hunting of birds such as the yellow-breasted bunting (*Emberiza aureola*; Fig. 1), which is a heavily targeted species.

The illegally hunted species included 25% of the 363 species of terrestrial vertebrates in China that are threatened with extinction (as assessed by the IUCN Red List) and more than 20% of vertebrates in the IUCN's near-threatened category. For mammals and reptiles, the percentage of threatened and near-threatened species in trade was substantially higher than the percentage of traded species that are not of conservation concern (IUCN least concern category), underscoring the observation that illegal hunting can be a driver of extinction risk[1,2].

Although the number of species affected are important, so too is understanding how the evolutionary tree is affected by illegal hunting of wildlife. By constructing evolutionary trees for each taxon that included all Chinese species and then marking these phylogenies with the species lineages associated with at least one court verdict, the authors revealed two key points. First, trade in China is widely distributed across the tree of life, with 80% of the nation's 45 orders of taxa affected. Second, there was a strong phylogenetic signal of illegal hunting especially in mammals and reptiles, indicating that hunting was particularly focused on certain families.

It is widely acknowledged that not all illegal hunting activity will be detected and result in arrest and conviction, owing to the difficulty in detecting crimes and identifying the species involved. Indeed, the authors point to field evidence that detected crimes might represent less than 1% of all such incidents. To quantify the degree of error that undersampling of the true number of crimes generates in the estimated number of species and the identity of those species, the authors applied two approaches that are more commonly used in research that quantifies the effects of land-use change on biodiversity — a scenario in which undersampling is also probable because of the sheer spatial scale of deforestation and degradation.

First, Liang and colleagues used the method of sample-based extrapolation to project how the 'accumulation curve' of species that were illegally hunted, based on the number of court verdicts, would change if more court verdicts had been made. The expectation is that a rise in the number of verdicts (increased sampling efficiency) would increase the number of species detected. Assuming that 10% of incidents result in conviction, the authors estimate that at least 866 vertebrate species (28% of China's vertebrate species) were hunted illegally during the six-year study period, pointing to 193 overlooked species. These 866 species include 39% of China's birds, 22% of mammals and 19% of reptiles.

Second, given the strong phylogenetic signal in illegal hunting, Liang and colleagues used the method of phylogenetic logistic regression to predict ecological traits that correlate with whether a species is hunted or not. Among other findings, they discovered that species with larger distributions and body mass tended to be more affected by illegal hunting, which is a conclusion supported by previous global-scale assessments[6,9]. The authors' approach of using a best-fitting model of key ecological traits and phylogenetic relatedness identified that 781 species were not mentioned in court verdicts but have a high likelihood of being hunted. These include 90 species classified by the IUCN as being globally threatened.

Taken together, this evidence indicates that it is highly probable that a substantial number of illegally hunted species are yet to come under the attention of law enforcement. Liang and colleagues' list of species with a high likelihood of being hunted is a crucial conservation resource because it can guide authorities towards monitoring these potentially overlooked species. So too is the fact that only 5% of convictions accounted for 90% of the individual animals taken — focused enforcement and strict penalties for the most egregious perpetrators could help to reduce the volume and diversity of illegally hunted species for commerce.

Moving forwards, I predict that the analysis of hunting-related convictions and the use of underpinning modelling approaches, as demonstrated by Liang and colleagues, will provide the blueprint from which to quantify the severity of detected and overlooked hunting crimes in other hotspots of national-level trade. Thereafter, as the authors affirm, we must quantify the degree to which illegal hunting reduces vertebrate populations — of both imperilled species and those considered to be (formerly) common. Then we should examine how the consequences of this affects aspects of ecosystem function, such as crop pollination (especially by bats), pest predation and maintenance of forest carbon stocks through seed dispersal.

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1. Benítez-López, A. *et al*. *Science* **356**, 180–183 (2017).
2. Morton, O., Scheffers, B. R., Haugaasen, T. & Edwards, D. P. *Nature Ecol. Evol.* **5**, 540–548 (2021).
3. Harfoot, M. *et al*. *Biol. Conserv*. **223**, 47–57 (2018).
4. Liang, D. *et al*. *Nature* **623**, 100–105 (2023).
5. Fan, P.-F., Yang, L., Liu, Y. & Lee, T. M. *Nature Ecol. Evol.* **4**, 1162–1167 (2020).
6. Scheffers, B. R., Oliveira, B., Lamb, I. & Edwards, D. P. *Science* **366**, 71–76 (2019).
7. Marshall, B. M., Strine, C. & Hughes, A. C. *Nature Commun.* **11**, 4738 (2020).
8. Hughes, L. J. *et al*. *Nature* **620**, 351–357 (2023).
9. Hughes, L. J., Morton, O., Scheffers, B. R. & Edwards, D. P. *Biol. Rev.* **98**, 775–791 (2023).

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Genetics

Master regulator of a mosquito X chromosome

Maggie P. Lauria Sneideman & Victoria H. Meller

In organisms with X and Y chromosomes, gene expression must be equalized between the sexes. A protein that causes upregulation of gene expression of the X chromosome in male mosquitoes has been discovered. **See p.175**

In organisms that have X and Y sex chromosomes, females have two X chromosomes but males have only one. The first and most essential aspect of sexual development in such organisms is the adjustment of X-chromosome gene expression — a process called dosage compensation — to ensure similar expression levels in both sexes. Dosage compensation is remarkable not only because it modulates hundreds of genes, of which the only common feature is residence on the X chromosome, but also because of the diversity of mechanisms used. On page 175, Kalita *et al*.[1] identify a key regulator of dosage compensation in the malaria-carrying mosquito *Anopheles gambiae*.

Sex chromosomes evolve rapidly, and dosage compensation has arisen independently in each lineage of organisms[2]. Only a few examples of compensation are well understood[3]: mammalian females inactivate expression of one of their two X chromosomes; hermaphrodites of the roundworm *Caenorhabditis elegans* downregulate expression of both of their two X chromosomes; and males of the fruit fly *Drosophila melanogaster* double the amount of expression from their single X chromosome.

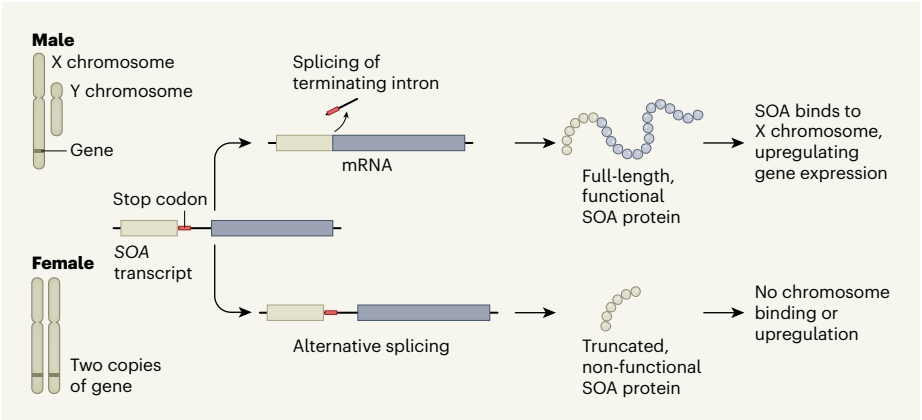

Figure 1 | Sex-specific alternative splicing in *Anopheles gambiae* regulates dosage compensation. Male *Anopheles gambiae* mosquitoes have one X chromosome, whereas females have two. The expression of X-chromosome genes is upregulated in males – a phenomenon known as dosage compensation – to ensure that both sexes express these genes equally. Kalita *et al.*[1] identify the *SOA* gene as a regulator of X-chromosome gene expression in *A. gambiae*. **a**, In males, the RNA transcript of *SOA* is processed (spliced) to remove a terminating intron – a non-coding region that includes a short sequence (a stop codon) that terminates protein synthesis. The resulting messenger RNA produces a full-length SOA protein that binds to the male X chromosome and upregulates gene expression. **b**, In females, alternative splicing produces an mRNA that retains the terminating intron. The resulting mRNA produces a truncated SOA protein that does not bind to the X chromosome or upregulate gene expression.

In each case, machinery that modifies chromatin – the nuclear complex composed of DNA and histone proteins – assembles on the X chromosome of the appropriate sex.

Like *D. melanogaster*, males of *A. gambiae* increase expression from their single X chromosome[4]. Kalita and colleagues sequenced RNA from male and female *A. gambiae* embryos of different ages to pinpoint the onset of compensation. They observed that RNA output from the male X chromosome is half of that of the two female X chromosomes 3.5 hours after egg laying, but equals that of females by 9 hours.

They then searched for genes that are expressed more in males than in females after 3.5 hours and, surprisingly, found only two. The first was *Yob*, a gene on the Y chromosome that induces male differentiation by blocking the activity of the femaleless (fle) protein; fle promotes female differentiation, prevents dosage compensation and is present in both sexes[5].The second was a previously uncharacterized gene that arose through gene duplication in the *Anopheles* lineage. Kalita *et al.* named this gene *sex chromosome activation* (*SOA*).

The authors found that the messenger RNA for *SOA* accumulates preferentially in males, but is present in both sexes. However, the mRNA in females retains a non-coding sequence (an intron) that is lost from the males' mRNA and contains a stop codon – a short sequence that terminates protein synthesis (Fig. 1). The production of different mRNA molecules from the same transcript is known as alternative splicing. In this case, the SOA protein produced from the females' mRNA is shorter than that produced by males and is probably non-functional.

Kalita and colleagues found that not only is *SOA* evolutionarily conserved in the *Anopheles* lineage, but so is female retention of the terminating intron. Gene regulation by alternative splicing is reminiscent of the sex-determination cascade in *D. melanogaster*[6], in which the *transformer 2* (*tra2*) gene regulates sexual differentiation and courtship behaviour through splicing of the evolutionarily conserved genes *doublesex* (*dsx*) and *fruitless* (*fru*). Interestingly, *fle* regulates splicing of *dsx* and *fru* in female mosquitoes and is related to the fly gene *tra2* (ref. 5). In flies, *tra2* is necessary for female sexual differentiation but dosage compensation is regulated by the upstream factor Sxl (ref. 3).

The SOA protein binds non-specifically to DNA *in vitro*, but the full-length version produced in males localizes to active genes on the X chromosome. When the authors generated genetically engineered male mosquitoes that lack *SOA*, they observed reduced expression of genes on the X chromosome compared with wild-type males. Although the mutant males were alive and fertile, they emerged several hours later than wild-type males. Conversely, when the authors engineered females that express full-length SOA, they observed SOA binding to the X chromosomes and increased X-chromosome expression compared with that in wild-type females. Taken together, these findings identify SOA as the first known component of the dosage-compensation machinery in *A. gambiae*.

How SOA activates gene expression remains unknown. In male *D. melanogaster*, an activating chromatin modification, known as H4K16ac, is enriched on the upregulated X chromosome[7]. This same mark is depleted on the inactive or repressed X chromosomes of mammals and *C. elegans*. Enrichment of H4K16ac has not been detected on the *A. gambiae* male X chromosome[8], suggesting that a different gene-regulation mechanism operates in this organism. Elucidation of the mode of action of SOA will expand the list of gene-regulation mechanisms used for dosage compensation.

Loss of dosage compensation in female mammals and *D. melanogaster* males is lethal[9]. It is therefore surprising that the loss of *SOA* in *A. gambiae* merely delays the emergence of adult males. Several factors might account for this. For example, the X chromosome in *Anopheles* is small, and thus only a limited number of genes are affected. Moreover, the loss of *SOA* reduces expression from the male X chromosome much less than would be expected from a complete loss of compensation – indicating that *Anopheles* males might use multiple compensation systems. Intriguingly, loss of *fle* is lethal in female *Anopheles* and produces a bigger increase in expression from the X chromosomes than does expression of full length SOA, suggesting that *fle* blocks more than just the SOA pathway[5]. Consistent with these ideas, compensation in *D. melanogaster* males is thought to require multiple regulatory systems[10].

A. gambiae provides a fascinating new model for studying sex determination and dosage compensation. Importantly, biological methods for controlling mosquitoes – such as releasing sterile males – require the production of single-sex populations. Mis-expression of *SOA* itself is not lethal in either sex, but the terminating *SOA* intron could be used to engineer mosquitoes in which the expression of certain genes sterilizes or kills one sex. The evolutionary conservation of sex-specific *SOA* splicing in the *Anopheles* lineage suggests that a malaria-control strategy based on this switch would be broadly useful in this genus.

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1. Kalita, A. I. *et al. Nature* **623**, 175–182 (2023).
2. Charlesworth, B. *Curr. Biol.* **6**, 149–162 (1996).
3. Lucchesi, J. C., Kelly, W. G. & Panning, B. *Annu. Rev. Genet.* **39**, 615–651 (2005).
4. Rose, G. *et al. Genome Biol. Evol.* **8**, 411–425 (2016).
5. Krzywinska, E. *et al. Curr. Biol.* **31**, 1084–1091 (2021).
6. Baker, B. S. *Nature* **340**, 521–524 (1989).
7. Wells, M. B., Csankovszki, G. & Custer, L. M. *Genet. Res. Int.* **2012**, 795069 (2012).
8. Keller Valsecchi, C. I., Marois, E., Basilicata, M. F., Georgiev, P. & Akhtar, A. *Life Sci. Alliance* **4**, e202000996 (2021).
9. Ercan, S. *J. Genomics* **3**, 1–19 (2015).
10. Chen, Z.-X. & Oliver, B. *G3 Genes Genomes Genet.* **5**, 1057–1063 (2015).

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