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Comment and Opinion

Hiding in plain sight: the Y chromosome and its reinvigorated role in evolutionary processes

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Abstract

Recent methodological approaches have expanded our understanding of Y chromosome sequence, revealed unexpected Y diversity, and sparked a growing realization of its importance in evolutionary processes. To fully understand the diversity and importance of the Y chromosome, we suggest the need to move from a holotype Y chromosome sequence, based on a single individual and meant to represent the species, to a thorough understanding of Y chromosome haplotype diversity, its phenotypic implications, and its phylogeographic distribution. Additionally, the Y chromosome may play an important role in two key rules of speciation that have otherwise been attributed to the X, namely Haldane's Rule and the Large-X Effect. Emerging genomic tools and analytical approaches are just now giving us the means to ask how important this small, often forgotten region of the genome is in evolutionary processes.

Keywords: evolutionary genomics, speciation, sex chromosome

Lay Summary

Recent advances in sequencing and GWAS methods have led to a growing realization that Y chromosomes are unexpectedly diverse and are important in the genetic architecture of male phenotypic variation. We outline several new approaches that are useful in characterizing the Y chromosome. We also highlight several questions about the Y itself and its role in evolution that these methods now make possible.

The Y as it relates to the X

There has been much discussion about the phenotypic role of Y chromosomes ever since Fisher suggested that they would accumulate genes important for male traits (Fisher, 1931). However, despite their theoretical role in resolving sexual conflict by offering a male-specific genomic region, Y chromosomes remained enigmatic, full of contradictions, and, until very recently, often largely ignored in modern genomic analysis (Sun et al., 2023). Efforts to fully sequence Y chromosomes, based on laborious chromosome-walking approaches, were time and cost-prohibitive, ensuring they were applied to only a handful of species (e.g., Skaletsky et al., 2003; Soh et al., 2014). The effort and cost required also usually meant that only one individual of those species was sequenced as a representative holotype Y, preventing broader population comparisons. Full sequences of female-specific W chromosomes have also been limited (e.g., Bellott et al., 2017), and although, for simplicity, we focus here on Y chromosomes, many of the same theoretical predictions and limitations apply conversely to W chromosomes.

Given these difficulties, much molecular work on the Y focused on its relationship to the X, specifically the region of the X and Y that are homologous. Once the X and Y chromosomes stop recombining with each other, outside of the pseudo-autosomal region, homologous recombination otherwise ceases for the Y, although the Y-specific region may recombine nonhomologously with itself on rare occasions, leading to gene conversion and copy number variation within ampliconic regions (Lange et al., 2009).

Because the nonrecombining Y-specific region segregates as a single haplotype, it experiences a sharp reduction in the power and efficacy of natural selection compared to recombining portions of the genome, as selection can only act on the net fitness effect of the entire haplotype rather than each constituent variant within it (Rice, 1987). As a result, Y chromosomes are expected to accumulate more deleterious mutations and to incorporate fewer beneficial mutations than the remainder of the genome, and many genes on the Y that are homologous with the X quickly lose gene activity and protein structure once recombination has ceased (Liu et al., 2004; Hughes et al., 2010; Papadopulos et al., 2015; Liu et al., 2004; Papadopulos et al., 2015). The image of the Y that emerged from sequence comparisons to the X chromosome was one of gene loss and decay (Bachtrog, 2013), and it is perhaps not surprising that as Genome Wide Association Studies (GWAS) gained traction, Y-linked genetic variation was rarely explicitly examined (Sun et al., 2023).

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Several recent methodological approaches have expanded our understanding of the Y chromosome sequence, its diversity and population-level patterns and led to a growing realization about its importance in evolutionary processes. Chromosome Quotient (CQ) comparisons between male and female genomes, originally developed to identify Y sequence (Hall et al., 2013), have been adapted to identify X chromosomes as well (Darolti et al., 2019; Vicoso & Bachtrog, 2015). CQ methods, combined with approaches based on Male:Female SNP differences or F_{ST} (M:F F_{ST}), rapidly expanded the study of sex chromosomes to a more diverse range of taxa. Both of these methods are based on a female (XX) reference genome and compare short-read sequence data collected from each sex to identify chromosomal regions with elevated SNP density (F_{ST}) and/or reduced coverage (CQ) in males compared to females, indicating Y divergence or degeneration respectively (Figure 1A) and delineating the X-specific region. This approach has been remarkably useful in identifying the sex chromosomes where they were not previously known and revealed both patterns of deep conservation of a single sex chromosome system in some clades (Li et al., 2024; Toups & Vicoso, 2023; Wu et al., 2024), but also clades with curiously rapid sex chromosome turnover (El Taher et al., 2021; Vicoso & Bachtrog, 2015).

The Y free from the X

M:F F_{ST} and CQ approaches are effective methods to identify autosomal regions that have homology to the Y and are not present on the X (Figure 1B), and the results have revealed unexpected levels of sequence similarity between autosomal regions and the Y chromosome (Bissegger et al., 2019; Kasimatis et al., 2020), much of it likely representing gene duplication from the autosomes to the Y (Lin et al., 2022; Tobler et al., 2017; van der Bijl et al., 2023). Although gene gain has been known on the *Drosophila* Y for some time (Koerich et al., 2008; Skateletsky et al., 2003), the extent of duplication to younger Y chromosomes has been surprising and suggests that the Y chromosome may indeed act as a hotspot for genes important for male phenotypes, just as predicted (Fisher, 1931).

Additionally, haplotype-phased genome assemblies, enabled initially by linked short-read sequencing (e.g., Almeida et al., 2021) and more recently through progress in long-read sequencing (e.g., Sacchi et al., 2024), have greatly improved our understanding of the Y itself, regardless of its relationship to the X. Importantly, these efforts have revealed far greater diversity than expected (Hallast et al., 2023; Kaufmann et al., 2023; Sandkam et al, 2021) in structure and genetic content of the Y chromosome.

Although few GWAS have even entertained the possibility of Y-linked variation contributing to phenotypic variation (Sun et al., 2023), those that looked for it have found ample evidence (McKinney et al., 2020; van der Bijl et al., 2023) and the Y chromosome has been found to affect a wide array of reproductive and nonreproductive traits (Cirulis et al., 2022). These limited reports suggest that much of the haplotype diversity observed on the Y chromosome has functional phenotypic consequences.

Y chromosome holotypes to haplotype diversity

The realization of extensive Y haplotype diversity and its potential role in male phenotypic variation raises a host of new questions about the Y chromosome itself and its role in broader evolutionary processes. To fully address those, we need to move from a single holotype Y chromosome sequence to a thorough understanding of Y chromosome haplotype diversity, its phenotypic implications, and its phylogeographic distribution. Expanding pangenomes to include Y chromosome variation would be helpful. More easily, simply recording the sex of sequenced samples would go a long way toward repurposing publicly released data for studying the Y.

GWAS could easily be designed to explicitly test whether the Y chromosome contributes to phenotypic variation. This is particularly true for GWAS based on sequence data, which can be used to directly determine read depth differences consistent with Y regions (Figure 1), compared to SNP chips, where estimates of signal intensity are less sensitive in estimating dose differences. K-mer-based GWAS approaches (Rahman et al., 2018) do not require a reference genome, and this approach can help identify functional variation, Y-linked or not, that might not be present in the reference genome (Figure 2). K-mers associated with traits in males but not females could be due to sex differences in autosomal or X genetic architecture (Khramtsova et al., 2019; van der Bijl & Mank, 2021), in which case those k-mers will be present in both male and female genomes, but only statistically associated with the phenotype of interest in males. Alternatively, significant male k-mers not present in the female genome likely represent Y-specific sequence (Y-mers), suggesting a role of the Y in the underlying genetic architecture.

Even if using traditional, reference-based GWAS, simply examining significantly associated SNPs for sex differences in coverage and frequency can reveal the potential role of the Y (Fig 1B). Given the evidence of gene duplication from the autosomes to the Y chromosome (Lin et al., 2022; Tobler et al., 2017), many SNPs associated with phenotypes that map to the autosomes may in fact be Y-linked.

Identifying the potential role of the Y in GWAS is an important first step. However, it remains difficult to determine the precise genetic variant and mechanism by which the Y affects phenotypic variation due to the nonrecombining nature of the chromosome. In this way, Y chromosomes are similar to some supergenes, regions of tightly linked loci, often within an inversion, where recombination is suppressed or even eliminated with the ancestral haplotype (Schwander et al, 2014). In some systems, like fire ants (Wang et al., 2014) and ruffs (Küpper et al., 2016), the alternate haplotype in only present in heterozygotes and so never recombines. In these systems, as with Y chromosomes, the entire haplotype will be associated statistically with phenotypic variation, and it remains challenging to determine the exact location that encodes the phenotype of interest.

Origin and maintenance of Y chromosome diversity

Moving from Y chromosome holotypes to a deeper understanding of Y chromosome haplotype diversity and its functional phenotypic role will make it possible to answer several key questions about the evolution of the Y itself. First and foremost, we have only the vaguest understanding of the rate and mechanism of Y chromosome gene acquisition and the extent to which genes gained by the Y persist and remain functional (Hughes et al., 2012).

Because the nonrecombining Y-specific region segregates as a single haplotype, understanding the haplotype diversity will be needed to fully understand the unexpected extent of Y chromosome diversity that has been recently observed

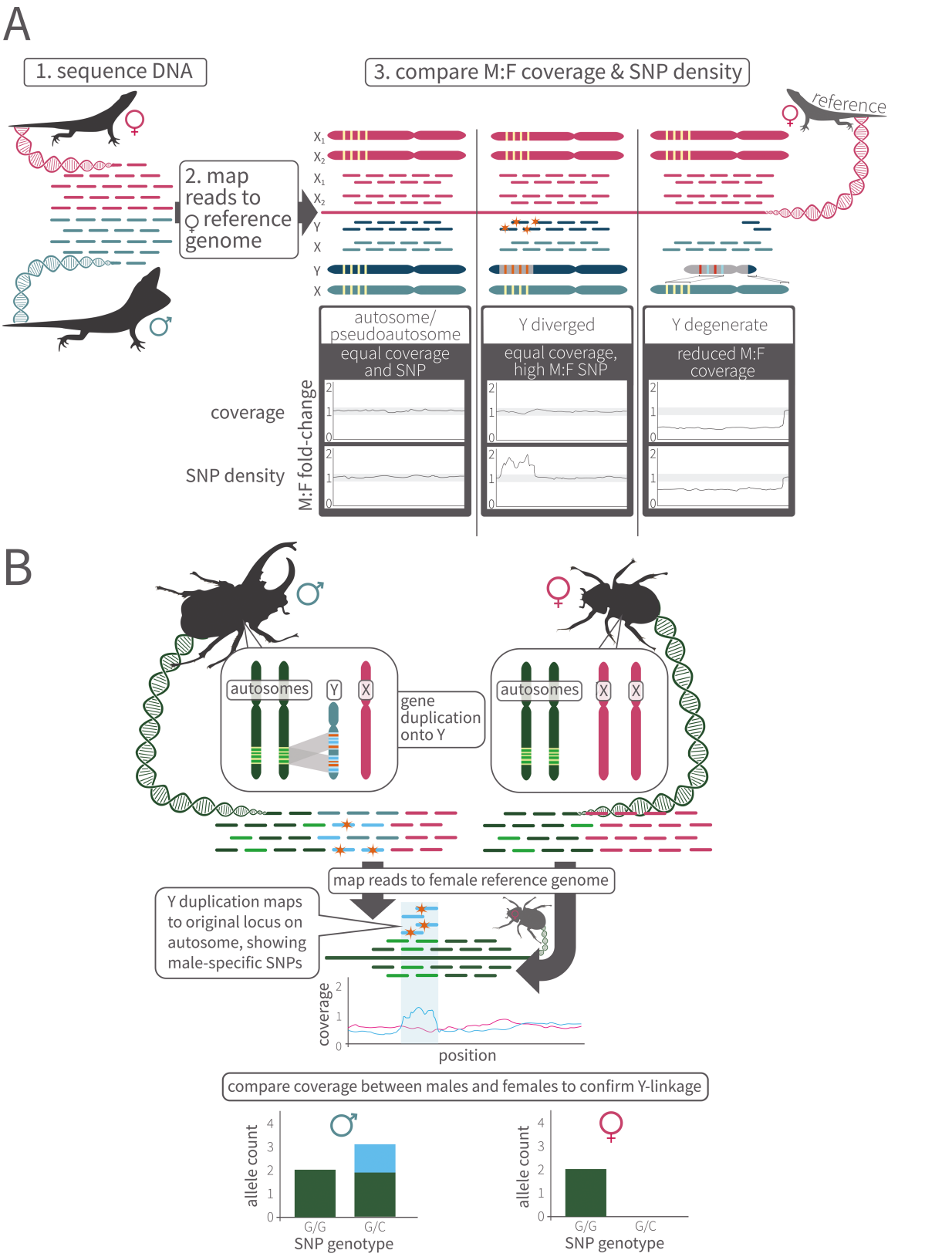

Figure 1. Understanding the Y in relation to the X. (A) The Y chromosome can be identified and characterized by sequencing males and females (1) and mapping the sequence reads to a female reference containing an X assembly (2). Differences in sequencing coverage (CQ) and SNPs density (F_{ST}) between male and female genomes can be used to identify different degrees of divergence between the X and Y chromosome (3). In these comparisons, a confidence interval (CI), typically based on the autosomal regions, is helpful (shown with gray band). Autosomes and pseudo-autosomal regions where the sex chromosomes are not diverged will generally fall within the CI, as they lack male:female differences in coverage or SNP density (left panel). Genomic regions where the Y has begun to diverge but retains strong similarity to the X exhibit similar coverage in both sexes but elevated SNP density in males, as Y reads still map to the X but contain male-specific SNPs (middle panel). Where the Y has diverged more substantially from the X or degenerated, few Y reads will map to the X, reducing CQ in males to nearly ½ compared to females. However, in these

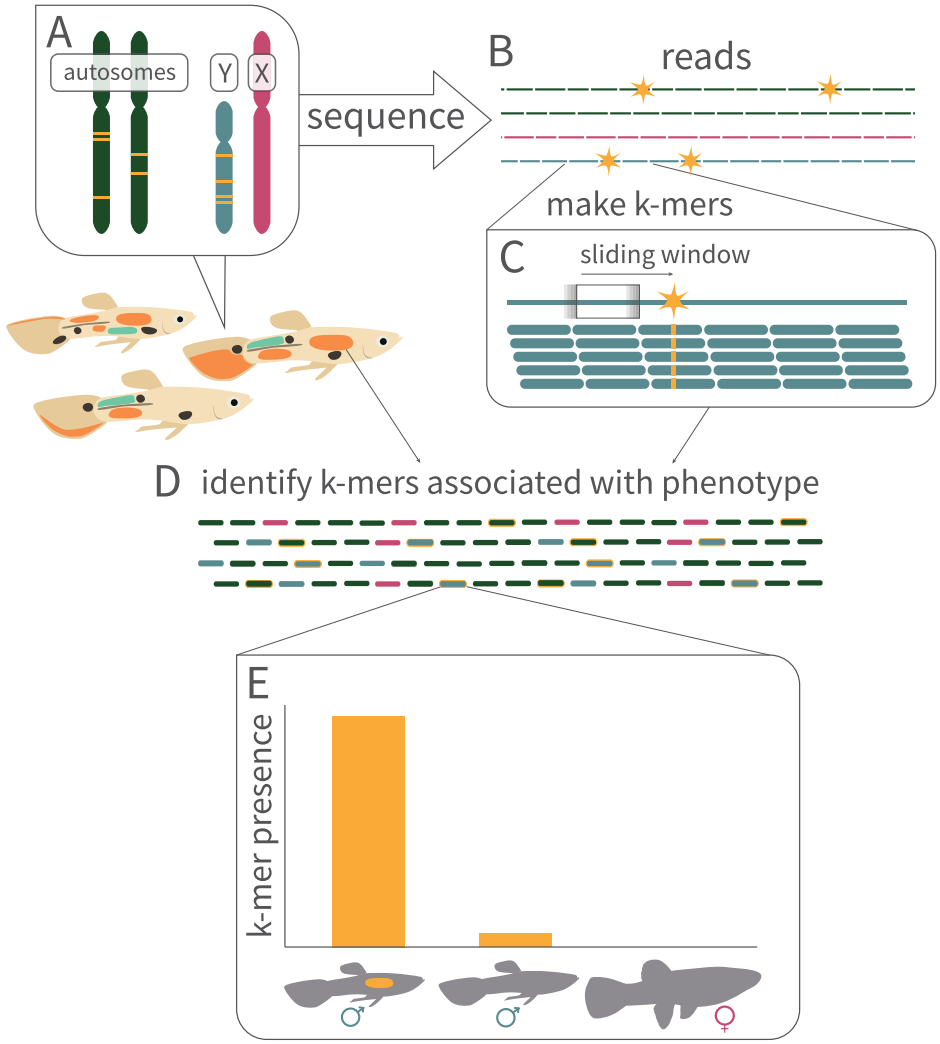

Figure 2. Discovery of functional variation on the Y using k-mer GWAS. Phenotypic traits, such as the guppy color ornaments shown here, maybe (partially) under the control of Y-linked genetic variation (yellow bars on chromosomes, A). K-mer GWAS starts with sequencing multiple individuals that vary for the trait of interest (B). The reads are then broken up into k-mers—short overlapping subsequences of the reads, usually 20–40 bp in length (C). Any genetic variants (yellow stars), such as SNPs, structural variants, or indels, create k-mers that are only present in the individuals carrying that variant. The association between k-mers and the trait of interest is statically tested (outlined in yellow, D). As in conventional GWAS, this test is repeated for each k-mer while controlling for population structure and multiple testing. This approach will discover variants throughout the genome, but Y-linkage can be inferred for k-mers which are present in males and absent in all females (bar chart, E). As this approach does not involve mapping reads to a reference genome, it can discover Y-linked functional variation in the absence of a Y-reference or discover variation absent from a single Y holotype.

(Almeida et al., 2021; Hallast et al., 2023; Kaufmann et al., 2023; Sandkam et al., 2021). It is clear that Y haplotypes within species differ far more in gene content, copy number, and structure than the autosomal and X-linked regions of the genome that still recombine. This diversity presents an evolutionary conundrum: nonrecombining regions are expected to lose genetic diversity (Ellegren & Galtier, 2016), as selection, positive or purifying, on any one site affects results in a selective sweep of the entire linked haplotype (Bachtrog, 2013; Singh et al., 2014; Wright et al., 2014). Although single-copy genes on the Y do indeed show reduced genetic diversity compared to other regions of the genome (Makova et al., 2024), in other ways, such as copy number variation and structural variation (Hallast et al., 2023), the nonrecombining Y is more diverse than the regions of the genome that

regions, male hemizygosity will reduce overall SNP density compared to females (right panel). (B) Duplications from the autosome to the Y can also be detected by mapping male and female reads to a female reference genome. Reads from the duplicated region on the Y will map to the homologous autosomal region. For genes present in a single copy on the autosomes and a single copy on the Y, males have 1.5x the coverage of females in that region, as they possess two autosomal copies and one Y copy. Genes with multiple Y copies will have increased M:F coverage. Y-linkage can be further confirmed by looking for apparent male-specific SNPs in the region. These are not true polymorphisms but rather represent fixed differences between the autosomal and Y gene copies so that all females are homozygous for the reference, while males carrying the duplication will appear heterozygous. These "heterozygous" males will have double the read depth for the reference (autosomal, green) allele compared to the alternative (Y chromosome, blue) allele. The alternative homozygous genotype does not exist, as this would represent an individual lacking the autosomal copy of the gene.

retain recombination. In the few cases where it has been examined, this variation has known functional consequences (e.g., Kaufmann et al., 2023; Zhou et al., 2012).

Genetic diversity is strongly influenced by selection, and it may be that selection against translocations, copy number variation, or structural rearrangement is very low compared to the remainder of the genome (Chang et al., 2022), perhaps because the Y-specific region is free of the selective constraints on gene order associated with recombination. This diversity may be ephemeral, generated rapidly and lost through neutral or selective processes just as rapidly. Alternatively, Y diversity may be geographically structured (Almeida et al., 2021; Larracuente & Clark, 2013), or other selective processes may be acting to retain Y diversity, such as balancing selection. It is entirely possible, even probable, that different species will have different Y chromosome dynamics depending on mating system and/or genome biology. Now that we have a toolkit to analyze the Y chromosome separately from the X, there are a host of fascinating questions just waiting to be asked.

The Y chromosome as driver of diversity

Studying Y chromosome diversity also offers a unique perspective on many fundamental evolutionary processes.

Two key rules of speciation may in fact have a major Y component. Haldane's Rule, the observation that the heterogametic sex more often tends to be sterile inviable than the homogametic sex in F1 hybrids (Haldane, 1922), is often conceptualized based on the interaction between the hemizygous X chromosome and the remainder of the genome in males (Turelli & Orr, 1995), and the X no doubt plays some role in it. Rarely is the Y considered in Haldane's Rule (Delph & Demuth, 2016), even though the interactions between a hemizygous sex chromosome and the autosomes apply just as well to the Y, and the Y is known in some species to carry genes critical to male fertility (Subrini & Turner, 2021; Zhang et al., 2020). Curiously, if the Y plays an important role in Haldane's Rule, we would expect that X0 species would be less often affected than XY species (e.g., Moran et al., 2017) and that XY species with greater functional Y genetic material would show the greatest effect of all.

Similarly, just as there is a Large-X Effect in speciation, where the X chromosome plays a disproportionately large role in hybrid dysfunction (Jiggins et al., 2001), we might similarly expect a large Y effect (Filatov, 2018; Johnson et al., 1993; Lamnissou et al., 1996). We might also expect the Y chromosome to less often cross species boundaries if it has negative phenotypic consequences in F1 hybrids.

These are just a few questions that we can now address with new approaches that let us understand the genetic variation of the Y and its functional consequences (Figures 1 and 2). It may be that the Y is largely inert, as often assumed, or it may be that this small, often forgotten region of the genome plays a far larger role in evolutionary processes than we would expect based on its size. These new tools now give us the means to ask.

Data and code availability

There is no data to be archived.

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