



Research

Cite this article: Rovatsos M, Vukić J, Lymberakis P, Kratochvíl L. 2015 Evolutionary stability of sex chromosomes in snakes. *Proc. R. Soc. B* **282**: 20151992. <http://dx.doi.org/10.1098/rspb.2015.1992>

Received: 18 August 2015

Accepted: 24 November 2015

Subject Areas:

evolution, genetics, molecular biology

Keywords:

sex chromosomes, sex determination, reptiles, snakes, evolution

Author for correspondence:

Lukáš Kratochvíl

e-mail: lukas.kratochvil@natur.cuni.cz

Electronic supplementary material is available at <http://dx.doi.org/10.1098/rspb.2015.1992> or via <http://rspb.royalsocietypublishing.org>.

Evolutionary stability of sex chromosomes in snakes

Michail Rovatsos¹, Jasna Vukić¹, Petros Lymberakis² and Lukáš Kratochvíl¹

¹Faculty of Science, Department of Ecology, Charles University in Prague, Viničná 7, 12844 Praha 2, Czech Republic

²Natural History Museum of Crete, University of Crete, Knossou Avenue, 71409 Irakleio, Crete, Greece

Amniote vertebrates possess various mechanisms of sex determination, but their variability is not equally distributed. The large evolutionary stability of sex chromosomes in viviparous mammals and birds was believed to be connected with their endothermy. However, some ectotherm lineages seem to be comparably conserved in sex determination, but previously there was a lack of molecular evidence to confirm this. Here, we document a stability of sex chromosomes in advanced snakes based on the testing of Z-specificity of genes using quantitative PCR (qPCR) across 37 snake species (our qPCR technique is suitable for molecular sexing in potentially all advanced snakes). We discovered that at least part of sex chromosomes is homologous across all families of caenophidian snakes (Acrochordidae, Xenodermatidae, Pareatidae, Viperidae, Homalopsidae, Colubridae, Elapidae and Lamprophiidae). The emergence of differentiated sex chromosomes can be dated back to about 60 Ma and preceded the extensive diversification of advanced snakes, the group with more than 3000 species. The Z-specific genes of caenophidian snakes are (pseudo)autosomal in the members of the snake families Pythonidae, Xenopeltidae, Boidae, Erycidae and Sanziniidae, as well as in outgroups with differentiated sex chromosomes such as monitor lizards, iguanas and chameleons. Along with iguanas, advanced snakes are therefore another example of ectothermic amniotes with a long-term stability of sex chromosomes comparable with endotherms.

1. Introduction

Despite the crucial importance of sex determination, animal lineages do not have a common standard mechanism for this process and differ in the way that the sex of an individual is decided. Amniotic vertebrates as a whole demonstrate two major sex determination systems: genotypic sex determination (GSD) and environmental sex determination (ESD). In GSD, the sex of an individual is set by its sex-specific genotype (i.e. by a combination of sex chromosomes). In contrast, in ESD, which is present for instance in crocodiles and many turtles, the sex of an individual is set in response to environmental conditions (mainly temperature) during the sensitive period of embryonic development, and there are no sex differences in genotype [1]. Since the transition from ESD (absence of sex chromosomes) to GSD (presence of sex chromosomes) seems to be mostly a one-way process, the evolutionary stability of GSD systems, particularly those with well-differentiated sex chromosomes, has been described as the ‘evolutionary trap’ [1–3]. However, for a long time, evolutionary stability of sex chromosomes was only documented by molecular data in endotherms (i.e. in viviparous mammals and birds), which led some authors to conclude that the stability versus non-stability of sex chromosomes corresponds to thermal strategies [4]. Nevertheless, cytogenetic data suggest that significant conservation in sex chromosomes can also be widespread in many lineages of ectothermic amniotes [2,3], but molecular data demonstrating the conservation of sex chromosomes comparable with endotherms in both time and taxonomical scale has been presented only recently and to date just for a single group of ectothermic amniotes (in iguanas) [5].

The aim of our current work is to reconstruct the evolutionary dynamics of sex chromosomes in an important and species-rich lineage of reptiles, the modern snakes (Ophidia: Serpentes). Modern snakes represent an ancient clade

of squamates separated from their putative sister group, the lizards of the clades Iguania and Anguimorpha, for approximately 127–172 Ma [6]. So far, more than 3400 extant snake species have been described [7], colonizing a vast diversity of habitats on all continents with the exception of Antarctica. As far as is known, snakes possess GSD, and female heterogamety has been revealed in many species [8,9]. However, knowledge of the presence and character of sex chromosomes is not equally distributed across all major snake lineages.

Well-differentiated, usually heteromorphic sex chromosomes or derived systems of multiple sex chromosomes have been described exclusively in members of advanced snakes (Colubroidea [9–11]). Molecular evidence for the homology of sex chromosomes based on knowledge of gene linkage to sex chromosomes is available only for four species from two families: Colubridae (*Elaphe quadrivirgata*, *Thamnophis elegans*) and Viperidae (*Protobothrops flavoviridis*, *Sistrurus miliarius*) [11,12]. The genetic content of the Z chromosome in these colubroid snakes reveals the strong homology of this chromosome with chromosome 6 of the green anole, *Anolis carolinensis* (ACA) [11], the model squamate species for reptile genomics with a fully sequenced and adequately annotated genome [13].

This evidence suggests that the sex chromosomes were already present in the common ancestor of the families Viperidae and Colubridae, and should thus be ancestral for the families Homalopsidae, Lamprophiidae and Elapidae, phylogenetically nested between viperids and colubrids. However, taking into account the recent evidence for evolutionary turnovers or losses of differentiated sex chromosomes in non-vertebrates [14,15] and reptiles [16,17], as well the fact that cytogenetically similar sex chromosomes may not be homologous [14,15], we cannot exclude a turnover of sex chromosomes even in this highly species-rich lineage. Moreover, it is not known whether snake lineages outside this clade also share homologous sex chromosomes. In order to reconstruct the evolutionary history and stability of sex chromosomes in snakes, it is therefore necessary to test the homology of sex chromosomes within this clade and its outgroups. Sex chromosomes have almost never been described in members of the snake lineages outside Colubroidea [9,11], with the single putative exception of the Dumeril's boa (*Acranthophis dumerili*), where a heteromorphic pair of chromosomes was observed in the female karyotype, but the linkage of this polymorphism to sex was not validated [9,18]. Also, the recent pioneering work by Vicoso *et al.* [11] applying a modern next-generation sequencing approach was not able to reveal any sex-linked loci in a non-colubroid snake (*Boa constrictor*). Hence, the presence/absence of sex chromosomes in non-colubroid snakes and their homology to sex chromosomes of advanced snakes remains unproven.

Here, we present a molecular test of the homology of sex chromosomes across all colubroid and many non-colubroid snake families, and in the closest outgroups to snakes. We present data demonstrating significant stability of sex chromosomes in advanced snakes and an estimation of emergence of highly differentiated sex chromosomes within snakes in general.

2. Material and methods

(a) Material

Tissue samples (blood or saliva from living individuals and muscle or tip of tail tissue from museum or other preserved specimens) were collected from at least one male and one female individual

of 37 representative snake species (see electronic supplementary material, S1). The studied lineages were selected based on their phylogenetic position (cf. [19–21]) and the availability of high-quality tissue samples of both sexes for DNA isolation. In addition, tissue samples were gathered from lizards representing the closest outgroups to snakes: families Dactyloidea (ACA), Chamaeleonidae (*Chamaeleo calyptratus*, *Furcifer oustaleti*) and Agamidae (*Pogona vitticeps*), all from the clade Iguania, and Varanidae (*Varanus indicus*; Anguimorpha).

(b) Comparison of gene copy numbers between sexes

Partial genetic content of the Z chromosome is known in colubrids and viperids, and is highly syntenic to chromosome 6 (ACA6) of ACA [11,12]. The W chromosome in these lineages is greatly degenerated and lacks the known Z-linked genes. Therefore, males (ZZ) have twice as many copies of genes linked to the Z-specific part of sex chromosomes than females (ZW), while genes in autosomal or pseudoautosomal regions should have equal copy numbers in both sexes. We designed specific primers for putative snake Z-specific genes and tested their sex linkage using qPCR. This approach provides a reliable comparison of the number of gene copies between sexes [5,17,22–24]. We recently applied it to iguanas, revealing a high conservation of sex chromosomes across most of their families and confirming X-linkage of hundreds of genes in the model species ACA [5,17].

Using PRIMER3 software [25], primer pairs were designed based on the anole genome [13] for the amplification of a 120–200 bp fragment of the single-copy gene elongation factor 1a (*ef1a1*), one autosomal 'control' gene located on chromosome 3 (*mecom*) and six putative Z-specific genes located on chromosome 6 (*adarb2*, *itgb1*, *armac4*, *sp4*, *tanc2*, *inmt*) of ACA. Genomic DNA was isolated from the available tissue using a Qiagen DNeasy Blood and Tissue kit (Qiagen). The qPCR was carried out in a LightCycler II 480 (Roche Diagnostics) with all samples run in triplicate. The detailed qPCR protocol and the description of the calculation of the relative gene dose between sexes are available in our previous reports [5,17,22]. Briefly, the gene dosage of each target gene was calculated from crossing point values and was subsequently normalized to the dose of the single-copy reference gene *ef1a1* from the same DNA sample. For this purpose, the target-to-reference gene dosage ratio (R) was calculated by the equation

$$R = (E_{ef1a1})^{C_P ef1a1} / (E_{gene})^{C_P gene}$$

using default amplification efficiencies (E) of 2. The relative gene dosage ratio (r) between females and males of each species for each target gene was obtained by dividing the gene dosage in a female by the gene dosage in a male of the same species (i.e. $r = R_{female} / R_{male}$). A relative gene dosage ratio (r) of 0.5 is expected for Z-specific genes, 1.0 for (pseudo)autosomal genes and potentially 2.0 for X-linked genes.

3. Results

Z-specificity versus autosomal or pseudoautosomal position was tested by qPCR in a control autosomal gene and six genes linked to ACA6 (homologous to Z in viperids and colubrids [11,12]) in every species. Due to the large phylogenetic divergences within snakes, and also between snakes and their tested outgroups, not all primers worked efficiently in qPCR. At least two Z-specific genes were successfully amplified for each species (electronic supplementary material, S3). The results confirmed the previously revealed homology of sex chromosomes in Viperidae and the subfamilies Colubrinae and Natricinae (family Colubridae), as the qPCR proved Z-specificity of the tested genes with homologues linked to

ACA6. A novel finding is that homologous sex chromosomes are present in members of the subfamily Dipsadinae (Colubridae) and in the families Elapidae, Lamprophiidae (tested in subfamilies Psammophiinae, Lamprophiinae and Pseudoxyrhopiinae) and Homalopsidae, demonstrating a conservation of sex chromosomes in this highly radiated clade of snakes. The tested genes with homologues on ACA6 are also Z-specific in the members of the families Pareatidae, Acrochordidae and Xenodermatidae, with the single exception of the gene *tanc2*, which seems to be (pseudo)autosomal in *Acrochordus granulatus*. All tested ACA6 genes showed an exclusively (pseudo)autosomal pattern in the members of the families Pythonidae, Xenopeltidae, Boidae, Erycidae and Sanziniidae.

The snake Z-specific genes proved to be autosomal in the dragon lizard (*P. vitticeps*; Iguania: Acrodonta: Agamidae), having a ZW sex chromosomal system with a highly degenerated W [26], and in ACA (Iguania: Pleurodonta: Dactyloidae), having an XY sex chromosomal system with a highly degenerated Y [22]. For the first time, we present the evidence that snake sex chromosomes are likely to be non-homologous in two other outgroups as well: in the chameleon, with highly differentiated ZW sex chromosomes and a largely heterochromatic W (*F. oustaleti*; Iguania: Acrodonta: Chamaeleonidae) [27], and in the monitor lizard (*V. indicus*; Anguimorpha: Varanidae). Monitor lizards and their putative sister lineage, the Gila monster [21], possess a ZW sex chromosomal system with a largely degenerated W [28,29]. In the case of the homology of snake and the monitor lizard sex chromosomes, a difference in the gene copy number between sexes of the monitor lizard would be expected. On the other hand, the veiled chameleon (*C. calypttratus*; Iguania: Acrodonta: Chamaeleonidae) is likely to possess poorly differentiated sex chromosomes and therefore a large pseudoautosomal region (own data), and the observed (pseudo)autosomal pattern in all tested snake Z-linked genes in this species is thus less informative for the comparison of homology between sex chromosomes of advanced snakes and this species.

The phylogenetic distribution (topology follows [19]) of the character states suggests that the largely differentiated sex chromosomes emerged in the common ancestor of Caenophidia (i.e. the clade comprising the families Acrochordidae, Xenodermatidae, Pareatidae, Viperidae, Homalopsidae, Colubridae, Elapidae and Lamprophiidae), and demonstrates a conservation of sex chromosomes in this highly radiated clade of snakes of more than 3000 species. There was no evidence of Z-linkage of any of the genes in the members of the families Pythonidae, Xenopeltidae, Boidae, Erycidae and Sanziniidae. The results from the snake outgroups suggest that sex chromosomes homologous with the sex chromosomes of advanced snakes were not present in the common ancestor of Toxicofera (the lineage comprising snakes, Iguania and Anguimorpha).

4. Discussion

We discovered that the highly differentiated sex chromosomes homologous to the sex chromosomes of viperids and colubrids are present not only in all families sharing the last common ancestor with vipers and colubrids (Homalopsidae, Elapidae, Lamprophiidae), but also in the families Pareatidae (Asian snail-eating snakes), Acrochordidae (file snakes) and Xenodermatidae forming the closest outgroup to the viperid–colubrid clade [19,21] (figure 1). In our sampling, we included members

from all the families of the enormously diversified group Caenophidia (around 3000 species), and we found that all tested species possessed (at least to some extent) homologous and highly differentiated sex chromosomes. This evidences the notable long-term evolutionary stability of these differentiated sex chromosomes and allows us to conclude that this stage of sex chromosomes was clearly present in the last common ancestor of caenophidian snakes, recently estimated to have lived about 60 Ma [21].

With the putative exception of the Madagascar Dumerili's boa (*A. dumerili*), sex chromosomes have not been identified in any snake clade outside Caenophidia [9]. This led to the conclusion that sex chromosomes in snakes outside Caenophidia are only poorly differentiated [11,12]. In agreement, we found exclusively pseudoautosomal or autosomal patterns for all tested genes in the members of non-caenophidian families: sand boas (Erycidae), true boas (Boidae), pythons (Pythonidae) and sunbeam snakes (Xenopeltidae). Interestingly, pseudoautosomal or autosomal patterns were also revealed in the Madagascar Dumerili's boa, despite heteromorphic sex chromosomes being previously reported in this snake [9,18]. It is possible that the Z and W sex chromosomes in this species differ in morphology, but not greatly in gene content (see [30] for a similar situation).

An important result of our study is the evidence for the non-homology of sex chromosomes between advanced snakes and their closest outgroup from the lineage Toxicofera, especially for monitor lizards and chameleons with ZZ/ZW sex chromosomes [27,28]. It seems that snake sex chromosomes evolved only after the divergence of snake and other toxicoferan lineages.

In conclusion, advanced snakes have highly evolutionarily stable sex chromosomes comparable with iguanas, viviparous mammals and birds [5,31,32]. This supports the hypothesis of the stability of GSD when compared with ESD ('GSD as an evolutionary trap' hypothesis), although ESD might be ancestral for squamates, and maybe all amniotic vertebrates [1,2,3]. The strong evolutionary stability of differentiated sex chromosomes in advanced snakes has important practical and theoretical consequences. For example, the qPCR technique used by us to test the homology of sex chromosomes can also be used as a reliable and relatively cheap method of molecular sexing of species from all caenophidian families. The genes *tanc2* and *adarb2* would be especially effective as they were successful in most of the tested species of advanced snakes (electronic supplementary material, S2 and S3). We observed that even stored DNA from saliva, which can be sampled non-destructively, can be used for this technique, which has importance for ecological studies in snakes and for animal conservation. From a theoretical point of view, it could be noted that in parallel with birds and mammals [31,32], the emergence of differentiated sex chromosomes in snakes came before the remarkable increase in diversification rate. Caenophidian snakes with highly differentiated sex chromosomes include more than 3000 recent species, while there are only around 400 species of non-caenophidian snakes [7], where sex chromosomes are likely to be only poorly differentiated. Nevertheless, it is important to note that identification of the key evolutionary novelty connected with enlarged diversification of a group is a difficult task, and that other candidate mechanisms such as the colonization of new areas and the evolution of advanced venom-delivery systems were suggested to be

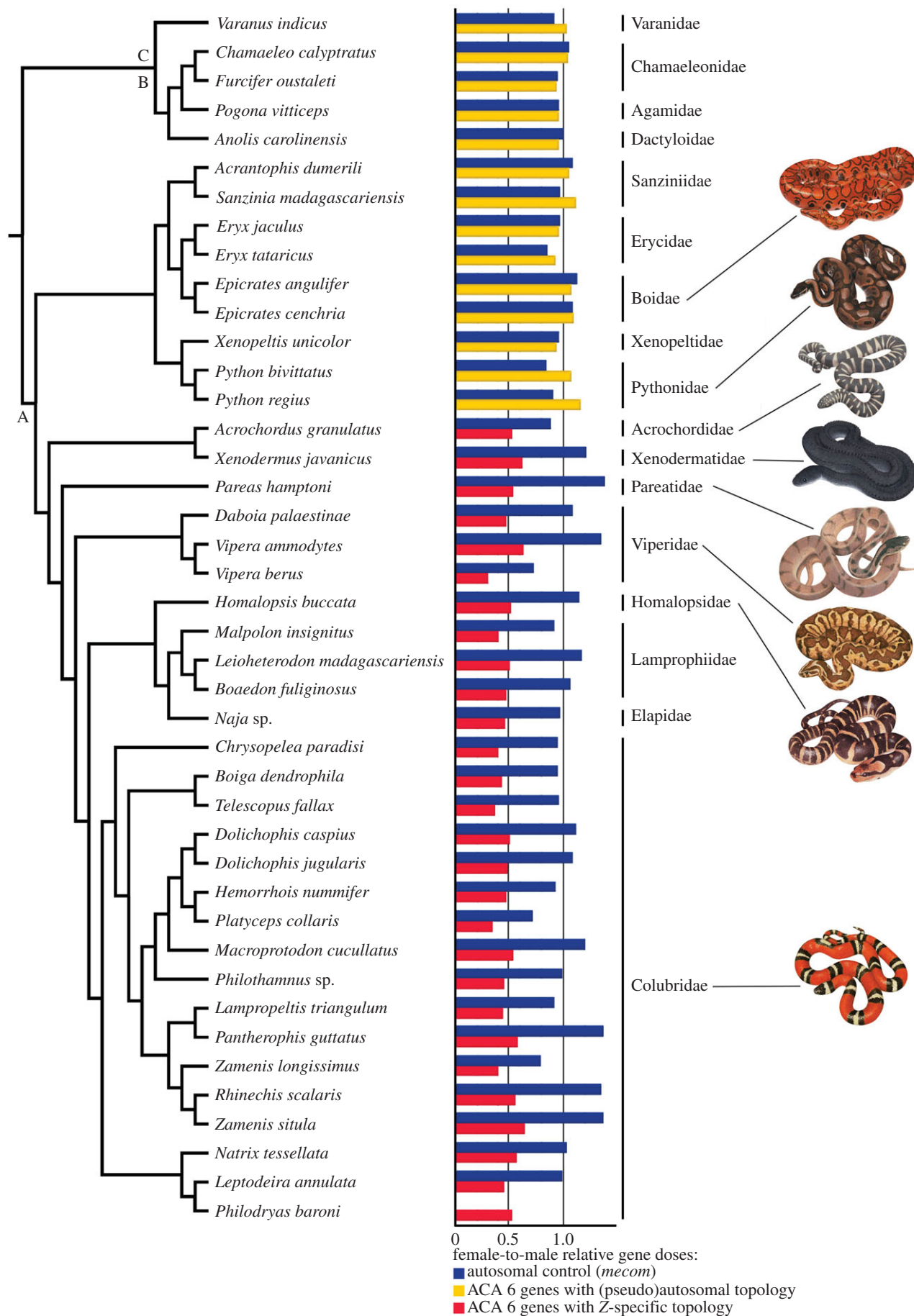


Figure 1. Relative gene dose ratios between a female and a male in 42 species of snakes (A) and lizard outgroups (B, Iguania; C, Anguimorpha). Values (means \pm s.d.) are depicted in red for the snake Z-linked genes, for (pseudo)autosomal loci homologous to ACA6 in yellow and for the control autosomal locus in blue. Value 1.0 is expected for autosomal or pseudoautosomal genes, while value 0.5 is consistent with Z-linkage. Our results suggest that sex chromosomes are highly differentiated and homologous across all caenophidian snakes. In contrast, the Z-specific genes of caenophidian snakes are pseudoautosomal or autosomal in non-caenophidian snakes and snake outgroups. (Online version in colour.)

responsible for the large diversity of caenophidian and colubroid snakes, respectively [33]. Future research should be carried out to determine to what extent the superradiation of this clade is connected to the so-called ‘fast Z’, and likewise ‘fast X’ phenomena (i.e. increased evolutionary rate of the Z-specific and likewise X-specific genes relative to their autosomal or pseudoautosomal homologues; in snakes, documented in [11]), potentially enabling higher diversification in lineages with differentiated sex chromosomes.

Ethics. The work was approved by the Ethical Committee of the Czech Republic.

Data accessibility. All results are uploaded as the electronic supplementary material.

References

- Johnson Pokorná M, Kratochvíl L. In press. What was the ancestral sex-determining mechanism in amniote vertebrates? *Biol. Rev.* (doi:10.1111/brv.12156)
- Pokorná M, Kratochvíl 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)
- 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)
- 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)
- Rovatsos M, Altmanová M, Pokorná M, Kratochvíl L. 2014 Conserved sex chromosomes across adaptively radiated *Anolis* lizards. *Evolution* **68**, 2079–2085. (doi:10.1111/evo.12357)
- Mulcahy DG, Noonan BP, Moss T, Townsend TM, Reeder TW, Sites JW, Wiens JJ. 2012 Estimating divergence dates and evaluating dating methods using phylogenomic and mitochondrial data in squamate reptiles. *Mol. Phylogenet. Evol.* **65**, 974–991. (doi:10.1016/j.ympev.2012.08.018)
- Uetz P, Hošek J (eds). 2014 The reptile database. See <http://www.reptile-database.org> (accessed 21 March 2015).
- Olmo E, Signorino GG. 2005 Chromorep: a reptile chromosomes database. See <http://chromorep.univpm.it> (accessed 21 March 2015).
- Oguiura N, Ferrarezzi H, Batistic RF. 2009 Cytogenetics and molecular data in snakes: a phylogenetic approach. *Cytogenet. Genome Res.* **127**, 128–142. (doi:10.1159/000295789)
- O’Meally D, Patel HR, Stigler R, Sarre SD, Georges A, Marshall Graves JA, Ezaz T. 2010 Non-homologous sex chromosomes of birds and snakes share repetitive sequences. *Chromosom. Res.* **18**, 787–800. (doi:10.1007/s10577-010-9152-9)
- 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)
- Matsubara K, Tarui H, Toriba M, Yamada K, Nishida-Umehara C, Agata K, Matsuda Y. 2006 Evidence for different origin of sex chromosomes in snakes, birds, and mammals and step-wise differentiation of snake sex chromosomes. *Proc. Natl Acad. Sci. USA* **103**, 18 190–18 195. (doi:10.1073/pnas.0605274103)
- Alföldi J *et al.* 2011 The genome of the green anole lizard and a comparative analysis with birds and mammals. *Nature* **477**, 587–591. (doi:10.1038/nature10390)
- Vicoso B, Bachtrog D. 2013 Reversal of an ancient sex chromosome to an autosome in *Drosophila*. *Nature* **499**, 332–335. (doi:10.1038/nature12235)
- Vicoso B, Bachtrog D. 2015 Numerous transitions of sex chromosomes in Diptera. *PLoS Biol.* **13**, e1002078. (doi:10.1371/journal.pbio.1002055. pmid:25575020)
- Koubová M, Johnson Pokorná M, Rovatsos M, Farkačová K, Altmanová M, Kratochvíl L. 2014 Sex determination in Madagascar geckos of the genus *Paroedura* (Squamata: Gekkonidae): are differentiated sex chromosomes indeed so evolutionary stable? *Chromosom. Res.* **22**, 441–452. (doi:10.1007/s10577-014-9430-z)
- 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)
- Mengden GA, Stock AD. 1980 Chromosomal evolution in Serpentes; a comparison of G and C chromosome banding patterns of some colubrid and boid genera. *Chromosoma* **79**, 53–64. (doi:10.1007/BF00328472)
- Pyron RA, Burbrink FT, Wiens JJ. 2013 A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. *BMC Evol. Biol.* **13**, 93. (doi:10.1186/1471-2148-13-93)
- Pyron RA, Reynolds RG, Burbrink FT. 2014 A taxonomic revision of boas (Serpentes: Boidae). *Zootaxa* **3846**, 249–260. (doi:10.11646/zootaxa.3846.2.5)
- Hsiang AY, Field DJ, Webster TH, Behlke ADB, Davis MB, Racicot RA, Gauthier JA. 2015 The origin of snakes: revealing the ecology, behavior, and evolutionary history of early snakes using genomics, phenomics, and the fossil record. *BMC Evol. Biol.* **15**, 87. (doi:10.1186/s12862-015-0358-5)
- Rovatsos M, Altmanová M, Johnson Pokorná M, Kratochvíl L. 2014 Novel X-linked genes revealed by quantitative polymerase chain reaction in the green anole, *Anolis carolinensis*. *Genes Genomes Genet.* **4**, 2107–2113. (doi:10.1534/g3.114.014084)
- Nguyen P, Sýkorová M, Šichová J, Kůta V, Dalíková M, Čapková Frydrychová R, Neven LG, Sahara K, Marec F. 2013 Neo-sex chromosomes and adaptive potential in tortricid pests. *Proc. Natl Acad. Sci. USA* **110**, 6931–6936. (doi:10.1073/pnas.1220372110)
- Gamble T, Geneva AJ, Glor RE, Zarkower D. 2014 *Anolis* sex chromosomes are derived from a single ancestral pair. *Evolution* **68**, 1027–1041. (doi:10.1111/evo.12328)
- Ye J, Coulouris G, Zaretskaya I, Cutcutache I, Rozen S, Madden TL. 2012 Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC Bioinform.* **13**, 134. (doi:10.1186/1471-2105-13-134)
- Young MJ, O’Meally D, Sarre SD, Georges A, Ezaz T. 2013 Molecular cytogenetic map of the central bearded dragon, *Pogona vitticeps* (Squamata: Agamidae). *Chromosom. Res.* **21**, 361–374. (doi:10.1007/s10577-013-9362-z)
- Rovatsos M, Johnson Pokorná M, Altmanová M, Kratochvíl L. 2015 Female heterogamety in Madagascar chameleons (Squamata: Chamaeleonidae: *Furcifer*): differentiation of sex and neo-sex chromosomes. *Sci. Rep.* **5**, 13196.
- King M, Mengden GA, King D. 1982 A pericentric-inversion polymorphism and s ZZ/ZW sex-chromosome system in *Varanus acanthurus* Boulenger analyzed by G- and C-banding and Ag staining. *Genetica* **58**, 39–45. (doi:10.1007/BF00056001)
- Johnson Pokorná M, Rovatsos M, Kratochvíl L. 2014 Sex chromosomes and karyotype of the (nearly)

- mythical creature, the Gila monster, *Heloderma suspectum* (Squamata: Helodermatidae). *PLoS ONE* **9**, e104716. (doi:10.1371/journal.pone.0104716)
30. Pokorná M, Rábová M, Ráb P, Ferguson-Smith MA, Rens W, Kratochvíl L. 2010 Differentiation of sex chromosomes and karyotypic evolution in the eye-lid geckos (Squamata: Gekkota: Eublepharidae), a group with different modes of sex determination. *Chromosome Res.* **18**, 809–820. (doi:10.1007/s10577-010-9154-7)
31. Zhou Q, Zhang J, Bachtrog D, An N, Huang Q, Jarvis ED, Gilbert MT, Zhang G. 2014 Complex evolutionary trajectories of sex chromosomes across bird taxa. *Science* **346**, 1246338. (doi:10.1126/science.1246338)
32. Waters PD, Wallis MC, Marshall Graves JA. 2007 Mammalian sex: origin and evolution of the Y chromosome and SRY. *Semin. Cell Dev. Biol.* **18**, 389–400. (doi:10.1016/j.semcdb.2007.02.007)
33. Pyron RA, Burbrink FT. 2012 Extinction, ecological opportunity, and the origins of global snake diversity. *Evolution* **66**, 163–178. (doi:10.1111/j.1558-5646.2011.01437.x)