] and evolutionary development [359]. Likewise, based on similarities and differences in SD and sexual differentiation in non-vertebrates,

such as insects and other arthropods, genomic and molecular links between these major taxonomic groups (e.g. the role of the *Dmrt* gene family [360]) may allow us to consider *sexomics*-like approaches in other organismal groups.

Beyond basic research, we emphasize that integrative *sexomics* research in vertebrates will also be of high relevance for many fields of applied research. For example, a major, still poorly understood and complex threat for aquatic and semi-terrestrial vertebrates is endocrine disruption [361]. Missing knowledge on the developmental biology of sex and the genetics of SD remains a major obstacle to study endocrine-disruptive effects in many non-model fishes and amphibians [175,362], and such applications would improve bioindication in freshwater bodies to sense threats for humans. A second field, with relevance to better protect wild fish populations, is sustainable aquaculture with an increasing demand to control sex ratios or to foster mono-sex production [363]. We hope that our review, which does not claim to be complete, will provide a stimulus for upcoming and future research.

Data accessibility. This article has no additional data.

Authors’ contributions. M.St. and L.K. drafted the paper to which first M.Sc. and Y.G., and then all co-authors contributed.

Competing interests. We declare we have no competing interests.

Funding. M.St. and M.Sc. were in part supported by COFASP/ERANET (STURGEoNOMICS) by the German Federal Ministry of Food and Agriculture through the Federal Office for Agriculture and Food (grant nos. 2816ERA04G, and 2816ERA05G); M.Sc. was also funded by the German Research Foundation (DFG), grant nos. SCHA408/14-1 und 15-1; H.K. was funded by the German Research Foundation (DFG), grant no. KU 3596/1-1 (project no. 324050651); L.K. and M.R. were supported by the Czech Science Foundation (project no. 17-22604S); B.J.E. was supported by the Natural Sciences and Engineering Research Council of Canada (RGPIN-2017-05770); A.S. was supported by the Swedish Research Council Vetenskapsrådet (grant nos. 2016-05139547 and 2020-04436); N.V. was supported by a grant from the National Science Foundation (grant no. IOS-1555999); B.C. was supported by a grant from the National Science Foundation (grant no. IOS-1256675); Q.Z. is supported by a European Research Council Starting Grant (grant no. 677696).

Acknowledgement. We thank Jana Thomayerová and Sylvia Kanzler for technical assistance in preparing the manuscript.

References

- Matson CK, Zarkower D. 2012 Sex and the singular DM domain: insights into sexual regulation, evolution and plasticity. *Nat. Rev. Genet.* **13**, 163–174. (doi:10.1038/nrg3161)
- Bachtrog D, Kirkpatrick M, Mank JE, Mcdaniel SF, Pires JC, Rice W, Valenzuela N. 2014 Sex determination: why so many ways of doing it? *PLoS Biol.* **12**, e1001899. (doi:10.1016/j.tig.2011.05.005)
- Herpin A, Scharl M. 2015 Plasticity of gene-regulatory networks controlling sex determination: of masters, slaves, usual suspects, newcomers, and usurpators. *EMBO Rep.* **16**, 1260–1274. (doi:10.15252/embr.201540667)
- Pan Q, Anderson J, Bertho S, Herpin A, Wilson C, Postlethwait JH, Scharl M, Guiguen Y. 2016 Vertebrate sex-determining genes play musical chairs. *C. R. Biol.* **339**, 258–262. (doi:10.1016/j.crvi.2016.05.010)
- Johnson Pokorná M, Kratochvíl L. 2016 What was the ancestral sex-determining mechanism in amniote vertebrates? *Biol. Rev.* **91**, 1–12. (doi:10.1111/brv.12156)
- Straková B, Rovatsos M, Kubička L, Kratochvíl L. 2020 Evolution of sex determination in amniotes: Did stress and sequential hermaphroditism produce environmental determination? *BioEssays* **42**, e2000050. (doi:10.1002/bies.202000050)
- Capel B. 2017 Vertebrate sex determination: evolutionary plasticity of a fundamental switch. *Nat. Rev. Genet.* **18**, 675–689. (doi:10.1038/nrg.2017.60)
- Scharl M. 2004 Sex chromosome evolution in non-mammalian vertebrates. *Curr. Opin. Genet. Dev.* **14**, 634–641. (doi:10.1016/j.gde.2004.09.005)
- Perrin N. 2009 Sex reversal: a fountain of youth for sex chromosomes? *Evolution* **63**, 3043–3049. (doi:10.1111/j.1558-5646.2009.00837.x)
- Cauret CMS, Gansauge M-T, Tupper AS, Furman BLS, Knytl M, Song XY, Greenbaum E, Meyer M, Evans BJ. 2020 Developmental systems drift and the drivers of sex chromosome evolution. *Mol. Biol. Evol.* **37**, 799–810. (doi:10.1093/molbev/msz268)
- Beukeboom LW, Perrin N. 2014 *The evolution of sex determination*. Oxford, UK: Oxford University Press.
- van Doorn GS, Kirkpatrick M. 2007 Turnover of sex chromosomes induced by sexual conflict. *Nature* **449**, 909–912. (doi:10.1038/nature06178)
- Ross JA, Urton JR, Boland J, Shapiro MD, Peichel CL. 2009 Turnover of sex chromosomes in the stickleback fishes (Gasterosteidae). *PLoS Genet.* **5**, e1000391. (doi:10.1371/journal.pgen.1000391)

14. O'Meally D, Ezaz T, Georges A, Sarre SD, Graves JAM. 2012 Are some chromosomes particularly good at sex? Insights from amniotes. *Chromosome Res.* **20**, 7–19. (doi:10.1007/s10577-011-9266-8)
15. Jeffries DL *et al.* 2018 A rapid rate of sex-chromosome turnover and nonrandom transitions in true frogs. *Nat. Comm.* **9**, 4088. (doi:10.1038/s41467-018-06517-2)
16. Montiel EE, Badenhorst D, Lee LS, Literman R, Trifonov V, Valenzuela N. 2016 Cytogenetic insights into the evolution of chromosomes and sex determination reveal striking homology of turtle sex chromosomes to amphibian autosomes. *Cytogenet. Genome Res.* **148**, 292–304. (doi:10.1159/000447478)
17. Kratochvil L, Gamble T, Rovatsos M. 2021 Sex chromosome evolution among amniotes: is the origin of sex chromosomes non-random? *Phil. Trans. R. Soc. B* **376**, 20200108. (doi:10.1098/rstb.2020.0108)
18. Pokorná M, Kratochvil L. 2009 Phylogeny of sex-determining mechanisms in squamate reptiles: are sex chromosomes an evolutionary trap? *Zool. J. Linn. Soc.* **156**, 168–183. (doi:10.1111/j.1096-3642.2008.00481.x)
19. Leonard J.L. 2018 The evolution of sexual systems in animals. In *Transitions between sexual systems* (ed. J Leonard), pp. 1–58. Cham, Switzerland: Springer.
20. Ponnikas S, Sigeman H, Abbott JK, Hansson B. 2018 Why do sex chromosomes stop recombining? *Trends Genet.* **34**, 492–503. (doi:10.1016/j.tig.2018.04.001)
21. Meisel RP. 2020 Evolution of sex determination and sex chromosomes: a novel alternative paradigm. *BioEssays* **42**, 1900212. (doi:10.1002/bies.20200152)
22. Lenormand T, Fyon F, Sun E, Roze D. 2020 Sex chromosome degeneration by regulatory evolution. *Curr. Biol.* **30**, 3001–3006. (doi:10.1016/j.cub.2020.05.052)
23. Charlesworth B, Charlesworth D. 2020 Evolution: a new idea about the degeneration of Y and W chromosomes. *Curr. Biol.* **30**, R871–R896. (doi:10.1016/j.cub.2020.06.008)
24. Charnov EL, Bull J. 1977 When is sex environmentally determined? *Nature* **266**, 828–830. (doi:10.1038/266828a0)
25. Warner D, Shine R. 2008 The adaptive significance of temperature-dependent sex determination in a reptile. *Nature* **451**, 566–568. (doi:10.1038/nature06519)
26. Organ CL, Janes DE, Meade A, Pagel M. 2009 Genotypic sex determination enabled adaptive radiations of extinct marine reptiles. *Nature* **461**, 389–392 (doi:10.1038/nature08350)
27. Geffroy B, Douhard M. 2019 The adaptive sex in stressful environments. *Trends Ecol. Evol.* **34**, 628–640 (doi:10.1016/j.tree.2019.02.012)
28. Putnam N *et al.* 2008 The amphioxus genome and the evolution of the chordate karyotype. *Nature* **453**, 1064–1071. (doi:10.1038/nature06967)
29. Simakov O *et al.* 2020 Deeply conserved synteny resolves early events in vertebrate evolution. *Nat. Ecol. Evol.* **4**, 820–830 (doi:10.1038/s41559-020-1156-z)
30. Satoh N, Rokhsar D, Nishikawa T. 2014 Chordate evolution and the three-phylum system. *Proc. R. Soc. B* **281**, 20141729. (doi:10.1098/rspb.2014.1729)
31. Shi C, Wu X, Su L, Shang C, Li X, Wang Y, Li G. 2020 A ZZ/ZW sex chromosome system in cephalochordate amphioxus. *Genetics* **214**, 617–622. (doi:10.1534/genetics.120.303051)
32. Howell WM, Boschung HT. 1971 Chromosomes of the lancelet, *Branchiostoma floridae* (order amphioxii). *Experientia* **27**, 1495–1496. (doi:10.1007/BF02154315)
33. Meyer A, Van de Peer Y. 2005 From 2R to 3R: evidence for a fish-specific genome duplication (FSGD). *BioEssays* **27**, 937–945. (doi:10.1002/bies.20293)
34. Sacerdot C, Louis A, Bon C, Berthelot C, Crollius HR. 2018 Chromosome evolution at the origin of the ancestral vertebrate genome. *Genome Biol.* **19**, 166. (doi:10.1186/s13059-018-1559-1)
35. Sawada H, Shirae-Kurabayashi M. 2020 Chapter 9. Self- and nonself-recognition of gametes in Ascidians. In *Reproduction in aquatic animals* (eds M Yoshida, J Asturiano), pp. 179–192. Singapore: Springer.
36. Holland LZ, Gorsky G, Fenaux R. 1988 Fertilization in *Oikopleura dioica* (Tunicata, Appendicularia): acrosome reaction, cortical reaction and sperm-egg fusion. *Zoomorphology* **108**, 229–243. (doi:10.1007/BF00312223)
37. Navratilova P, Danks GB, Long A, Butcher S, Manak JR, Thompson EM. 2017 Sex-specific chromatin landscapes in an ultra-compact chordate genome. *Epigenetics Chromatin* **10**, 3. (doi:10.1186/s13072-016-0110-4)
38. Henschke N, Everet JD, Anthony J, Richardson AJ, Suthers IM. 2016 Rethinking the role of salps in the ocean. *Trends Ecol. Evol.* **31**, 720–733. (doi:10.1016/j.tree.2016.06.007)
39. Holland LZ. 2016 Tunicates. *Curr. Biol.* **26**, R146–R152. (doi:10.1016/j.cub.2015.12.024)
40. Smith JJ *et al.* 2013 Sequencing of the sea lamprey (*Petromyzon marinus*) genome provides insights into vertebrate evolution. *Nat. Genet.* **45**, 415–421. (doi:10.1038/ng.2568)
41. Smith JJ *et al.* 2018 The sea lamprey germline genome provides insights into programmed genome rearrangement and vertebrate evolution. *Nat. Genet.* **50**, 270–277 (doi:10.1038/s41588-017-0036-1).
42. NCBI. 2020 www.ncbi.nlm.nih.gov (accessed December 2020).
43. Gregory TR. 2015 Animal genome size database. See <http://www.genomesize.com>.
44. Gorbman A. 1990 Sex differentiation in the hagfish *Eptatretus stouti*. *Gen. Comp. Endocrinol.* **77**, 309–323. (doi:10.1016/0016-6480(90)90315-D)
45. Adolphi MC, Nakajima RT, N'Nobrega RH, Scharf M. 2019 Intersex, hermaphroditism, and gonadal plasticity in vertebrates: evolution of the Müllerian duct and *Amh/Amhr2* signaling. *Annu. Rev. Anim. Biosci.* **7**, 7.1–7.24. (doi:10.1146/annurev-animal-020518-114955)
46. Smith JJ, Timoshevskiy VA, Saraceno C. 2021 Programmed DNA elimination in vertebrates. *Annu. Rev. Anim. Biosci.* **9**, 173–201. (doi:10.1146/annurev-animal-061220-023220)
47. Hendon JM, Koester DM, Hoffmayer ER, Driggers III WB, Cicia AM. 2013 Occurrence of an intersexual blacktip shark in the northern Gulf of Mexico, with notes on the standardization of classifications for this condition in elasmobranchs. *Mar. Coast. Fish.* **5**, 174–180. (doi:10.1080/19425120.2013.799618)
48. Mims SD, Shelton WL, Linhart O, Wang C. 1997 Induced meiotic gynogenesis of paddlefish *Polyodon spathula*. *J. World Aquacult. Soc.* **28**, 334–343. (doi:10.1111/j.1749-7345.1997.tb00280.x)
49. Shelton WL, Mims SD. 2012 Evidence for female heterogametic sex determination in paddlefish *Polyodon spathula* based on gynogenesis. *Aquaculture* **356–357**, 116–118. (doi:10.1016/j.aquaculture.2012.05.029)
50. Bogart JP. 2019 Unisexual salamanders in the genus *Ambystoma*. *Herpetologica* **75**, 259–267 (doi:10.1655/Herpetologica-D-19-00043.1)
51. Macgregor HC, Uzzell TM. 1964 Gynogenesis in salamanders related to *Ambystoma jeffersonianum*. *Science* **143**, 1043–1045. (doi:10.1126/science.143.3610.1043)
52. Olsen MW. 1975 Avian parthenogenesis. *Agricultural Research Service USDA, ARS-NE* **65**, 1–82.
53. Ramachandran R, McDaniel CD. 2018 Parthenogenesis in birds: a review. *Reproduction* **155**, R245–R257. (doi:10.1530/REP-17-0728)
54. Bickham JW, Hanks BG, Hale DW, Martin JE. 1993 Ploidy diversity and the production of balanced gametes in male twist-necked turtles (*Platemys platycephala*). *Copeia* **1993**, 723 (doi:10.2307/1447233)
55. Jørgensen JM, Lomholt JP, Weber RE, Malte H. 1998 *The biology of hagfishes*. London, UK: Chapman & Hall.
56. Powell ML, Kavanaugh SI, Sower SA. 2005 Current knowledge of hagfish reproduction: implications for fisheries management. *Integr. Comp. Biol.* **45**: 158–165. (doi:10.1093/icb/45.1.158)
57. Johnson NS, Swink WD, Brenden TO. 2017 Field study suggests that sex determination in sea lamprey is directly influenced by larval growth rate. *Proc. R. Soc. B* **284**, 20170262. (doi:10.1098/rspb.2017.0262)
58. Docker MF, Beamish FWH, Yasmin T, Bryan MB, Khan A. 2019 Chapter 1: the lamprey gonad. In *Lampreys: biology, conservation and control* (ed. MF Docker), pp. 1–186. Dordrecht, The Netherlands: Springer.
59. Irisarri I *et al.* 2017 Phylotranscriptomic consolidation of the jawed vertebrate timetree. *Nat. Ecol. Evol.* **1**, 1370–1378. (doi:10.1038/s41559-017-0240-5)
60. Hara Y *et al.* 2018 Shark genomes provide insights into elasmobranch evolution and the origin of vertebrates. *Nat. Ecol. Evol.* **2**, 1761–1771. (doi:10.1038/s41559-018-0673-5)

61. King BL, Gillis JA, Carlisle HR, Dahn RD. 2011 A natural deletion of the HoxC cluster in elasmobranch fishes. *Science* **334**, 1517. (doi:10.1126/science.1210912)
62. Venkatesh B *et al.* 2014 Elephant shark genome provides unique insights into gnathostome evolution. *Nature* **505**, 174–179. (doi:10.1038/nature12826)
63. Musick JA, Ellis J. 2005 Reproductive evolution of chondrichthyans. In *Reproductive biology and phylogeny of chondrichthyans* (ed. WC Hamlett), pp. 45–79. Enfield, NH: Scientific Publication.
64. Dudgeon CL, Coulton L, Bone R, Ovenden JR, Thomas S. 2017 Switch from sexual to parthenogenetic reproduction in a zebra shark. *Sci. Rep.* **7**, 40537. (doi:10.1038/srep40537)
65. Maddock MB, Schwartz FJ. 1996 Elasmobranch cytogenetics: methods and sex chromosomes. *Bull. Mar. Sci.* **58**, 147–155.
66. Donahue WHA. 1974 A karyotypic study of three species of rajiformes (Chondrichthyes, Pisces). *Can. J. Genet. Cytol.* **16**, 203–211. (doi:10.1139/g74-020)
67. da Cruz VP, Shimabukuro-Dias CK, Oliveira C, Fausto Foresti F. 2011 Karyotype description and evidence of multiple sex chromosome system $X_1X_1X_2X_2/X_1X_2Y$ in *Potamotrygon* aff. *motoro* and *P. falkneri* (Chondrichthyes: Potamotrygonidae) in the upper Paraná River basin, Brazil. *Neotrop. Ichthyol.* **9**, 201–208. (doi:10.1590/S1679-62252011000100020)
68. Valentim FCS, Porto JIR, Bertollo LAC, Gross MC, Feldberg E. 2013 XX/X0, a rare sex chromosome system in *Potamotrygon* freshwater stingray from the Amazon Basin, Brazil. *Genetica* **141**, 381–387. (doi:10.1007/s10709-013-9737-2)
69. O'Shaughnessy KL, Dahn RD, Cohn MJ. 2015 Molecular development of chondrichthyan claspers and the evolution of copulatory organs. *Nat. Comm.* **6**, 6698. (doi:10.1038/ncomms7698)
70. Braasch I, Postlethwait JH. 2012 Polyploidy in fish and the teleost genome duplication. In *Polyploidy and genome evolution* (eds P Soltis, D Soltis), pp. 341–383. Berlin, Germany: Springer.
71. Hughes LC *et al.* 2018 Comprehensive phylogeny of ray-finned fishes (Actinopterygii) based on transcriptomic and genomic data. *Proc. Natl Acad. Sci. USA* **24**, 6249–6254. (doi:10.1073/pnas.1719358115)
72. Raincrow JD, Dewar K, Stocsits C, Prohaska SJ, Amemiya CT, Stadler PF, Chiu CH. 2011 Hox clusters of the bichir (Actinopterygii, *Polypterus senegalus*) highlight unique patterns of sequence evolution in gnathostome phylogeny. *J. Exp. Zool. (Mol. Dev. Evol.)* **316**, 451–464. (doi:10.1002/jez.b.21420)
73. Morescalchi MA, Stingoa V, Capriglione T. 2011 Cytogenetic analysis in *Polypterus ornatipinnis* (Actinopterygii, Cladistia, Polypteridae) and 5S rDNA. *Mar. Genomics* **4**, 25–31. (doi:10.1016/j.margen.2010.12.002)
74. Morescalchi MA, Liguori I, Rocco L, Archimandritis A, Stingo V. 2008 Karyotypic characterization and genomic organization of the 5S rDNA in *Polypterus senegalus* (Osteichthyes, Polypteridae). *Genetica* **132**, 179–186. (doi:10.1007/s10709-007-9160-7)
75. Morescalchi MA, Liguori I, Rocco L, Stingo V. 2007 Karyotypic characterization and genomic organization of the 5S rDNA in *Erpetoichthys calabaricus* (Osteichthyes, Polypteridae). *Genetica* **131**, 209–216. (doi:10.1007/s10709-006-9119-0)
76. Hochleithner M, Gessner J. 2001 The sturgeons and paddlefishes of the world — biology and aquaculture. *Aquacult. Publ.* **106**, 81–82.
77. Du K *et al.* 2020 The sterlet sturgeon genome sequence and the mechanisms of segmental rediploidization. *Nat. Ecol. Evol.* **4**, 841–852. (doi:10.1038/s41559-020-1166-x)
78. Havelka M, Hulák M, Bailie D, Prodöhl P, Flajšhans M. 2013 Extensive genome duplications in sturgeons: new evidence from microsatellite data. *J. Appl. Ichthyol.* **29**, 704–708. (doi:10.1111/jai.12224)
79. Cheng P *et al.* 2020 The American paddlefish genome provides novel insights into chromosomal evolution and bone mineralization in early vertebrates. *Mol. Biol. Evol.* **38**, 1595–1607. (doi:10.1093/molbev/msaa326)
80. Romanenko SA *et al.* 2019 Segmental paleotetraploidy revealed in sterlet (*Acipenser ruthenus*) genome by chromosome painting. *Mol. Cytogenet.* **8**, 90. (doi:10.1186/s13039-015-0194-8)
81. Saito T, Pšenička M, Goto R, Adachi S, Inoue K, Arai K, Yamaha E. 2014 The origin and migration of primordial germ cells in sturgeons. *PLoS ONE* **9**, e86861. (doi:10.1371/journal.pone.0086861)
82. Keyvanshokoh S, Gharaei A. 2010 A review of sex determination and searches for sex-specific markers in sturgeon. *Aquacult. Res.* **41**, e1–e7. (doi:10.1111/j.1365-2109.2009.02463.x)
83. Fopp-Bayat D, Kolman R, Woznicki P. 2007 Induction of meiotic gynogenesis in sterlet (*Acipenser ruthenus*) using UV-irradiated baster sperm. *Aquaculture* **264**, 54–58. (doi:10.1016/j.aquaculture.2006.12.006)
84. Kuhl H *et al.* 2021 A 180 Myr-old female-specific genome region in sturgeon reveals the oldest known vertebrate sex determining system with undifferentiated sex chromosomes. *Phil. Trans. R. Soc. B* **376**, 20200089. (doi:10.1098/rstb.2020.0089)
85. Majtánová Z, Symonová R, Arias-Rodríguez L, Sallan L, Ráb P. 2017 'Holostei versus halecostomi' problem: insight from cytogenetics of ancient nonteleost actinopterygian fish, bowfin *Amia calva*. *J. Exp. Zool. (Mol. Dev. Evol.)* **328B**, 620–628. (doi:10.1002/jez.b.22720)
86. Nelson JS, Grande TC, Wilson MVH. 2016 *Fishes of the world*, 5th edn. New York, NY: John Wiley and Sons.
87. Braasch I *et al.* 2016 The spotted gar genome illuminates vertebrate evolution and facilitates human-teleost comparisons. *Nat. Genet.* **48**, 427–437. (doi:10.1038/ng.3526)
88. Thompson A *et al.* 2020 The genome of the bowfin (*Amia calva*) illuminates the developmental evolution of ray-finned fish. Preprint. (doi:10.21203/rs.3.rs-92055/v1)
89. Herrington SJ, Hettiger KN, Heist EJ, Keeney DB. 2008 Hybridization between longnose and alligator gars in captivity, with comments on possible gar hybridization in nature. *Transact. Am. Fish. Soc.* **137**, 158–164. (doi:10.1577/T07-044.1)
90. McGinn Daniels KL. 1993 Reproductive biology of the bowfin, *Amia calva* Linnaeus, from the green bottom wildlife management area, Cabell County, West Virginia. *Theses, Dissertations Capstones* **309**. (https://mds.marshall.edu/etd/309)
91. Froese R, Pauly D (eds). 2019 FishBase World Wide Web electronic publication. See www.fishbase.org.
92. Hasley A, Chavez S, Danilchik M, Wühr M, Pelegri F. 2017 Vertebrate embryonic cleavage pattern determination. In *Vertebrate development. Advances in experimental medicine and biology*, vol. 953 (eds F Pelegri, M Danilchik, A Sutherland), pp. 117–171. Cham, Switzerland: Springer.
93. Smith C, Wootton RJ. 2016 The remarkable diversity of teleost fishes. *Fish Fish.* **17**, 1208–1215. (doi:10.1111/faf.12116)
94. Avise JC. 2008 *Clonality. The genetics, ecology and evolution of sexual abstinence in vertebrate animals*, pp. 1–237. New York, NY: Oxford University Press.
95. Warren W *et al.* 2018 Clonal polymorphism and high heterozygosity in the celibate genome of the Amazon molly. *Nat. Ecol. Evol.* **2**, 669–679. (doi:10.1038/s41559-018-0473-y)
96. Avise JC, Mank JE. 2009 Evolutionary perspectives on hermaphroditism in fishes. *Sex. Dev.* **3**, 152–163. (doi:10.1159/000223079)
97. Fricke H, Fricke S. 1977 Monogamy and sex change by aggressive dominance in coral reef fish. *Nature* **266**, 830–832. (doi:10.1038/266830a0)
98. Todd EV *et al.* 2019 Stress, novel sex genes, and epigenetic reprogramming orchestrate socially controlled sex change. *Sci. Adv.* **5**, eaaw7006. (doi:10.1126/sciadv.aaw7006)
99. Erisman BE, Petersen CW, Hastings PA, Warner RR. 2013 Phylogenetic perspectives on the evolution of functional hermaphroditism in teleost fishes. *Integ. Comp. Biol.* **53**, 736–754. (doi:10.1093/icb/ict077)
100. Hart MK, Kratter AW, Crowley PH. 2016 Partner fidelity and reciprocal investments in the mating system of a simultaneous hermaphrodite. *Behav. Ecol.* **27**, 1471–1479. (doi:10.1093/beheco/arw065)
101. Kanamori A, Sugita Y, Yuasa Y, Suzuki T, Kawamura K. 2016 A Genetic map for the only self-fertilizing vertebrate. *G3: Genes Genom. Genet.* **4**, 1095–1106. (doi:10.1534/g3.115.022699)
102. Liu H, Todd E, Lokman M, Laamm MS, Godwin JR, Gemmell NJ. 2017 Sexual plasticity: a fishy tale. *Mol. Reprod. Dev.* **84**, 171–194. (doi:10.1002/mrd.22691)
103. Baroiller JF, D'Cotta H. 2016 The reversible sex of gonochoristic fish: insights and consequences. *Sex. Dev.* **10**, 242–266. (doi:10.1159/000452362)
104. Martyniuk CJ, Feswick A, Munkittrick KR, Dreier DA, Denslow ND. 2020 Twenty years of transcriptomics, 17alpha-ethinylestradiol, and fish. *Gen. Comp.*

- Endocrinol.* **286**, 113325. (doi:10.1016/j.ygcn.2019.113325)
105. Nishimura T, Tanaka M. 2016 The mechanism of germ line sex determination in vertebrates. *Biol. Reprod.* **95**, 30. (doi:10.1095/biolreprod.115.138271)
 106. Nakamura S, Watakabe I, Nishimura T, Picard J-Y, Toyoda A, Taniguchi Y, Di Clemente N, Tanaka M. 2012 Hyperproliferation of mitotically active germ cells due to defective anti-Müllerian hormone signaling mediates sex reversal in medaka. *Development* **139**, 2283–2287. (doi:10.1242/dev.076307)
 107. Penman DJ, Piferrer F. 2008 Fish gonadogenesis. Part I: genetic and environmental mechanisms of sex determination. *Rev. Fisher. Sci.* **16**(Suppl. 1), 16–34. (doi:10.1080/10641260802324610)
 108. Ser JR, Roberts RB, Kocher T. 2010 Multiple interacting loci control sex determination in lake Malawi cichlids. *Evolution* **64**, 486–501. (doi:10.1111/j.1558-5646.2009.00871.x)
 109. Moore EC, Roberts RB. 2013 Polygenic sex determination. *Curr. Biol.* **23**, R510–R513. (doi:10.1016/j.cub.2013.04.004)
 110. Sember A, Nguyen P, Perez MF, Altmanová M, Ráb P, Cioffi M de B. 2021 Multiple sex chromosomes in teleost fishes from a cytogenetic perspective: state of the art and future challenges. *Phil. Trans. R. Soc. B* **376**, 20200098. (doi:10.1098/rstb.2020.0098)
 111. Yamamoto Y, Zhang Y, Sarida M, Hattori RS, Strüssmann CA. 2014 Coexistence of genotypic and temperature-dependent sex determination in pejerrey *Odontesthes bonariensis*. *PLoS ONE* **9**, e102574. (doi:10.1371/journal.pone.0102574)
 112. Guiguen Y, Fostier A, Herpin A. 2019 Sex determination and differentiation in fish. In *Sex control in aquaculture*. vol. 1 (eds H-P Wang, F Piferrer, S-L Chen, Z-G Shen), pp. 35–63. Hoboken, NJ: John Wiley & Sons.
 113. Yano A *et al.* 2012 An immune-related gene evolved into the master sex-determining gene in rainbow trout, *Oncorhynchus mykiss*. *Curr. Biol.* **22**, 1423–1428. (doi:10.1016/j.cub.2012.05.045)
 114. Rafati N *et al.* 2020 Reconstruction of the birth of a male sex chromosome present in Atlantic herring. *Proc. Natl Acad. Sci. USA* **117**, 24 359–24 368. (doi:10.1073/pnas.2009925117)
 115. Kamiya T *et al.* 2012 A trans-species missense SNP in *Amhr2* is associated with sex determination in the tiger pufferfish, *Takifugu rubripes* (fugu). *PLoS Genet.* **8**, e1002798. (doi:10.1371/journal.pgen.1002798)
 116. Lamatsch DK, Stöck M. 2009 Sperm-dependent parthenogenesis and hybridogenesis in teleost fishes. Chapter 19. In *Lost sex – the evolutionary biology of parthenogenesis* (eds I Schoen, K Martens, P van Dijk), pp. 399–432. Berlin, Germany: Springer.
 117. Biscotti M, Gerdol M, Canapa A, Forconi M, Olmo E, Pallavicini A, Barucca M, Scharf M. 2016 The lungfish transcriptome: a glimpse into molecular evolution events at the transition from water to land. *Sci. Rep.* **6**, 21571. (doi:10.1038/srep21571)
 118. Pouyaud L, Wirjoatmodjo S, Rachmatika I, Tjakrawidjaja A, Hadiaty R, Hadie W. 1999 Une nouvelle espèce de coelacanth. Preuves génétiques et morphologiques. *C. R. Acad. Sci.* **322**, 261–267. (doi:10.1016/S0764-4469(99)80061-4)
 119. Smith CL, Rand CS, Schaeffer B, Atz JW. 1975. *Latimeria*, the living coelacanth, is ovoviparous. *Science* **190**, 1105–1106. (doi:10.1126/science.190.4219.1105)
 120. Wourms JP, Atz JW, Stribling MD. 1991 Viviparity and the maternal-embryonic relationship in the coelacanth *Latimeria chalumnae*. In *The biology of Latimeria chalumnae and evolution of coelacanths. Developments in environmental biology of fishes*, vol. 12 (eds JA Musick, MN Bruton, EK Balon), pp. 225–248. Dordrecht, The Netherlands: Springer.
 121. Amemiya CT *et al.* 2013 The African coelacanth genome provides insights into tetrapod evolution. *Nature* **496**, 311–316. (doi:10.1038/nature12027)
 122. Forconi M *et al.* 2013 Characterization of sex determination and sex differentiation genes in *Latimeria*. *PLoS ONE* **8**, e56006. (doi:10.1371/journal.pone.0056006)
 123. Biscotti MA, Adolphi MC, Barucca M, Forconi M, Pallavicini A, Gerdol M, Canapa A, Costantini M. 2018 A comparative view on sex differentiation and gametogenesis genes in lungfish and coelacanths. *Genome Biol. Evol.* **10**, 1430–1444. (doi:10.1093/gbe/evy101)
 124. Brinkmann H, Venkatesh B, Brenner S, Meyer A. 2004 Nuclear protein-coding genes support lungfish and not the coelacanth as the closest living relatives of land vertebrates. *Proc. Natl Acad. Sci. USA* **101**, 4900–4905. (doi:10.1073/pnas.0400609101)
 125. Kershaw F, Joss GH, Joss JMP. 2009 Early development in sarcopterygian fishes. In *Development of non-teleost fishes* (eds YW Kunz, CA Luer, BG Kapoor), pp. 275–289. Enfield, NJ: Scientific Publication.
 126. Meyer A *et al.* 2021 Giant lungfish genome elucidates the conquest of land by vertebrates. *Nature* **590**, 284–299. (doi:10.1038/s41586-021-03198-8)
 127. Wang K *et al.* 2021 African lungfish genome sheds light on the vertebrate water-to-land transition. *Cell* **184**, 1362–1376. (doi:10.1016/j.cell.2021.01.047)
 128. Betancur RR *et al.* 2013 The tree of life and a new classification of bony fishes. *PLoS Curr. Tree Life* **1**. (doi:10.1371/currents.tol.53ba26640df0ccae75bb165c8c26288)
 129. Pyron A. 2011 Divergence time estimation using fossils as terminal taxa and the origins of Lissamphibia. *Syst. Biol.* **60**, 4466–4481. (doi:10.1093/sysbio/syr047)
 130. Bossuyt F, Roelants K. 2009 Anura. In *The timetree of life* (eds SB Hedges, S Kumar), pp. 357–364. New York, NY: Oxford University Press.
 131. Pyron RA, Wiens JJ. 2011 A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. *Mol. Phylogenet. Evol.* **61**, 543–583. (doi:10.1016/j.ympev.2011.06.012)
 132. Feng Y-J, Blackburn DC, Liang D, Hillis DM, Wake DB, Cannatella DC, Zhang P. 2017 Phylogenomics reveals rapid, simultaneous diversification of three major clades of Gondwanan frogs at the Cretaceous–Paleogene boundary. *Proc. Natl Acad. Sci. USA* **114**, E5864–E5870. (doi:10.1073/pnas.1704632114)
 133. San Mauro D, Gower DJ, Müller H, Loader SP, Zardoya R, Nussbaum RA, Wilkinson M. 2014 Life-history evolution and mitogenomic phylogeny of caecilian amphibians. *Mol. Phylogenet. Evol.* **73**, 177–189. (doi:10.1016/j.ympev.2014.01.009)
 134. Dünker N, Wake MH, Olson WM. 2000 Embryonic and larval development in the caecilian *Ichthyophis kohtaoensis* (Amphibia, Gymnophiona) a staging table. *J. Morph.* **243**, 3–34. (doi:10.1002/(SICI)1097-4687(200001)243:1<3::AID-JMOR2>3.0.CO;2-D)
 135. AmphibiaWeb. See www.amphibiaweb.org (accessed December 2020).
 136. Burgin CJ, Colella JP, Kahn PL, Upham NS. 2018 How many species of mammals are there? *J. Mammal.* **99**, 1–11. (doi:10.1093/jmammal/gyz052)
 137. Mammal Diversity Database. 2020 Mammal Diversity Database (Version 1.2) (Dataset). Zenodo. Accessed December 2020. (doi:10.5281/zenodo.4139818)
 138. Liedtke HC, Gower DJ, Wilkinson M, Gomez-Mestre I. 2018 Macroevolutionary shift in the size of amphibian genomes and the role of life history and climate. *Nat. Ecol. Evol.* **2**, 1792–1799. (doi:10.1038/s41559-018-0674-4)
 139. Li J, Yu H, Wang W, Fu C, Zhang W, Han F, Wu H. 2019 Genomic and transcriptomic insights into molecular basis of sexually dimorphic nuptial spines in *Leptobranchium leishanense*. *Nat. Comm.* **10**, 5551. (doi:10.1038/s41467-019-13531-5)
 140. Gomez-Mestre I, Pyron RA, Wiens JJ. 2011 Phylogenetic analyses reveal unexpected patterns in the evolution of reproductive modes in frogs. *Evolution* **66**, 3687–3700. (doi:10.1111/j.1558-5646.2012.01715.x)
 141. Buckley D, Alcobendas M, Garcia-Paris M, Wake MH. 2007 Heterochrony, cannibalism, and the evolution of viviparity in *Salamandra salamandra*. *Evol. Dev.* **9**, 105–115. (doi:10.1111/j.1525-142X.2006.00141.x)
 142. Wake MH. 1977 The reproductive biology of caecilians. An evolutionary perspective. In *The reproductive biology of amphibians* (eds DH Taylor, SI Guttman), pp. 73–100. Oxford, OH: Miami University.
 143. Exbrayat J-M. 2009 Oogenesis and female reproductive system in Gymnophiona. In *Reproduction in amphibians* (ed. M Ogielska), pp. 305–342. Enfield, NH: Scientific Publication.
 144. Wake MH. 1989 Phylogenesis of direct development and viviparity in vertebrates. In *Complex organismal functions: integration and evolution in vertebrates* (eds D Wake, G Roth), pp. 235–250. New York, NY: John Wiley & Sons Ltd.

145. Hoffman A *et al.* 2015 Genetic diversity and distribution patterns of diploid and polyploid hybrid water frog populations (*Pelophylax esculentus* complex) across Europe. *Mol. Ecol.* **24**, 4371–4391. (doi:10.1111/mec.13325)
146. Schmid M, Evans BJ, Bogart JP. 2015 Polyploidy in amphibia. *Cytogenet. Genome Res.* **145**, 315–330. (doi:10.1159/000431888)
147. Grafe TU, Linsenmair KE. 1989 Protogynous sex change in the reed frog (*Hyperolius viridiflavus*). *Copeia* **1989**, 1024–1029. (doi:10.2307/1445989)
148. Schmid M, Nanda I, Steinlein C, Kausch K, Epplen JT, Haaf T. 1991 Sex-determining mechanisms and sex chromosomes in amphibia. In *Amphibian cytogenetics and evolution* (eds DM Green, SK Sessions), pp. 393–430. San Diego, CA: Academic Press.
149. Eggert C. 2004 Sex determination: the amphibian models. *Reprod. Nutr. Dev.* **44**, 539–549. (doi:10.1051/rnd:2004062)
150. Hillis DM, Green DM. 1990 Evolutionary changes of heterogametic sex in the phylogenetic history of amphibians. *J. Evol. Biol.* **3**, 49–64. (doi:10.1046/j.1420-9101.1990.3010049.x)
151. Ito M. 2018 Sex determination and differentiation in frogs. *Reproductive and developmental strategies. Diversity and commonality in animals* (eds K Kobayashi, T Kitano, Y Iwao, M Kondo), pp. 349–366. Tokyo, Japan: Springer.
152. Nakamura M. 2010 The mechanism of sex determination in vertebrates: are sex steroids the key-factor? *J. Exp. Zool.* **313A**, 381–398. (doi:10.1002/jez.616)
153. Green DM. 1988 Cytogenetics of the endemic New Zealand frog, *Leiopelma hochstetteri*: extraordinary supernumerary chromosome variation and a unique sex-chromosome system. *Chromosoma* **97**, 55–77. (doi:10.1007/BF00331795)
154. Roco AS, Olmstead AW, Degitz SJ, Amano T, Zimmerman LB, Bullejos M. 2015 Coexistence of Y, W, and Z sex chromosomes in *Xenopus tropicalis*. *Proc. Natl Acad. Sci. USA* **112**, E4752–E4761. (doi:10.1073/pnas.1505291112)
155. Gazoni T, Haddad CFB, Narimatsu H, Cabral-de-Mello DC, Lyra ML, Parise-Maltempi PP. 2018 More sex chromosomes than autosomes in the Amazonian frog *Leptodactylus pentadactylus*. *Chromosoma* **127**, 269–278. (doi:10.1007/s00412-018-0663-z)
156. Schmid M, Steinlein C. 2001 Sex chromosomes, sex-linked genes, and sex determination in the vertebrate class Amphibia. In *Genes and mechanisms in vertebrate sex determination* (eds G Scherer, M Schmid), pp. 143–176. Basel, Switzerland: Birkhäuser Verlag.
157. Sessions SK, Bizjak Malib L, Green DM, Trifonov V, Ferguson-Smith M. 2016 Evidence for sex chromosome turnover in proteid salamanders. *Cytogenet. Genome Res.* **148**, 305–313. (doi:10.1159/000446882)
158. Denton RD, Kudra RS, Malcom JW, Du Preez L, Malone JH. 2018 The African bullfrog (*Pyxicephalus adspersus*) genome unites the two ancestral ingredients for making vertebrate sex chromosomes. *BioRxiv* (doi:10.1101/329847)
159. Furman BLS, Cauret CMS, Knytl M, Song X-Y, Premachandra T, Ofori-Boateng C, Jordan DC, Horb ME, Evans BJ. 2020 A frog with three sex chromosomes that co-mingle together in nature: *Xenopus tropicalis* has a degenerate W and a Y that evolved from a Z chromosome. *PLoS Genet.* **16**, e1009121. (doi:10.1371/journal.pgen.1009121)
160. Schmid M, Steinlein C. 2018 Chromosome banding in Amphibia. XXXVII. Y-Autosome translocations in Anura. *Cytogenet. Genome Res.* **154**, 153–180. (doi:10.1159/000487907)
161. Wallace H. 1994 The balanced lethal system of crested newts. *Heredity* **73**, 41–46. (doi:10.1038/hdy.1994.96)
162. Sessions SK, Macgregor HC, Schmid M, Haaf T. 1988 Cytology, embryology and evolution of the developmental arrest syndrome in newts of the genus *Triturus* (Caudata: Salamandridae). *J. Exp. Zool.* **248**, 321–334. (doi:10.1002/jez.1402480311)
163. Green DM, Sessions SK (eds). 1991 *Amphibian cytogenetics and evolution*. San Diego, CA: Academic Press.
164. Keinath, MC, Timoshevskaya N, Timoshevskiy VA, Voss R, Smith JJ. 2018 Miniscule differences between sex chromosomes in the giant genome of a salamander. *Sci. Rep.* **8**, 17882. (doi:10.1038/s41598-018-36209-2)
165. Smith JJ, Voss SR. 2009 Amphibian sex determination: segregation and linkage analysis using members of the tiger salamander species complex (*Ambystoma mexicanum* and *A. t. tigrinum*). *Heredity* **102**, 542–548. (doi:10.1038/hdy.2009.15)
166. Hu Q, Chang C, Wang Q, Tian H, Qiao Z, Wang L, Meng Y, Xu C, Xiao H. 2019 Genome-wide RAD sequencing to identify a sex-specific marker in Chinese giant salamander *Andrias davidianus*. *BMC Genomics* **20**, 415. (doi:10.1186/s12864-019-5771-5)
167. Hime PM, Briggler JT, Reece JS, Weisrock DW. 2019 Genomic data reveal conserved female heterogamety in giant salamanders with gigantic nuclear genomes. *G3: Genes Genom. Genet.* **9**, 3467–3476. (doi:10.1534/g3.119.400556)
168. Stöck M *et al.* 2019 Shedding light on a secretive Tertiary urodelean relict: Hynobiid salamanders (*Paradactylodon persicus* s.l.) from Iran, illuminated by phylogeographic, developmental, and transcriptomic data. *Genes* **10**, 306 (doi:10.3390/genes10040306)
169. Biscotti MA, Carducci F, Barucca M, Gerdol M, Pallavicini A, Scharl M, Canapa A, Adolphi MC. 2020 The transcriptome of the newt *Cynops orientalis* provides new insights into evolution and function of sexual gene networks in sarcopterygians. *Sci. Rep.* **10**, 5445. (doi:10.1038/s41598-020-62408-x)
170. Venu G, Venkatachalaiah G. 2005 Karyology of two species of caecilians (Caeciliidae: Gymnophiona): evolution through tandem fusion and sex chromosome dimorphism. *Caryologia* **58**, 140–151. (doi:10.1080/00087114.2005.10589444)
171. Evans BJ, Pyron RA, Wiens JJ. 2012 Polyploidization and sex chromosome evolution in amphibians. In *Polyploidy and genome evolution* (eds PS Soltis, DE Soltis), pp. 385–410. Berlin, Germany: Springer.
172. Stöck M *et al.* 2011 Ever-young sex chromosomes in European tree frogs. *PLoS Biol.* **9**, e1001062. (doi:10.1371/journal.pbio.1001062)
173. Rodrigues N, Studer T, Dufresnes C, Perrin N. 2018 Sex-chromosome recombination in common frogs brings water to the fountain-of-youth. *Mol. Biol. Evol.* **35**, 942–948. (doi:10.1093/molbev/msy057)
174. Stöck M, Savary R, Betto-Colliard C, Biollay S, Jourdan-Pineau H, Perrin N. 2013 Low rates of X-Y recombination, not turnovers, account for homomorphic sex chromosomes in several diploid species of Palearctic green toads (*Bufo viridis* subgroup). *J. Evol. Biol.* **3**, 674–682.
175. Tamschick S, Rozenblut-Kościsty B, Ogińska M, Lehmann A, Lymberakis P, Hoffmann F, Lutz I, Kloas W, Stöck M. 2016 Sex reversal assessments reveal different vulnerability to endocrine disruption between deeply diverged anuran lineages. *Sci. Rep.* **6**, 23825. (doi:10.1038/srep23825)
176. Ponce K. 1941 La proportion sexuelle dans la descendance issue des œufs produits par l'organe de Bidder des crapauds femelles. *Rev. Suisse Zool.* **48**, 541–544.
177. Ponce K. 1949 *La différenciation du sexe et l'intersexualité chez les vertébrés*. Lausanne, Switzerland: F. Rouge.
178. Olmstead AW, Lindberg-Livingston A, Degitz SJ. 2010 Genotyping sex in the amphibian, *Xenopus (Silurana) tropicalis*, for endocrine disruptor bioassays. *Aquatic Toxicol.* **98**, 60–66. (doi:10.1016/j.aquatox.2010.01.012)
179. Bewick AJ, Anderson DW, Evans BJ. 2013 A large pseudoautosomal region on the sex chromosomes of the frog *Silurana tropicalis*. *Genome Biol. Evol.* **5**, 1087–1098. (doi:10.1093/gbe/evt073)
180. Mitros T *et al.* 2019 A chromosome-scale genome assembly and dense genetic map for *Xenopus tropicalis*. *Dev. Biol.* **452**, 8–20. (doi:10.1016/j.ydbio.2019.03.015)
181. Yoshimoto S *et al.* 2008 A W-linked DM-domain gene, DM-W, participates in primary ovary development in *Xenopus laevis*. *Proc. Natl Acad. Sci. USA* **105**, 2469–2474. (doi:10.1073/pnas.0712244105)
182. Bewick AJ, Anderson DW, Evans BJ. 2011 Evolution of the closely related, sex-related genes *dm-w* and *Dmrt1* in African clawed frogs (*Xenopus*). *Evolution* **65**, 698–712. (doi:10.1111/j.1558-5646.2010.01163.x)
183. Furman BLS, Evans BJ. 2016 Sequential turnovers of sex chromosomes in African clawed frogs (*Xenopus*) suggest some genomic regions are good at sex determination. *G3: Genes Genom. Genet.* **6**, 3625–3633. (doi:10.1534/g3.116.033423)
184. Mawaribuchi S *et al.* 2017 Sex chromosome differentiation and the W- and Z-specific loci in *Xenopus laevis*. *Dev. Biol.* **426**, 393–400. (doi:10.1016/j.ydbio.2016.06.015)

185. Brelsford A *et al.* 2013 Homologous sex chromosomes in three deeply divergent anuran species. *Evolution* **67**, 2434–2440. (doi:10.1111/evo.12151)
186. Brelsford A, Dufresnes C, Perrin N. 2016 Trans-species variation in *Dmrt1* is associated with sex determination in four European tree-frog species. *Evolution* **70**, 840–847. (doi:10.1111/evo.12891)
187. Ogita Y *et al.* 2020 Parallel evolution of two *dmrt1*-derived genes, *dmy* and *dm-W*, for vertebrate sex determination. *iScience* **23**, 100757. (doi:10.1016/j.isci.2019.100757)
188. Miura I. 2008 An evolutionary witness: the frog *Rana rugosa* underwent change of heterogametic sex from XY male to ZW female. *Sex. Dev.* **1**, 323–331. (doi:10.1159/000111764)
189. Uno Y, Nishida C, Oshima Y, Yokoyama S, Miura I. 2008 Comparative chromosome mapping of sex-linked genes and identification of sex chromosomal rearrangements in the Japanese wrinkled frog (*Rana rugosa*, Ranidae) with ZW and XY sex chromosome systems. *Chromosome Res.* **16**, 637–647. (doi:10.1007/s10577-008-1217-7)
190. Miura I. 2017 Sex determination and sex chromosomes in Amphibia. *Sex. Dev.* **11**, 298–306. (doi:10.1159/000485270)
191. Gemmell NJ *et al.* 2020 The tuatara genome: insights into vertebrate evolution from the sole survivor of an ancient reptilian order. *Nature* **584**, 403–409. (doi:10.1038/s41586-020-2561-9)
192. Janes DE *et al.* 2014 Molecular evolution of *Dmrt1* accompanies change of sex-determining mechanisms in reptilia. *Biol. Lett.* **10**, 20140809. (doi:10.1098/rsbl.2014.0809)
193. Cree A, Thompson MB, Daugherty CH. 1995 Tuatara sex determination. *Nature* **375**, 543. (doi:10.1038/375543a0)
194. Uetz P (ed.) 2020 The Reptile Database. See <http://www.reptile-database.org> (accessed December 2020).
195. Zheng Y, Wiens JJ. 2016 Combining phylogenomic and supermatrix approaches, and a time-calibrated phylogeny for squamate reptiles (lizards and snakes) based on 52 genes and 4162 species. *Mol. Phylogenet. Evol.* **94**, 537–547. (doi:10.1016/j.ympev.2015.10.009)
196. Organ CL, Moreno GR, Edwards SV. 2008 Three tiers of genome evolution in reptiles. *Integr. Comp. Biol.* **48**, 494–504. (doi:10.1093/icb/ict046)
197. Shine R. 1983 Reptilian reproductive modes: the oviparity-viviparity continuum. *Herpetologica* **39**, 1–8.
198. Darevsky IS. 1958 Natural parthenogenesis in certain subspecies of rock lizards (*Lacerta saxicola* Eversmann). *Dokl. Akad. Nauk SSSR Biol. Sci.* **122**, 730–732.
199. Kearney M, Fujita MK, Ridenour J. 2009 Lost sex in the reptiles: constraints and correlations. In *Lost sex: the evolutionary biology of parthenogenesis* (eds I Schön, K Martens, P van Dijk), pp. 447–474. Dordrecht, The Netherlands: Springer.
200. Fujita MK, Moritz C. 2009 Origin and evolution of parthenogenetic genomes in lizards: current state and future directions. *Cytogenet. Genome Res.* **127**, 261–272. (doi:10.1159/000295177)
201. Lutes AA, Baumann DP, Neaves WB, Baumann P. 2011 Laboratory synthesis of an independently reproducing vertebrate species. *Proc. Natl Acad. Sci. USA* **108**, 9910–9915. (doi:10.1073/pnas.1102811108)
202. Moritz C, Bi K. 2011 Spontaneous speciation by ploidy elevation: laboratory synthesis of a new clonal vertebrate. *Proc. Natl Acad. Sci. USA* **108**, 9733–9734. (doi:10.1073/pnas.1106455108)
203. Ezaz T, Sarre SD, O'Meally D, Graves JAM, Georges A. 2009 Sex chromosome evolution in lizards: independent origins and rapid transitions. *Cytogenet. Genome Res.* **127**, 249–260. (doi:10.1159/000300507)
204. Valenzuela N. 2018 Causes and consequences of evolutionary transitions in the level of phenotypic plasticity of reptilian sex determination. In *Transitions between sexual systems*, (ed. J Leonard), pp. 345–363. Cham, Switzerland: Springer.
205. Gamble T, Coryell J, Ezaz T, Lynch J, Scantlebury DP, Zarkower D. 2015 Restriction site-associated DNA sequencing (RAD-seq) reveals an extraordinary number of transitions among gecko sex-determining systems. *Mol. Biol. Evol.* **32**, 1296–1309. (doi:10.1093/molbev/msv023)
206. Kostmann A, Kratochvíl L, Rovatsos M. 2021 Poorly differentiated XX/XY sex chromosomes are widely shared across skink radiation. *Proc. R. Soc. B* **288**, 20202139. (doi:10.1098/rspb.2020.2139)
207. Pokorná M, Altmanová M, Kratochvíl L. 2014 Multiple sex chromosomes in the light of female meiotic drive in amniote vertebrate. *Chromosome Res.* **22**, 35–44. (doi:10.1007/s10577-014-9403-2)
208. Pennell MW, Kirkpatrick M, Otto SP, Vamori JC, Peichel CL, Valenzuela N, Kitano J. 2015 Y Fuse? Sex chromosome fusions in fishes and reptiles. *PLoS Genet.* **11**, e1005237. (doi:10.1371/journal.pgen.1005237)
209. Leonard JL. 2013 Williams' paradox and the role of phenotypic plasticity in sexual systems. *Integr. Comp. Biol.* **53**, 671–688. (doi:10.1093/icb/ict088)
210. Booth W, Schuett GW. 2016 The emerging phylogenetic pattern of parthenogenesis in snakes. *Biol. J. Linn. Soc. Lond.* **118**, 172–186. (doi:10.1111/bij.12744)
211. Kratochvíl L, Vukić J, Červenka J, Kubička L, Johnson Pokorná M, Kukačková D, Rovatsos M, Piálek L. 2020 Mixed-sex offspring produced via cryptic parthenogenesis in a lizard. *Mol. Ecol.* **9**, 4118–4127 (doi:10.1111/mec.15617)
212. Rovatsos M, Pokorná M, Altmanová M, Kratochvíl L. 2014 Cretaceous park of sex determination: sex chromosomes are conserved across iguanas. *Biol. Lett.* **10**, 20131093. (doi:10.1098/rsbl.2013.1093)
213. Rovatsos M, Vukić J, Altmanová M, Johnson Pokorná M, Moravec J, Kratochvíl L. 2016 Conservation of sex chromosomes in lacertid lizards. *Mol. Ecol.* **25**, 3120–3126.
214. Augstenová B, Johnson Pokorná M, Altmanová M, Frynta D, Rovatsos M, Kratochvíl L. 2018 ZW, XY, and yet ZW: sex chromosome evolution in snakes even more complicated. *Evolution* **72**, 1701–1707. (doi:10.1111/evo.13543)
215. Rovatsos M, Rehák I, Velenský P, Kratochvíl L. 2019 Shared ancient sex chromosomes in varanids, beaded lizards, and alligator lizards. *Mol. Biol. Evol.* **36**, 1113–1120. (doi:10.1093/molbev/msz024)
216. Iannucci A *et al.* 2019 Conserved sex chromosomes and karyotype evolution in monitor lizards (Varanidae). *Heredity* **123**, 215–227. (doi:10.1038/s41437-018-0179-6)
217. Nielsen SV, Banks JL, Diaz Jr RE, Trainor PA, Gamble T. 2018 Dynamic sex chromosomes in Old World chameleons (Squamata: Chamaeleonidae). *J. Evol. Biol.* **31**, 484–490. (doi:10.1111/jeb.13242)
218. Gamble T. 2010 A review of sex determining mechanisms in geckos (Gekkota: Squamata). *Sex. Dev.* **4**, 88–103. (doi:10.1159/000289578)
219. Gamble T, Castoe TA, Nielsen SV, Banks JL, Card DC, Schield DR, Schuett GW, Booth W. 2017 The discovery of XY sex chromosomes in a *Boa* and *Python*. *Curr. Biol.* **27**, 2148–2153. (doi:10.1016/j.cub.2017.06.010)
220. Vicoso B, Emerson JJ, Zektser Y, Mahajan S, Bachtrog D. 2013 Comparative sex chromosome genomics in snakes: differentiation, evolutionary strata, and lack of global dosage compensation. *PLoS Biol.* **11**, e1001643. (doi:10.1371/journal.pbio.1001643)
221. Lind AL *et al.* 2019 Genome of the Komodo dragon reveals adaptations in the cardiovascular and chemosensory systems of monitor lizards. *Nat. Ecol. Evol.* **3**, 1241–1252. (doi:10.1038/s41559-019-0945-8)
222. Rupp SM, Webster TH, Olney KC, Hutchins ED, Kusumi K, Wilson Sayres MA. 2017 Evolution of dosage compensation in *Anolis carolinensis*, a reptile with XX/XY chromosomal sex determination. *Genome Biol. Evol.* **9**, 231–240. (doi:10.1093/gbe/evw263)
223. Marin R *et al.* 2017 Convergent origination of a *Drosophila*-like dosage compensation mechanism in a reptile lineage. *Genome Res.* **27**, 1974–1987. (doi:10.1101/gr.223727.117)
224. Acosta A *et al.* 2019 Corytophanids replaced the pleurodont XY system with a new pair of XY chromosomes. *Genome Biol. Evol.* **9**, 666–2677. (doi.org/10.1093/gbe/evz196)
225. Rovatsos M, Gamble T, Nielsen SV, Georges A, Ezaz T, Kratochvíl L. 2021 Do male and female heterogamety really differ in expression regulation? Lack of global dosage balance in pygopodid geckos. *Phil. Trans. R. Soc. B* **376**, 20200102. (doi:10.1098/rstb.2020.0102)
226. Nielsen SV, Guzmán-Méndez IA, Gamble T, Blumer M, Pinto BJ, Kratochvíl L, Rovatsos M. 2015 Escaping the evolutionary trap? Sex chromosome turnover in basilisks and related lizards (Corytophanidae:

- Squamata). *Biol. Lett.* **15**, 20190498. (doi:10.1098/rsbl.2019.0498)
227. Wang Z *et al.* 2013 The draft genomes of softshell turtle and green sea turtle yield insights into the development and evolution of the turtle-specific body plan. *Nat. Gen.* **45**, 701–708. (doi:10.1038/ng.2615)
228. Shaffer B, McCartney-Melstad E, Near TJ, Mountac GG, Spinks PQ. 2017 Phylogenomic analyses of 539 highly informative loci dates a fully resolved time tree for the major clades of living turtles (Testudines). *Mol. Phylogenet. Evol.* **115**, 7–15. (doi:10.1016/j.ympev.2017.07.006)
229. Kasai F, O'Brien PCM, Ferguson-Smith MA. 2012 Reassessment of genome size in turtle and crocodile based on chromosome measurement by flow karyotyping: close similarity to chicken. *Biol. Lett.* **8**, 631–635. (doi:10.1098/rsbl.2012.0141)
230. Kawagoshi T, Nishida C, Matsuda Y. 2012 The origin and differentiation process of X and Y chromosomes of the black marsh turtle (*Siebenrockiella crassicolis*, Geomydidae, Testudines). *Chromosome Res.* **20**, 95–110. (doi:10.1007/s10577-011-9267-7)
231. Kawagoshi T, Uno Y, Matsubara K, Matsuda Y, Nishida C. 2009 The ZW micro-sex chromosomes of the Chinese soft-shelled turtle (*Pelodiscus sinensis*, Trionychidae, Testudines) have the same origin as chicken chromosome 15. *Cytogenet. Genome Res.* **125**, 125–131. (doi:10.1159/000227837)
232. Badenhorst D, Stanyon R, Engstrom T, Valenzuela N. 2013 A ZZ/ZW microchromosome system in the spiny softshell turtle, *Apalone spinifer*, reveals an intriguing sex chromosome conservation in Trionychidae. *Chromosome Res.* **21**, 137–147. (doi:10.1007/s10577-013-9343-2)
233. Bista B, Valenzuela N. 2020 Turtle insights into the evolution of the reptilian karyotype and the genomic architecture of sex determination. *Genes* **11**, 416 (doi:10.3390/genes11040416)
234. Mazzoleni S *et al.* 2020 Sex is determined by XX/XY sex chromosomes in Australasian side-necked turtles (Testudines: Chelidae). *Sci. Rep.* **10**, 4276. (doi:10.1038/s41598-020-61116-w)
235. Rovatsos M, Praschag P, Fritz U, Kratochvil L. 2017 Stable Cretaceous sex chromosomes enable molecular sexing in softshell turtles (Testudines: Trionychidae). *Sci. Rep.* **7**, 42150. (doi:10.1038/srep42150)
236. Lee LS, Montiel EE, Valenzuela N. 2019 Discovery of putative XX/XY male heterogamety in *Emydura subglobosa* turtles exposes a novel trajectory of sex chromosome evolution in *Emydura*. *Cytogenet. Genome Res.* **158**, 160–169. (doi:10.1159/000501891)
237. Rovatsos M, Kratochvil L. 2021 Evolution of dosage compensation does not depend on genomic background. *Mol. Ecol.* **30**, 1836–1845. (doi:10.1111/2020.08.14.251801)
238. Bista B, Wu Z, Literman R, Valenzuela N. 2021 Thermosensitive sex chromosome dosage compensation in ZZ/ZW softshell turtles, *Apalone spinifer*. *Phil. Trans. R. Soc. B* **376**, 20200101. (doi:10.1098/rstb.2015.2020.0101)
239. Radhakrishnan S, Valenzuela N. 2017 Chromosomal context affects the molecular evolution of sex-linked genes and their autosomal counterparts in turtles and other vertebrates. *J. Hered.* **108**, 720–730.
240. Literman R, Burret A, Bista B, Valenzuela N. 2018 Putative independent evolutionary reversals from genotypic to temperature-dependent sex determination are associated with accelerated evolution of sex-determining genes in turtles. *J. Mol. Evol.* **86**, 11–26.
241. Eggers S, Ohnesorg T, Sinclair A. 2014 Genetic regulation of mammalian gonad development. *Nat. Rev. Endocrinol.* **10**, 673–683. (doi:10.1038/nrendo.2014.163)
242. Smith CA. 2010 Sex determination in birds: a review. *Emu* **110**, 364–377.
243. Lee LS, Montiel Jiménez EE, Navarro-Domínguez BM, Valenzuela N. 2019 Chromosomal rearrangements during turtle evolution altered the synteny of genes involved in vertebrate sex determination. *Cytogenet. Genome Res.* **157**, 77–88. (doi:10.1159/000497302)
244. Czerwinski M, Natarajan A, Barske L, Looger LL, Capel B. 2016 A timecourse analysis of systemic and gonadal effects of temperature on sexual development of the red-eared slider turtle *Trachemys scripta elegans*. *Dev. Biol.* **420**, 166–177. (doi:10.1016/j.ydbio.2016.09.018)
245. Radhakrishnan S, Literman R, Neuwald J, Severin A, Valenzuela N. 2017 Transcriptomic responses to environmental temperature by turtles with temperature-dependent and genotypic sex determination assessed by RNAseq inform the genetic architecture of embryonic gonadal development. *PLoS ONE* **12**, e0172044. (doi:10.1371/journal.pone.0172044)
246. Radhakrishnan S, Literman R, Neuwald JL, Valenzuela N. 2018 Thermal response of epigenetic genes informs turtle sex determination with and without sex chromosomes. *Sex. Dev.* **12**, 308–319. (doi:10.1159/000492188)
247. Radhakrishnan S, Literman R, Mizoguchi BA, Valenzuela N. 2017 MeDIPseq and nCpG analyses illuminate sexually dimorphic methylation of gonadal development genes with high historic methylation in turtle hatchlings with temperature-dependent sex determination. *Epigenetics Chromatin* **10**, 1–16. (doi:10.1186/s13072-017-0136-2)
248. Ge C, Ye J, Weber C, Sun W, Zhang H, Zhou Y, Cai C, Qian G, Capel B. 2018 The histone demethylase *Kdm6b* regulates temperature-dependent sex determination in a turtle species. *Science* **360**, 645–648. (doi:10.1126/science.aap8328)
249. Weber C, Capel B. 2021 Sex determination without sex chromosomes. *Phil. Trans. R. Soc. B* **376**, 20200109. (doi:10.1098/rstb.2020.0109)
250. Chiari Y, Cahais V, Galtier N, Delsuc F. 2012 Phylogenomic analyses support the position of turtles as the sister group of birds and crocodiles (Archosauria). *BMC Biol.* **10**, 1–14. (doi:10.1186/1741-7007-10-65)
251. Green RE, Braun EL, Armstrong J, Earl D, Nguyen N. 2014 Three crocodilian genomes reveal ancestral patterns of evolution among archosaurs. *Science* **346**, 1254449. (doi:10.1126/science.1254449)
252. Brochu CA. 2003 Phylogenetic approaches toward crocodylian history. *Annu. Rev. Earth Planet. Sci.* **31**, 357–397. (doi:10.1146/annurev.earth.31.100901.141308)
253. Wan Q-H *et al.* 2013 Genome analysis and signature discovery for diving and sensory properties of the endangered Chinese alligator. *Cell Res.* **23**, 1091–1105. (doi:10.1038/cr.2013.104)
254. Lewis JL, FitzSimmons NN, Jamerlan ML, Buchan JC, Grigg GC. 2013 Mating systems and multiple paternity in the estuarine crocodile (*Crocodylus porosus*). *J. Herpetol.* **47**, 24–33. (doi:10.1670/10-303)
255. González EJ, Martínez-López M, Morales-Garduza MA, García-Morales R, Charruau P, Gallardo-Cruz JA. 2019 The sex-determination pattern in crocodilians: a systematic review of three decades of research. *J. Anim. Ecol.* **88**, 1417–1427. (doi:10.1111/1365-2656.13037)
256. Smith CA, Joss JMP. 1994 Steroidogenic enzyme activity and ovarian differentiation in the saltwater crocodile, *Crocodylus porosus*. *Gen. Comp. Endocrinol.* **93**, 232–245. (doi:10.1006/gcen.1994.1027)
257. Smith CA, Elf PK, Lang JW, Joss JMP. 1995 Aromatase enzyme activity during gonadal sex differentiation in alligator embryos. *Differentiation* **58**, 281–290. (doi:10.1046/j.1432-0436.1995.5840281.x)
258. Yatsu R *et al.* 2016 RNA-seq analysis of the gonadal transcriptome during *Alligator mississippiensis* temperature-dependent sex determination and differentiation. *BMC Genomics* **17**, 1. (doi:10.1186/s12864-016-2396-9)
259. Deveson IW, Holleley CE, Blackburn J, Graves JAM, Mattick JS, Waters PD, Georges A. 2017 Differential intron retention in Jumonji chromatin modifier genes is implicated in reptile temperature-dependent sex determination. *Sci. Adv.* **3**, e1700731. (doi:10.1126/sciadv.1700731)
260. Lin JQ, Zhou Q, Yang HQ, Fang LM, Tang K-Y, Sun L, Wan Q-H, Fang S-G. 2018 Molecular mechanism of temperature-dependent sex determination and differentiation in Chinese alligator revealed by developmental transcriptome profiling. *Sci. Bull.* **63**, 209–212. (doi:10.1016/j.scib.2018.01.004)
261. AVIBASE. 2020 See <https://avibase.bsc-eoc.org/avibase.jsp?lang=EN>.
262. Zhang G. 2018 The bird's-eye view on chromosome evolution. *Genome Biol.* **19**, 201. (doi:10.1186/s13059-018-1585-z)
263. Feng S *et al.* 2020 Dense sampling of bird diversity increases power of comparative genomics. *Nature* **587**, 252–257. (doi:10.1038/s41586-020-2873-9)
264. Fridolfsson AK *et al.* 1998 Evolution of the avian sex chromosomes from an ancestral pair of autosomes. *Proc. Natl Acad. Sci. USA* **95**, 8147–8152. (doi:10.1073/pnas.95.14.8147)
265. Hirst CE, Major AT, Ayes KL, Brown RJ, Mariette M, Sackton TB, Smith CA. 2017 Sex reversal and comparative data undermine the W chromosome and support Z-linked Dmrt1 as the regulator of gonadal sex differentiation in birds.

- Endocrinology* **158**, 2970–2987. (doi:10.1210/en.2017-00316)
266. Estermann MA, Williams S, Hirst CE, Roly, ZY, Serralbo O, Adhikari D, Powell D, Major AT, Smith CA. 2020 Insights into gonadal sex differentiation provided by single-cell transcriptomics in the chicken embryo. *Cell Rep.* **31**, 107491. (doi:10.1016/j.celrep.2020.03.055)
267. Smith CA, Roeszler KN, Ohnesorg T, Cummins DM, Farlie FG, Doran TJ, Sinclair AH. 2009 The avian Z-linked gene *Dmrt1* is required for male sex determination in the chicken. *Nature* **461**, 267–271. (doi:10.1038/nature08298)
268. Zhou Q, Zhang J, Bachtrog D, An N, Huang Q, Jarvis ED, Gilbert MTP, Zhang G. 2014 Complex evolutionary trajectories of sex chromosomes across bird taxa. *Science* **346**, 1246338. (doi:10.1126/science.1246338)
269. Shetty S, Kirby P, Zarkower D, Graves JAM. 2002 *Dmrt1* in a ratite bird: evidence for a role in sex determination and discovery of a putative regulatory element. *Cytogenet. Genome Res.* **99**, 245–251. (doi:10.1159/000071600)
270. Yazdi HP, Ellegren H. 2019 A genetic map of ostrich Z chromosome and the role of inversions in avian sex chromosome evolution. *Genome Biol. Evol.* **10**, 2049–2060. (doi:10.1093/gbe/evy163)
271. Vicoso B, Kaiser VB, Bachtrog D. 2013 Sex-biased gene expression at homomorphic sex chromosomes in emus and its implication for sex chromosome evolution. *Proc. Natl Acad. Sci. USA* **110**, 6453–6458. (doi:10.1073/pnas.1217027110)
272. Ioannidis J *et al.* 2020 Primary sex determination in birds depends on *DMRT1* dosage, but gonadal sex does not determine secondary sexual characteristics. *Proc. Natl Acad. Sci. USA* **118**, e2020909118. (doi:10.1073/pnas.2020909118)
273. Zhao D, McBride D, Nandi S, McQueen HA, McGrew MJ. 2010 Somatic sex identity is cell autonomous in the chicken. *Nature* **464**, 237–242. (doi:10.1038/nature08852)
274. Lin M, Thorne MH, Martin IC, Sheldon BL, Jones RC. 1995 Development of the gonads in the triploid (ZZW and ZZZ) fowl, *Gallus domesticus*, and comparison with normal diploid males (ZZ) and females (ZW). *Reprod. Fertility Dev.* **7**, 1185–1197. (doi:10.1071/RD9951185)
275. Graves JAM. 2003 Sex and death in birds: a model of dosage compensation that predicts lethality of sex chromosome aneuploids. *Cytogenet. Genome Res.* **101**, 278–282. (doi:10.1159/000074349)
276. Kuroiwa A. 2017 Sex-determining mechanism in avians. In *Avian reproduction. Advances in experimental medicine and biology*, vol. 1001 (ed. T Sasanami), pp. 19–31. Singapore: Springer.
277. Clinton M. 1998 Sex determination and gonadal development: a bird's eye view. *J. Exp. Zool.* **281**, 457–465.
278. Bloom SE. 1972 Chromosome abnormalities in chicken (*Gallus domesticus*) embryos: types, frequencies and phenotypic effects. *Chromosoma* **37**, 309–326. (doi:10.1007/bf00319873)
279. Otto SP, Whitton J. 2000 Polyploidy: incidence and evolution. *Annu. Rev. Genet.* **34**, 401–437. (doi:10.1146/annurev.genet.34.1.401)
280. Gunsli RJ, Cañedo AD, Garner ADV, Ledesma MA, Coria N, Montalti D, Degrandi TM. 2017 Multiple sex chromosome system in penguins (*Pygoscelis*, Spheniscidae). *Comp. Cytogenet.* **11**, 541–552. (doi:10.3897/CompCytogen.v11i3.13795)
281. Pala I, Naurin S, Stervander M, Hasselquist D, Bensch S, Hansson B. 2012 Evidence of a neo-sex chromosome in birds. *Heredity* **108**, 264–272. (doi:10.1038/hdy.2011.70)
282. Pala I, Hasselquist D, Bensch S, Hansson B. 2012 Patterns of molecular evolution of an avian neo-sex chromosome. *Mol. Biol. Evol.* **12**, 3741–3754. (doi:10.1093/molbev/mss177)
283. Sigeman H, Ponnikas S, Chauhan P, Dierickx E, Brooke MD, Hansson B. 2019 Repeated sex chromosome evolution in vertebrates supported by expanded avian sex chromosomes. *Proc. R. Soc. B* **286**, 20192051. (doi:10.1098/rspb.2019.2051)
284. Gan HM, Falk S, Morales HE, Austin CM, Sunnucks P, Pavlova A. 2019 Genomic evidence of neo-sex chromosomes in the eastern yellow robin. *GigaScience* **8**, giz111. (doi:10.1093/gigascience/giz111)
285. Graves JAM. 2016 Evolution of vertebrate sex chromosomes and dosage compensation. *Nat. Rev. Gen.* **17**, 33–46. (doi:10.1038/nrg.2015.2)
286. Yazdi HP, Silva WTAF, Suh A. 2020 Why do some sex chromosomes degenerate more slowly than others? The odd case of ratite sex chromosomes. *Genes* **11**, 1153. (doi:10.3390/genes11101153)
287. Bellott D *et al.* 2017 Avian W and mammalian Y chromosomes convergently retained dosage-sensitive regulators. *Nat. Genet.* **49**, 387–394. (doi:10.1038/ng.3778)
288. Xu L, Zhou Q. 2020 The female-specific W chromosomes of birds have conserved gene contents but are not feminized. *Genes* **11**, 1126. (doi:10.3390/genes11101126)
289. Uebbing S, Küstner A, Makinen H, Ellegren H. 2013 Transcriptome sequencing reveals the character of incomplete dosage compensation across multiple tissues in flycatchers. *Genome Biol. Evol.* **5**, 1555–1566. (doi:10.1093/gbe/evt114)
290. Itoh Y *et al.* 2007 Dosage compensation is less effective in birds than in mammals. *J. Biol.* **6**, 2. (doi:10.1186/jbiol53)
291. Irwin DE. 2018 Sex chromosomes and speciation in birds and other ZW systems. *Mol. Ecol.* **27**, 3831–3851. (doi:10.1111/mec.14537)
292. Warnefors M, Mossinger K, Halbert J, Studer T, VandeBerg JL. 2017 Sex-biased microRNA expression in mammals and birds reveals underlying regulatory mechanisms and a role in dosage compensation. *Genome Res.* **27**, 1961–1973. (doi:10.1101/gr.225391.117)
293. Graves JAM. 2014 Avian sex, sex chromosomes, and dosage compensation in the age of genomics. *Chromosome Res.* **22**, 45–57. (doi:10.1007/s10577-014-9409-9)
294. Xu L, Wa Sin SY, Grayson P, Edwards SV, Sackton TB. 2019 Evolutionary dynamics of sex chromosomes of paleognathous birds. *Genome Biol. Evol.* **11**, 2376–2390. (doi:10.1093/gbe/evz154)
295. Kapusta A, Suh A. 2016 Evolution of bird genomes – a transposon's-eye view. *Annu. NY Acad. Sci.* **1389**, 164–185. (doi:10.1111/nyas.13295)
296. Peona V *et al.* 2021 The avian W chromosome is a refugium for endogenous retroviruses with likely effects on female-biased mutational load and genetic incompatibilities. *Phil. Trans. R. Soc. B* **376**, 20200186. (doi:10.1098/rstb.2020.0186)
297. Pigozzi MI, Solari AJ. 1998 Germ cell restriction and regular transmission of an accessory chromosome that mimics a sex body in the zebra finch, *Taeniopygia guttata*. *Chromosome Res.* **6**, 105–113. (doi:10.1023/A:1009234912307)
298. Itoh Y, Kampf K, Pigozzi MI, Arnold AP. 2009 Molecular cloning and characterization of the germline-restricted chromosome sequence in the zebra finch. *Chromosoma* **118**, 527–536. (doi:10.1007/s00412-009-0216-6)
299. Malinovsky LP *et al.* 2020 Germline-restricted chromosome (GRC) in the sand martin and the pale martin (Hirundinidae, Aves): synopsis, recombination and copy number variation. *Sci. Rep.* **10**, 1058. (doi:10.1038/s41598-020-58032-4)
300. Biederman MK, Nelson MM, Asalone KC, Pedersen AL, Saldanha CJ, Bracht JR. 2018 Discovery of the first germline-restricted gene by subtractive transcriptomic analysis in the zebra finch, *Taeniopygia guttata*. *Curr. Biol.* **28**, 1620–1627. (doi:10.1016/j.cub.2018.03.067)
301. Kinsella CM *et al.* 2019 Programmed DNA elimination of germline development genes in songbirds. *Nat. Comm.* **10**, 5468. (doi:10.1038/s41467-019-13427-4)
302. Torgasheva AA *et al.* 2019 Germline-restricted chromosome (GRC) is widespread among songbirds. *Proc. Natl Acad. Sci. USA* **116**, 11 845–11 850. (doi:10.1073/pnas.1817373116)
303. Pei Y *et al.* 2021 Occasional paternal inheritance of the germline-restricted chromosome in songbirds. *BioRxiv*. See <https://www.biorxiv.org/content/10.1101/2021.01.28.428604v1>. (doi:10.1101/2021.01.28.428604)
304. Cortez D, Marin R, Toledo-Flores D, Froidevaux L, Liechti A, Waters PD, Grützner F, Kaessmann H. 2014 Origins and functional evolution of Y chromosomes across mammals. *Nature* **508**, 488–493. (doi:10.1038/nature13151)
305. Warren WC *et al.* 2008 Genome analysis of the platypus reveals unique signatures of evolution. *Nature* **453**, 175–183. (doi:10.1038/nature06936)
306. Zhou Y *et al.* 2021 Platypus and echidna genomes reveal mammalian biology and evolution. *Nature* **592**, 756–762. (doi:10.1038/s41586-020-03039-0)
307. Rens W *et al.* 2007 The multiple sex chromosomes of platypus and echidna are not completely identical and several share homology with the avian Z. *Genome Biol.* **8**, R243. (doi:10.1186/gb-2007-8-11-r243)

308. Grützner F, Rens W, Tsend-Ayush E, El-Mogharbel N, O'Brien PCM, Jones RC, Ferguson-Smith MA, Graves JAM. 2004 In the platypus a meiotic chain of ten sex chromosomes shares genes with the bird Z and mammal X chromosomes. *Nature* **432**, 913–917. (doi:10.1038/nature03021)
309. Veyrunes F *et al.* 2008 Bird-like sex chromosomes of platypus imply recent origin of mammal sex chromosomes. *Genome Res.* **18**, 965–973. (doi:10.1101/gr.7101908)
310. El-Mogharbel N, Wakefield M, Deakin JE, Tsend-Ayush E, Grützner F, Alsop A, Ezaz T, Marshall Graves JA. 2007 *Dmr1* gene cluster analysis in the platypus: new insights into genomic organization and regulatory regions. *Genomics* **89**, 10–21. (doi:10.1016/j.ygeno.2006.07.017)
311. Deakin JE. 2017 Implications of monotreme and marsupial chromosome evolution on sex determination and differentiation. *Gen. Comp. Endocrinol.* **244**, 130–138. (doi:10.1016/j.ygcen.2015.09.029)
312. Deakin JE, Hore TA, Koina E, Graves JAM. 2008 The status of dosage compensation in the multiple X chromosomes of the platypus. *PLoS Genet.* **4**, e1000140. (doi:10.1371/journal.pgen.1000140)
313. Julien P, Brawand D, Soumillon M, Necseulea A, Liechti A, Schütz F, Daish T, Grützner F, Kaessmann H. 2012 Mechanisms and evolutionary patterns of mammalian and avian dosage compensation. *PLoS Biol.* **10**, e1001328. (doi:10.1371/journal.pbio.1001328)
314. Whitworth DJ, Pask AJ. 2016 The X factor: X chromosome dosage compensation in the evolutionarily divergent monotremes and marsupials. *Semin. Cell Dev. Biol.* **56**, 117–121. (doi:10.1016/j.semcdb.2016.01.006)
315. Redia CA, Capanna E. 2012 Genome size evolution: sizing mammalian genomes. *Cytogenet. Genome Res.* **137**, 97–112. (doi:10.1159/000338820)
316. Deakin JE, O'Neill RJ. 2020 Evolution of marsupial genomes. *Annu. Rev. Anim. Biosci.* **8**, 25–45. (doi:10.1146/annurev-animal-021419-083555)
317. Frankenberg S. 2018 Pre-gastrula development of non-eutherian mammals. *Curr. Top. Dev. Biol.* **128**, 237–265. (doi:10.1016/bs.ctdb.2017.10.013)
318. Hayman D. 1989 Marsupial cytogenetics. *Aust. J. Zool.* **37**, 331–349. (doi:10.1071/ZO9890331)
319. Deakin JE. 2018 Chromosome evolution in marsupials. *Genes* **9**, 72. (doi:10.3390/genes9020072)
320. Grant J *et al.* 2012 Rxs is a metatherian RNA with Xist-like properties in X-chromosome inactivation. *Nature* **487**, 254–258. (doi:10.1038/nature11171)
321. Deakin JE. 2013 Marsupial X chromosome inactivation: past, present and future. *Aust. J. Zool.* **61**, 13–23.
322. Rens W, Wallduck MS, Lovell FL, Ferguson-Smith MA, Ferguson-Smith AC. 2010 Epigenetic modifications on X chromosomes in marsupial and monotreme mammals and implications for evolution of dosage compensation. *Proc. Natl Acad. Sci. USA* **107**, 17 657–17 662. (doi:10.1073/pnas.0910322107)
323. Johnson PG, Watson CM, Adams M, Paull DJ. 2002 Sex chromosome elimination, X chromosome inactivation and reactivation in the southern brown bandicoot *Isodon obesulus* (Marsupialia: Peramelidae). *Cytogenet. Genome Res.* **99**, 119–124. (doi:10.1159/000071583)
324. Sharp P. 1982 Sex chromosome pairing during male meiosis in marsupials. *Chromosoma* **86**, 27–47. (doi:10.1007/BF00330728)
325. Page J, Berrios S, Parra MT, Viera A, Suja JA, Prieto I, Barbero JL, Rufas JS, Fernández-Donoso R. 2005 The program of sex chromosome pairing in meiosis is highly conserved across marsupial species: implications for sex chromosome evolution. *Genetics* **170**, 793–799. (doi:10.1534/genetics.104.039073)
326. Foster JW *et al.* 1992 Evolution of sex determination and the Y chromosome: *SRY*-related sequences in marsupials. *Nature* **359**, 531–533. (doi:10.1038/359531a0)
327. Evans BJ, Upham NS, Golding GB, Ojeda RA, Ojeda AA. 2017 Evolution of the largest mammalian genome. *Genome Biol. Evol.* **9**, 1711–1724. (doi:10.1093/gbe/evx113)
328. Georgiades P, Watkins M, Burton GJ, Ferguson-Smith AC. 2001 Roles for genomic imprinting and the zygotic genome in placental development. *Proc. Natl Acad. Sci. USA* **98**, 4522–4527. (doi:10.1073/pnas.081540898)
329. Svartman MT, Stone G, Stanyon R. 2005 Molecular cytogenetics discards polyploidy in mammals. *Genomics* **85**, 425–430. (doi:10.1016/j.ygeno.2004.12.004)
330. Bachtrog D. 2013 Y-chromosome evolution: emerging insights into processes of Y-chromosome degeneration. *Nat. Rev. Genet.* **14**, 113–124. (doi:10.1038/nrg3366)
331. De la Fuente R, Parra MT, Viera A, Calvente A, Gomez R, Suja J, Rufas JS, Page J. 2007 Meiotic pairing and segregation of achiasmate sex chromosomes in eutherian mammals: the role of SYCP3 protein. *PLoS Genet.* **3**, e198. (doi:10.1371/journal.pgen.0030198)
332. Tasman Daish T, Grützner F. 2019 Evolution and meiotic organization of heteromorphic sex chromosomes. *Curr. Topics Dev. Biol.* **134**, 1–48. (doi:10.1016/bs.ctdb.2019.009)
333. Gil-Fernandez G-F *et al.* 2020 Meiosis reveals the early steps in the evolution of a neo-XY sex chromosome pair in the African pygmy mouse *Mus minutoides*. *PLoS Genet.* **16**, e1008959. (doi:10.1371/journal.pgen.1008959)
334. Wutz A, Rasmussen TP, Jaenisch R. 2002 Chromosomal silencing and localization are mediated by different domains of Xist RNA. *Nat. Genet.* **30**, 167–174. (doi:10.1038/ng820)
335. Jégu T, Aebly E, Lee JT. 2017 The X chromosome in space. *Nat. Rev. Genet.* **18**, 377–389.
336. Koopman P, Gubbay J, Vivian N, Goodfellow P, Lovell-Badge R. 1991 Male development of chromosomally female mice transgenic for *Sry*. *Nature* **351**, 117–121.
337. Miyawaki S, Kuroki S, Maeda R, Okashita N, Koopman P, Tachibana M. 2020 The mouse *Sry* locus harbors a cryptic exon that is essential for male sex determination. *Science* **370**, 121–124.
338. Parma P, Veyrunes F, Pailhoux E. 2016 Sex reversal in non-human placental mammals. *Sex. Dev.* **10**, 326–344. (doi:10.1159/000448361)
339. 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**, 519–526.
340. Just W, Baumstark A, Süß A, Graphodatsky A, Rens W, Schäfer N, Bakloushinskaya I, Hameister H, Vogel W. 2007 *Ellobius lutescens*: sex determination and sex chromosome. *Sex. Dev.* **1**, 211–221. (doi:10.1159/000104771)
341. 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. R. Soc. B* **277**, 1049–1056. (doi:10.1098/rspb.2009.1925)
342. Real FM *et al.* 2020 The mole genome reveals regulatory rearrangements associated with adaptive intersexuality. *Science* **370**, 208–214. (doi:10.1126/science.aaz2582)
343. Uhlenhaut NH *et al.* 2009 Somatic sex reprogramming of adult ovaries to testes by *foxl2* ablation. *Cell* **139**, 1130–1142.
344. Matson CK, Murphy MW, Sarver AL, Griswold MD, Bardwell VJ, Zarkower D. 2011 *Dmr1* prevents female reprogramming in the postnatal mammalian testis. *Nature* **476**, 101–104. (doi:10.1038/nature10239)
345. Naqvi S, Godfrey AK, Hughes JF, Goodheart ML, Mitchell RN, Page DC. 2019 Conservation, acquisition, and functional impact of sex-biased gene expression in mammals. *Science* **365**, eaaw7317. (doi:10.1126/science.aaw7317)
346. Davis EJ, Lobach I, Dubal DB. 2019 Female XX sex chromosomes increase survival and extend lifespan in aging mice. *Aging Cell* **18**, e12871. (doi:10.1111/ace1.12871)
347. VGP. 2020 See <https://vertebrategenomesproject.org>.
348. EBG. 2020 See <https://www.earthbiogenome.org/>.
349. Fish1 k. 2020 See <https://db.cngb.org/fisht1k>.
350. Birds10 K. 2020 See <https://b10k.genomics.cn>.
351. Skaletsky H *et al.* 2003 The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. *Nature* **423**, 825–837. (doi:10.1038/nature01722)
352. Carvalho AB, Clark AG. 2013 Efficient identification of Y chromosome sequences in the human and *Drosophila* genomes. *Genome Res.* **23**, 1894–1907. (doi:10.1101/gr.156034.113)
353. Clark AG. 2014 The vital Y chromosome. *Nature* **508**, 463–465. (doi:10.1038/508463a)
354. Rhie A *et al.* 2021 Towards complete and error-free genome assemblies of all vertebrate species. *Nature* **592**, 737–746. (doi:10.1038/s41586-021-03451-0)
355. Koepfli K-P, Paten B, Antunes A, Belov K, Bustamante C *et al.* 2015 The genome 10 K project: a way forward. *Ann. Rev. Anim. Biosci.* **3**, 57–111. (doi:10.1146/annurev-animal-090414-014900)

356. Pennell MW, Mank JE, Peichel CL. 2018 Transitions in sex determination and sex chromosomes across vertebrate species. *Mol. Ecol.* **2**, 3950–3963. (doi:10.1111/mec.14540)
357. Payseur BA, Presgraves DC, Filatov DA. 2018 Sex chromosomes and speciation. *Mol. Ecol.* **27**, 3745–3748. (doi:10.1111/mec.14828)
358. Runemark A, Eroukhmanoff F, Nava-Bolaños A, Hermansen JS, Meier JI. 2018 Hybridization, sex-specific genomic architecture and local adaptation. *Phil. Trans. R. Soc. B* **373**, 20170419. (doi:10.1098/rstb.2017.0419)
359. Capel B (ed). 2019 *Sex determination in vertebrates. Current topics in developmental biology*, vol. 104, pp. 1–376. New York, NY: Academic Press.
360. Kopp A. 2012 *Dmrt* genes in the development and evolution of sexual dimorphism. *Trends Genet.* **28**, 175–184. (doi:10.1016/j.tig.2012.02.002)
361. Darbre PD. 2019 The history of endocrine-disrupting chemicals. *Curr. Opin. Endocrinol. Metab. Res.* **7**, 26–33. (doi:10.1016/j.coemr.2019.06.007)
362. Kloas W *et al.* 2009 Endocrine disruption in aquatic vertebrates. *Ann. NY Acad. Sci.* **1163**, 187–200. (doi:10.1111/j.1749-6632.2009.04453.x)
363. Wang H, Piferrer F, Chen S (eds). 2019 *Sex control in aquaculture*, vol. 1. New York, NY: John Wiley & Sons.

Glossary

<i>Autosomes:</i>	all chromosomes of the nucleus, which are not sex chromosomes (or B chromosomes).	<i>Evolutionary stratum:</i>	a region of the sex chromosomes, which ceased recombination in a single step and thus evolutionary period (plural: ‘evolutionary strata’).
<i>Budding:</i>	a type of asexual reproduction, where the new organism emerges gradually as a bud from the cellular membrane or a body part of the parent.	<i>Exotrophic larvae:</i>	larvae feeding on external resources (including maternal trophic eggs) (opposite term ‘endotrophic’).
<i>Deduplication:</i>	the process by which duplicated genes revert to single-copy genes owing to loss or silencing of one copy.	<i>Facultative parthenogenesis:</i>	occasional parthenogenetic reproduction in a species that typically reproduces sexually.
<i>Diploidization:</i>	the process by which a polyploid genome turns functionally and structurally (e.g. by gene expression changes, chromosome loss or divergence between homologous chromosomes in autopolyploids) into a diploid state.	<i>Fast(er)-X/Z effect:</i>	the accelerated evolutionary rate of X- or Z-linked sequences relative to that of autosomal loci.
<i>Dosage balance:</i>	(also ‘male-to-female expression balance’, or ‘parity in the expression between sexes’): the molecular mechanisms that equalize the expression of X-/Z-linked genes missing copies on the Y/W chromosomes between sexes, regardless of whether the ancestral expression levels are restored.	<i>Female heterogamety:</i>	species with sex determination controlled by a ZZ/ZW sex chromosome system (and similar derived systems, e.g. with multiple sex chromosomes).
<i>Dosage compensation:</i>	the molecular mechanisms that restore the expression of X-/Z-linked genes with missing copies on the Y/W chromosome equalizing the expression of these genes between sexes, according to some definitions, to the ancestral expression levels.	<i>Gametogenesis:</i>	the developmental process to produce (usually sex-specific and usually haploid) cells specialized for reproduction (gametes), commonly referred to as ovum/egg (in females) and sperm (in males).
<i>Environmental sex determination:</i>	sex is determined by environmental factors, most commonly temperature (TSD) during a sensitive embryonic stage of gonadal development, in species without sex-specific sequence differences in their genome.	<i>Gametologues:</i>	homologous genes shared by sex chromosomes (e.g. between X and Y chromosomes) in their non-recombining parts.
<i>Epigenetic modification:</i>	reversible changes that modify the genetic material and regulate expression without affecting the DNA sequence (e.g. DNA methylation, histone modification).	<i>Genome elimination:</i>	regulated loss of genomic regions during development of an organism.
		<i>Genotypic sex determination (GSD):</i>	sex is determined by a sex-specific genomic region (at least a single-nucleotide polymorphism), most commonly by a sex-specific combination of chromosomes (i.e. sex chromosomes).
		<i>Germline-restricted chromosomes (GRCs):</i>	type of partial genome elimination where one or more entire chromosomes, the GRCs, are lost during germline-soma differentiation.
		<i>Gonochorism:</i>	having just one of at least two distinct sexes in any one individual organism throughout its lifetime.

<i>Gynogenesis:</i>	reproductive mode, requiring the activation of embryogenesis by sperm without contribution of paternal DNA (often also called 'sperm-dependent parthenogenesis' or sometimes 'pseudogamy').	<i>Kleptogenesis:</i>	reproductive mode of a hybrid unisexual species, requiring sperm from a related, often parental, species to trigger the embryonic development; the sperm can either be eliminated (see Gynogenesis) or its genome can be partially or completely incorporated.
<i>Hemizygous genes:</i>	genes present in a single copy in an otherwise diploid cell, typically Y- and W-specific genes, and likewise X-specific genes in males (i.e. genes present on the X and missing on the Y chromosome or X-linked genes in X0-males of XX/X0 systems), and Z-specific genes in females.	<i>Male heterogamety:</i>	species with sex determination controlled by an XX/XY sex chromosome system (and similar derived systems, e.g. with multiple sex chromosomes).
<i>Hermaphroditism:</i>	developing both male and female gametes during the life cycle of an organism.	<i>Menopause:</i>	post-reproductive life period after the end of female reproduction; known from few cetaceans and hominins.
<i>Heterogametic sex:</i>	the sex that produces two types of gametes that each contain one of two different types of sex chromosomes, e.g. XY-males or ZW-females.	<i>Meroblastic (partial) embryonal cleavage:</i>	the cleavage furrows do not completely divide the fertilized egg (usually in eggs with large amounts of yolk).
<i>Heteromorphic sex chromosomes:</i>	sex chromosomes that are morphologically distinguishable when viewed with a light microscope (opposite term: 'homomorphic').	<i>Mosaicism:</i>	the presence of more than one population of somatic or germline cells with different genotypes or ploidies within an individual.
<i>Holoblastic (total) embryonal cleavage:</i>	the zygote and blastomeres are completely divided during the cleavage.	<i>Neo-sex chromosome:</i>	derived sex chromosome, formed by fusion of the ancestral sex chromosome with an autosome.
<i>Homeologues:</i>	orthologous genes derived from different lineages or species, combined by hybridization in the same diploid or polyploid genome.	<i>Neoteny:</i>	reproduction while retaining juvenile characteristics, e.g. in amphibians (urodela) without full metamorphosis.
<i>Homeologous chromosomes:</i>	homologous chromosomes derived from different lineages or species, combined in the same genome of hybrid origin (diploid or polyploid).	<i>Obligate parthenogenesis:</i>	offspring are produced exclusively by parthenogenesis.
<i>Homeothermic:</i>	having stable, usually physiologically maintained, internal temperature.	<i>Ohnologues:</i>	paralogous genes originated from whole-genome duplications, in vertebrate research usually understood as diversified paralogues, evolved by ancient whole-genome duplications.
<i>Homogametic sex:</i>	the sex that produces gametes that all contain the same type of sex chromosomes, e.g. XX females or ZZ males.	<i>Orthologues:</i>	homologous genes originated from a single common ancestor, now present in different genomes (usually separated by a speciation event).
<i>Homomorphic sex chromosomes:</i>	sex chromosomes that are morphologically indistinguishable in size and shape (opposite term: 'heteromorphic').	<i>Oviparity:</i>	the egg is expelled and the embryo largely develops and hatches outside the body of the mother.
<i>Hybridogenesis:</i>	reproductive mode with selective (usually clonal: 'hemiclonal', i.e. 'half clonal') transmission of one of the two parental genomes of hybrids to their offspring; more complex inheritance patterns may occur in hybrid polyploid organisms, sometimes collectively termed 'hybridogenesis' (or 'meroclinal', i.e. 'partly clonal', inheritance).	<i>Ovo-viviparity:</i>	the egg develops until hatching within the mother, where the embryo is feeding exclusively on nutrients pre-deposited in the egg.
<i>Introgression:</i>	the transfer/moving of genetic/genomic material from one population or species into the gene pool of another, by hybridization.	<i>Ovotestis:</i>	a gonad with both ovarian and testicular tissues (irrespective of their functionality).
		<i>Palindromic sequence:</i>	complementary short DNA or RNA sequence motifs, arranged in close proximity but with opposite orientation; they can potentially form secondary structures, such as hairpins.
		<i>Parthenogenesis:</i>	reproductive mode by which offspring (or at least an embryo) is produced from an egg without genetic contribution from sperm.

<i>Paralogues:</i>	homologous genes in the same genome, originated by duplication (local gene duplication, whole-genome duplication).	<i>Sex chromosome differentiation:</i>	the process leading to changes in content and structure between the homologous X and Y (or Z and W) sex chromosomes, involving one or more of the following events: accumulation of sexually antagonistic alleles, loss of functional genes, recombination arrest, chromosomal rearrangements, heterochromatinization and/or accumulation of repetitive elements.
<i>Poikilothermic:</i>	having variable, usually environmentally dependent, internal temperature.		
<i>Ploidy:</i>	the number of the complete sets of chromosomes in a eukaryotic cell (i.e. one set = haploidy, two sets = diploidy).	<i>Sex chromosome turnover:</i>	evolutionary switch from one sex chromosome system to another, e.g. by the emergence of new master SD genes on new chromosomes or translocation of the sex-determining locus to another chromosome.
<i>Polygenic sex determination:</i>	sex is controlled by multiple genes (also called 'multilocus sex determination').		
<i>Polyploidy:</i>	more than two complete sets of chromosomes occur in a eukaryotic cell.	<i>Sex reversal:</i>	the change of sex during the development of an organism, evident by a mismatch between gonadal phenotype (phenotypic sex) and sex-specific genotype (genotypic sex).
<i>Protandrous hermaphroditism:</i>	producing male gametes in the first stage of the life cycle, and female gametes in a later stage of an organism.	<i>Simultaneous hermaphroditism:</i>	producing both female and male gametes at the same time in one organism.
<i>Protogynous hermaphroditism:</i>	producing female gametes in the first stage of the life cycle, and male gametes in a later stage of an organism.	<i>Synaptonemal complex:</i>	a protein structure that connects paired homologous chromosomes during the meiotic prophase in eukaryotes.
<i>Pseudoautosomal region:</i>	recombining part of sex chromosomes.	<i>Transcription factor:</i>	protein that regulates the transcription of genes.
<i>Recombination:</i>	the exchange of genetic material between homologous chromosomes, occurring during meiosis (most frequent and regularly) or mitosis in eukaryotes.	<i>Transposable element:</i>	genetic elements capable of mobilizing via copy-and-paste (retrotransposons, including endogenous retroviruses) or cut-and-paste mechanisms (most DNA transposons).
<i>Sequential hermaphroditism:</i>	producing female and male gametes at different periods of the life cycle of an organism.	<i>Viviparity:</i>	the egg develops exclusively within the mother, where the embryo is feeding on nutrients, regularly provided by the mother.
<i>Sex determination:</i>	the developmental process deciding the sex of the individual.		
<i>Sex-determining locus:</i>	a locus determining the sex of an individual, in vertebrates triggering the differentiation of the initially bipotential gonad either towards testis or ovary.		
<i>Sex chromosomes:</i>	chromosomes that carry a sex-determining locus (or loci) and segregate in a sex-specific manner.		