ribosomal protein genes is to calculate the average length of ribosomal protein nascent chains still in production. Even after deciding exactly how many genes ribosomal protein content will be split between, this average is sensitive to the exact way that amino acids are divided between these genes. In particular, genes that are longer than the mean contribute a disproportionate increase to the average length of all nascent chains. This nonlinearity stems from the facts that the messenger RNAs encoded by longer genes are not only occupied by more ribosomes, but also have correspondingly longer nascent chains attached to these ribosomes. By a straightforward mathematical argument, this means that any unequal division of gene length results in less efficient return on investment than an even split. Selective pressure is therefore expected to drive ribosomal protein organization towards the observed tight distribution of similar lengths.

Reuveni *et al.* [7] also explore more speculative implications of the selective pressure on ribosomes to efficiently self-synthesize, including on interpretation of the higher protein content of (non-self-synthesized) mitochondrial ribosomes. Of course, efficiency of self-synthesis is not the only selective force at play in ribosomal evolution, and the genetic organization of ribosomal proteins is undoubtedly influenced by a wide range of more specific functional constraints. Ribosome biogenesis is an intricately choreographed process [10] that proceeds in a modular fashion [11], suggesting that assembly is facilitated by separation into discrete polypeptides. Splitting ribosomal proteins into discrete genes also provides the opportunity to include or exclude particular proteins from a given ribosome. This flexibility could allow cells to produce a heterogeneous pool of specialized ribosomes [12] with distinct functional roles [13], for example in response to environmental stresses [14]. A complete understanding of the contributions of these processes to the complex evolutionary landscape of ribosomal architecture remains challenging, but the selective framework of efficient self-production proposed by Reuveni *et al.* represents an exciting new lens through which to view this challenge.

REFERENCES

1. Tocilj, A., Schlunzen, F., Janell, D., Gluhmann, M., Hansen, H.A.S., Harms, J., Bashan, A., Bartels, H., Agmon, I., Franceschi, F., *et al.* (1999). The small ribosomal subunit from Thermus thermophilus at 4.5 A resolution: Pattern fittings and the identification of a functional site. Proc. Natl. Acad. Sci. USA *96*, 14252–14257.

2. Nissen, P., Hansen, J., Ban, N., Moore, P.B., and Steitz, T.A. (2000). The structural basis of ribosome activity in peptide bond synthesis. Science *289*, 920–930.

3. Schuwirth, B.S., Borovinskaya, M.A., Hau, C.W., Zhang, W., Vila-Sanjurjo, A., Holton, J.M., and Cate, J.H.D. (2005). Structures of the bacterial ribosome at 3.5 A resolution. Science *310*, 827–834.

4. Noller, H.F. (2012). Evolution of protein synthesis from an RNA world. Cold Spring Harb. Perspect. Biol. *4*, a003681.

5. Traut, R.R., Delius, H., Ahmad-Zadeh, C., Bickle, T.A., Pearson, P., and Tissières, A. (1969). Ribosomal proteins of *E. Coli*: stoichiometry and implications for ribosome structure. Cold Spring Harb. Symp. Quant. Biol. *34*, 25–38.

6. Melnikov, S., Ben-Shem, A., Garreau de Loubresse, N., Jenner, L., Yusupova, G., and Yusupov, M. (2012). One core, two shells: bacterial and eukaryotic ribosomes. Nat. Struct. Mol. Biol. *19*, 560–567.

7. Reuveni, S., Ehrenburg, M., and Paulsson, J. (2017). Ribosomes are optimized for autocatalytic production. Nature *547*, 293–297.

8. Oh, E., Becker, A.H., Sandikci, A., Huber, D., Chaba, R., Gloge, F., Nichols, R.J., Typas, A., Gross, C.A., Kramer, G., *et al.* (2011). Selective ribosome profiling reveals the cotranslational chaperone action of trigger factor in vivo. Cell *147*, 1295–1308.

9. Brandt, F., Etchells, S.A., Ortiz, J.O., Elcock, A.H., Hartl, F.U., and Baumeister, W. (2009). The native 3D organization of bacterial polysomes. Cell *136*, 261–271.

10. Kaczanowska, M., and Rydén-Aulin, M. (2007). Ribosome biogenesis and the translation process in *Escherichia coli*. Microbiol. Mol. Biol. Rev. *71*, 477–494.

11. Davis, J.H., Tan, Y.Z., Carragher, B., Potter, C.S., Lyumkis, D., and Williamson, J.R. (2016). Modular assembly of the bacterial large ribosomal subunit. Cell *167*, 1610–1622.

12. Sauert, M., Temmel, H., and Moll, I. (2014). Heterogeneity of the translational machinery: Variations on a common theme. Biochimie *114*, 39–47.

13. Shi, Z., Fujii, K., Kovary, K.M., Genuth, N.R., Rost, H.L., Teruel, M.N., and Barna, M. (2017). Heterogeneous ribosomes preferentially translate distinct subpools of mRNAs genome-wide. Mol. Cell *32*, 710–714.

14. Byrgazov, K., Vesper, O., and Moll, I. (2013). Ribosome heterogeneity: Another level of complexity in bacterial translation regulation. Curr. Opin. Microbiol. *16*, 133–139.

Evolution: A Paradigm Shift in Snake Sex Chromosome Genetics

J.J. Emerson[1,2]
[1]Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92697, USA
[2]Center for Complex Biological Systems, University of California, Irvine, CA 92697, USA
Correspondence: jje@uci.edu
http://dx.doi.org/10.1016/j.cub.2017.06.079

The belief that all snakes possess ZW sex chromosomes has prevailed for decades, despite no evidence of this in boas, pythons, and their relatives. A recent discovery of male-specific genetic markers reveals that these snakes instead possess XY sex chromosomes.

Sex chromosomes were discovered by Nettie Stevens in 1905 [1] by noting that in certain insects, sperm comes in two types (i.e. males are heterogametic), with a single small chromosome (called a Y) in one type and a large chromosome (called an X) in the other. However, eggs come in only one type possessing the X chromosome (i.e. females are homogametic). Combined with the observation that female somatic cells contain two X chromosomes whereas

those of males contain an X and a Y, Stevens suggested that eggs fertilized by Y-bearing sperm become male whereas those fertilized by X-bearing sperm develop into females. This situation is reversed in many species, like birds, butterflies, and moths, where the male is homogametic (i.e. ZZ) and the female heterogametic (i.e. ZW) [2].

In snakes, the dogma is clear: the genetic makeup of the egg, not the sperm, determines the sex of the offspring. When a sperm, which always bears a Z chromosome, fertilizes an egg bearing a W chromosome, the resulting embryo is female, but when one fertilizes an egg bearing another Z chromosome, it is male. This defining quality of snakes — namely that females are ZW and males are ZZ — has dominated our assumptions about snake sex determination for more than half a century, though the discovery of putative Y-linked DNA in a recent study in *Current Biology* is poised to upend this dogma [3]. Indeed, snakes have served as a case study motivating a popular model for the acquisition and differentiation of sex chromosomes from ancestral autosomes [4]. In the current version of this model [5], one pair of autosomes acquires a sex-determining factor, but otherwise remains identical. As time passes, if mutations favoring one sex over the other (i.e. sexually antagonistic, or SA, mutations) occur near the sex-determining locus, then subsequent mutations are favored that prevent these SA mutations from occurring in the sex that they antagonize. This can be achieved by cessation of recombination between the loci, as can happen when a chromosomal inversion spans both loci. While recombination ceases in the heterogametic sex (XY males or ZW females) because they are heterozygous for both inversion genotypes, it continues in the homogametic sex (XX females or ZZ males). This cessation of recombination may favor degeneration of the female-specific region through the reduction in the efficacy of natural selection [6,7]. Over time, this process leads to increasing degeneration of the Y or W chromosome.

It was proposed that snake ZW chromosome evolution has paused at different stages of differentiation [4]. Henophidian snakes (including boas and pythons) possess indistinguishable sex chromosomes, whereas its sister group, the caenophidian snakes (including vipers, garter snakes, and cobras) bear clearly heteromorphic sex chromosomes with apparently varying degrees of differentiation (Figure 1). This variation among W chromosomes may correlate with many other aspects of its evolution, including the proliferation of transposable elements, acquisition of dosage compensation, and acceleration of evolution on the Z [8]. One attractive feature of this model is that it permits us to think of the henophidian snakes as exemplars of the early stages of sex chromosome differentiation and caenophidian snakes as representatives of the intermediate and later stages.

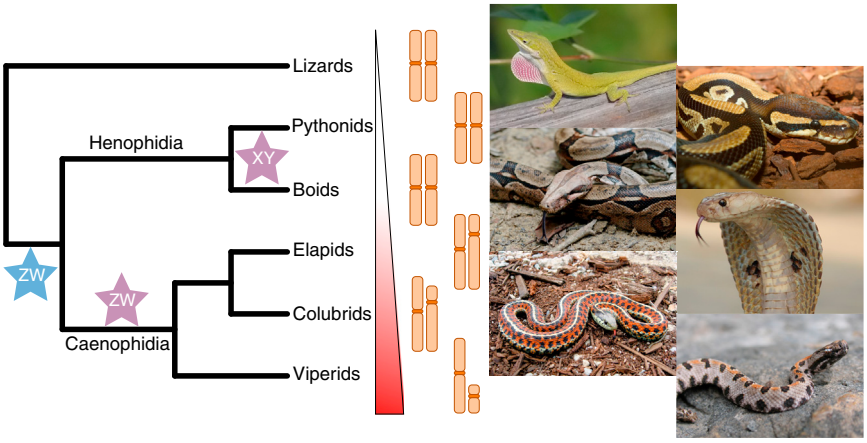

Figure 1. Sex chromosome evolution in snakes.
The phylogeny shows the relationships between selected major groups of snakes and the chromosome morphology of what is typically the fourth largest pair of chromosomes in male snakes [9], which is homologous to chromosome 6 in the green anole [8]. The chromosome ideograms indicate variation in chromosome size and centromere placement (following [8,20]). The triangle indicates the orthodox continuum model for evolution of sex chromosomes from their autosomal ancestors [4]. The narrow end indicates no differentiation between Z and W and the wider end indicates substantial differentiation. The blue star indicates the timing of acquisition of ZW chromosomes under the orthodox model. The purple stars indicate an alternative made plausible by Gamble *et al.* [3], though further study is needed to corroborate the model.

To the authors of the recent report in *Current Biology* [3], something about this model didn't add up: if the sex chromosomes of pythons and boas exhibit so few differences, then how were they identified in the first place? Indeed, how would we really know that henophidians even have ZW sex chromosomes at all? In opposition to more than fifty years of conventional thinking about snake genetics, Gamble *et al.* [3] make the argument that henophidian snakes do not actually share the ZW possessed by caenophidian snakes as previously thought [4,9], but rather exhibit XY sex determination (Figure 1). Their argument is an example of thorough scholarship and a few, key experimental observations. First, they note that despite extensive work on the karyotypes of henophidian snakes, there is only a single report of a heteromorphism (an inversion) in an unsexed individual for the putative ZW chromosome pair [10], a result that is not dispositive to ZW being characteristic of henophidians. Since only one karyotype was reported from an unsexed individual, the association of this inversion with sex cannot be determined. Moreover, since the observation is only in a single species, given the lability of karyotype evolution in other species [11,12], this example could be ZW even if other henophidians are not.

Next, Gamble *et al.* [3] point out that previous reports of parthenogenesis in henophidians have all resulted in female offspring (reviewed in [13,14]). These examples all yield parthenogens with less heterozygosity than their mothers, consistent with the fusion of a polar body with the ovum (meiotic terminal fusion). The original authors interpreted this as production of viable WW 'super' females without production of normal ZZ males, going so far as to speculate that the mother might actually have been WO

instead of ZW to explain the absence of males [15]. This would make henophidian parthenogenesis very strange for ZW snakes indeed, as WW female production is unattested in other snakes whereas production of ZZ males is the expected outcome in caenophidians [13]. In XY parthenogenesis, however, the production of only female parthenogens from an XX mother is the expected outcome.

Additionally, the authors note a recent observation of a heritable trait in ball pythons (*Python regius*) that appears to be sex-linked [14,16]. The trait, called coral glow (CG), is incompletely dominant and confers a yellow–orange color to the normally brown and black species. Heterozygous CG males with CG mothers and wild-type fathers sire mostly CG daughters and wild-type sons. On the other hand, heterozygous males with CG fathers and wild-type mothers sire mostly CG sons and wild-type daughters. This strongly suggests that the coral glow trait is determined by a single gene incompletely linked to an XY sex determining locus. Under this model, about 6–7% of crosses involve recombinants, indicating the presence of a pseudoautosomal region and incomplete degeneration of the Y chromosome.

Despite the literature pointing away from ZW and towards XY, there remains no direct evidence of sex chromosome heteromorphism of any kind in henophidian snakes. Indeed, recent genomic work on the supposed ZW in snakes has indicated a thoroughly homomorphic Z chromosome in Boa constrictor even at the sequence level [8]. To search for heteromorphism, Gamble *et al.* [3] genotyped thousands of anonymous markers associated with restriction digests (i.e. RAD markers) across many individuals of both sexes [12,17] in two henophidians (the boa constrictor, *Boa constrictor imperator* and the Burmese python, *Python bivattatus*) as well as one caenophidian (the Western diamond-backed rattlesnake, *Crolatus atrox*). As expected of a confirmed ZW species, the rattlesnake displayed more female-specific markers than expected by chance but no male-specific markers. Interestingly, across two different RAD sequencing experiments in boa, 117 male-specific and 4 female-specific markers were recovered. Since up to 14 sex-specific markers are expected by chance, this result makes sense only if boas have XY sex determination. Unfortunately, due to a small ascertainment sample size, the maximum number of sex-specific markers expected by chance in python was quite large, exceeding the already large number of observed male-specific markers. To address this, the authors applied PCR and PCR-RFLP assays to their markers in an independent validation panel consisting of twelve male and twelve female pythons, confirming male specificity for two loci. Similarly, validation of a RAD marker from boa was also male-specific. Interestingly, this locus was also male-specific in another boa (*Boa constrictor constrictor*), indicating a shared sex-linked region between species. However, the primers from Burmese python failed to amplify in a sex-specific fashion for either the Carpet python (*Morelia spilota*) or the Ball python (*Python regius*), suggesting rapid variation in sex linkage in pythons. Finally, the authors used sequence information derived from male-specific markers in both henophidian species to search for homology to Anole chromosome scaffolds, the most contiguous and well-annotated genome closely related to snakes. In boa, RAD markers identified several scaffolds in the published boa genome [18]. Transcripts from another henophidian [19] were used to identify genes on the scaffolds. These genes were mostly homologous to genes on microchromosome LGf in anole (19q in human). In contrast, one male-specific RAD marker in the Burmese python mapped to chromosome 6 in anole, which is homologous to the Z chromosome in caenophidians. Additionally, the authors identified five python transcripts with male-specific single nucleotide polymorphisms. Three of these also mapped to anole chromosome 6, suggesting that pythons have recruited the caenophidian Z homolog as an X chromosome.

If correct, these results will transform the field of sex chromosome evolution and genetics in snakes. Not only do henophidians have XY sex chromosomes, the age of the ZW chromosome pair in caenophidians could be potentially much younger than previously thought (Figure 1). The prospect of rapid sex chromosome transitions in snakes will ensure that snakes remain a model system for studying the evolution of sex chromosomes, especially in henophidians that appear to have evolved XY chromosomes independently. And while boas and pythons are no longer models for the incipient stages of snake ZW evolution, they may very well serve that role for XY evolution.

REFERENCES

1. Stevens, N.M. (1905). Studies in Spermatogenesis: With Especial Reference to the "Accessory Chromosome" (Carnegie Institution of Washington).
2. Bachtrog, D., Mank, J.E., Peichel, C.L., Kirkpatrick, M., Otto, S.P., Ashman, T.-L., Hahn, M.W., Kitano, J., Mayrose, I., Ming, R., *et al*. (2014). Sex determination: why so many ways of doing it? PLoS Biol. *12*, e1001899.
3. Gamble, T., Castoe, T.A., Nielsen, S.V., Banks, J.L., Card, D.C., Schield, D.R., Schuett, G.W., and Booth, W. (2017). The discovery of XY sex chromosomes in a boa and python. Curr. Biol. *27*, 2148–2153.e4.
4. Ohno, S. (1967). Sex Chromosomes and Sex-Linked Genes, A. Labhart, T. Mann, L.T. Samuels, and J. Zander, eds. (Berlin Heidelberg: Springer).
5. Bachtrog, D. (2013). Y chromosome evolution: emerging insights into processes of Y chromosome degeneration. Nat. Rev. Genet. *14*, 113–124.
6. Muller, H.J. (1914). A gene for the fourth chromosome of Drosophila. J. Exp. Zool. *17*, 325–336.
7. Charlesworth, B., and Charlesworth, D. (2000). The degeneration of Y chromosomes. Phil. Trans. R. Soc. B Biol. Sci. *355*, 1563–1572.
8. Vicoso, B., Emerson, J.J., Zektser, Y., Mahajan, S., and Bachtrog, D. (2013). Comparative sex chromosome genomics in snakes: differentiation, evolutionary strata, and lack of global dosage compensation. PLoS Biol. *11*, e1001643.
9. Beçak, W., Beçak, M.L., Nazareth, H.R.S., and Ohno, S. (1964). Close karyological kinship between the reptilian suborder serpentes and the class aves. Chromosoma *15*, 606–617.
10. Mengden, G.A., and Stock, A.D. (1980). Chromosomal evolution in serpentes; a comparison of G and C chromosome banding patterns of some colubrid and boid genera. Chromosoma *79*, 53–64.
11. Vicoso, B., and Bachtrog, D. (2015). Numerous transitions of sex chromosomes in Diptera. PLoS Biol. *13*, e1002078.
12. Gamble, T., Coryell, J., Ezaz, T., Lynch, J., Scantlebury, D.P., and 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.

13. Booth, W., and Schuett, G.W. (2016). The emerging phylogenetic pattern of parthenogenesis in snakes. Biol. J. Linn. Soc. *118*, 172–186.

14. Mallery, C.S., Jr., and Carrillo, M.M. (2016). A case study of sex-linkage in Python regius (Serpentes: Boidae), with new insights into sex determination in Henophidia. Phyllomedusa. J. Herpetol. *15*, 29–42.

15. Booth, W., Johnson, D.H., Moore, S., Schal, C., and Vargo, E.L. (2010). Evidence for viable, non-clonal but fatherless Boa constrictors. Biol. Lett., rsbl20100793.

16. Mallery, C.S., Jr. (2014). Following the chromosomes of inheritance and sex-linkage: an explanation of coral glow/banana. In The Ultimate Ball Python: Morph Maker Guide.

17. Gamble, T. (2016). Using RAD-seq to recognize sex-specific markers and sex chromosome systems. Mol. Ecol. *25*, 2114–2116.

18. Bradnam, K.R., Fass, J.N., Alexandrov, A., Baranay, P., Bechner, M., Birol, I., Boisvert, S., Chapman, J.A., Chapuis, G., Chikhi, R., *et al*. (2013). Assemblathon 2: evaluating de novo methods of genome assembly in three vertebrate species. GigaScience *2*, 10.

19. Castoe, T.A., de Koning, A.P.J., Hall, K.T., Card, D.C., Schield, D.R., Fujita, M.K., Ruggiero, R.P., Degner, J.F., Daza, J.M., Gu, W., *et al*. (2013). The Burmese python genome reveals the molecular basis for extreme adaptation in snakes. Proc. Natl. Acad. Sci. USA *110*, 20645–20650.

20. Ezaz, T., Stiglec, R., Veyrunes, F., and Marshall Graves, J.A. (2006). Relationships between vertebrate ZW and XY sex chromosome systems. Curr. Biol. *16*, R736–R743.

Energy Balance: Lateral Hypothalamus Hoards Food Memories

Kavya Devarakonda and Paul J. Kenny*
Department of Neuroscience, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, New York, NY 10029-6574, USA
*Correspondence: paul.kenny@mssm.edu
http://dx.doi.org/10.1016/j.cub.2017.06.082

The lateral hypothalamus is known to drive food consumption during periods of hunger. A new study suggests that the lateral hypothalamus may also participate in the formation and storage of memories about events in the environment that predict the availability of food.

One of the most important functions of the brain is to coordinate behavioral output during periods of hunger to obtain and consume food, thereby replenishing otherwise depleted energy stores to avoid starvation. Frequently, food is not readily available to hungry animals (including humans) for consumption and instead they must successfully forage. Hence, it is critical that an organism learns to recognize cues in the environment that predict food availability, and to use this information during periods of hunger to procure food. Precisely how food-predictive information in the environment is encoded in the brain is unclear. In a paper published recently in *Current Biology*, Sharpe *et al.* [1] present new findings suggesting that neurons in the lateral hypothalamus, previously thought to simply initiate feeding behaviors in hungry animals, are directly involved in learning about food-predictive environmental stimuli that guide foraging behaviors.

Nuclei in the hypothalamus play critical roles in feeding behavior. In particular, the lateral hypothalamus is considered a major feeding center in the brain [2]. Activity of neurons in the lateral hypothalamus is increased during feeding behavior [3]. Moreover, classic electrical brain stimulation experiments have shown that activation of the lateral hypothalamus can trigger voracious eating in animals even when they are fully fed [4,5]. Conversely, lesions of this brain structure result in markedly decreased food consumption and starvation [6]. Using modern tools to target genetically defined populations of neurons, it was recently shown that GABAergic neurons are the major class of cells in the lateral hypothalamus that stimulate feeding behaviors [7,8]. Based on these and related findings, the lateral hypothalamus is generally thought to serve as an 'actuator' of feeding behavior that links hunger signals to the initiation of the motor programs that support food-taking behaviors.

In their new paper, Sharpe *et al.* [1] offer a new perspective on the function of the lateral hypothalamus. The authors speculated that feeding-relevant GABAergic neurons in the lateral hypothalamus may serve as more than simple actuators of feeding behavior: instead, they hypothesized that these cells play an active role in the complex processes by which an organism learns about stimuli in the environment that predict the availability of food. To test their hypothesis, the authors first used bacterial artificial chromosome (BAC) technology to generate a new line of genetically modified rats in which Cre recombinase is expressed under the control of the promoter for the glutamate decarboxylase 1 gene (*Gad1*-Cre rats). In these rats, the recombinase protein Cre is expressed with high fidelity in GABAergic neurons in the lateral hypothalamus, but is not detected in surrounding orexin or melanin-concentrating hormone (MCH) neurons. This new line of *Gad1*-Cre rats enabled the authors to manipulate the activity of GABAergic neurons in the lateral hypothalamus and explore their contribution to food-related new learning.

Next, Sharpe *et al.* [1] designed a behavioral task in which *Gad1*-Cre rats learned to discriminate between two