Origin and evolution of Y chromosomes: *Drosophila* tales

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Classically, Y chromosomes are thought to originate from X chromosomes through a process of degeneration and gene loss. Now, the availability of 12 *Drosophila* genomes provides an opportunity to study the origin and evolution of Y chromosomes in an informative phylogenetic context. Surprisingly, the majority of *Drosophila* Y-linked genes are recent acquisitions from autosomes and Y chromosome gene gains are more frequent than gene losses. Moreover, the *Drosophila pseudoobscura* Y chromosome lacks homology with the Y of most *Drosophila* species. Thus, the *Drosophila* Y has a different evolutionary history from canonical Y chromosomes (such as the mammalian Y) and it also might have a different origin.

Sex-chromosome origins

Sex-chromosome evolution provides a particularly coherent and satisfying blend of empirical data and theory. According to canonical theory, X and Y chromosomes (for the sake of simplicity we refer to the ‘W’ chromosome of birds and butterflies as Y [1]) originate from an autosomal pair via a three-step process that begins with the acquisition of one or more strong sex-determining genes by one autosome, giving rise to nascent X and Y chromosomes. Natural selection then favors the suppression of recombination between the two chromosomes. The lack of recombination, together with the joint effects of mutation, natural selection and genetic drift, then leads to progressive degeneration and loss of Y chromosome genes until only the sex-determining gene(s) and a few relic genes survive [1–5]. As the X chromosome become progressively haploid in males (hemizygous), natural selection favors increased transcription of X-linked genes in males through several dosage-compensation mechanisms [1,2]. In the later stages, the Y usually becomes heterochromatic, accumulating large amounts of repetitive DNA. It also frequently acquires male-specific genes from the autosomes [6,7] (or female-specific genes in the case of the W chromosome, where ZW is female and ZZ is male). Empirical data in a variety of organisms including plants and birds support this scenario, the evidence being particularly clear in mammals. The most compelling evidence is provided by the observation that, among the 27 different proteins encoded by the human Y, 18 have an ancestral, close counterpart on the X chromosome [6]. This generality, coupled with the beautiful fit between theory and data, has led to the widespread assumption that all Y chromosomes arise through this path (or by the related neo-Y pathway; Box 1), invariably being homologous to X chromosomes.

Before 2000, an extensive identification of Y-linked genes had been performed only for humans [7]. The genome sequencing of *Drosophila melanogaster* in 2000, followed by *Drosophila pseudoobscura* (2003) and now by ten additional *Drosophila* genomes (2007), uncovered several unexpected phenomena, including the lack of X–Y shared genes, a wholesale replacement of the Y chromosome in the *D. pseudoobscura* lineage, and the preponderance of gene gains (compared with gene losses) in the 12 sequenced *Drosophila* species. Here, we review these data and explore their consequences in the broader context of Diptera sex-chromosome evolution. We concentrate on single-copy genes on the *Drosophila* Y, with an emphasis on results [8,9] appearing since the last review on this subject [10]. Readers interested in the role of repetitive DNA in the evolution of the *Drosophila* Y chromosome and on evolutionary models of Y-degeneration should consult Refs [4,11–13].

*D. melanogaster* Y chromosome: a lack of X–Y homology

The *D. melanogaster* Y chromosome comprises mainly repetitive DNA and is heterochromatic. It does not determine sex; instead, sex determination in *Drosophila* is basically accomplished by a count of the number of X chromosomes [14,15]. However, males devoid of Y chromosomes (‘X0 males’) are sterile [16] and formal genetic studies identified six genes that are essential for male fertility in the *D. melanogaster* Y [17–20]. Now, genome sequencing and the development of proper bioinformatics methods enables a thorough molecular identification of the Y chromosome gene content [21–23]. Despite its large size (~40 Mbp), it contains few single-copy protein-coding genes: 12 are currently known and indirect evidence suggests an upper bound of ~20 genes [21–24]. These genes are unusually large, owing to Mbp-sized introns comprising repetitive DNA [25]. *D. melanogaster* Y-linked genes have two additional important features; many (and probably all) have male-related functions (e.g. encoding sperm flagella motor proteins) and all arose by duplication from autosomal genes [9,21–24]. The initial step of their origin must have been a gene duplication that created a mutant Y carrying a copy of an autosomal gene. Then, in some cases, the mutant Y became fixed in the population, either by genetic drift or because it conferred a selective advantage.
D. melanogaster genes are autosomal in mosquitoes. Thus, the gene content of the autosomal part of the sex chromosome and high conservation in the other chromosomes, would produce the present pattern of low conservation of gene content in the Y chromosome and high conservation in the other chromosomes. This pattern dates back to mosquitoes, which diverged from Drosophila ~260 Mya; although there are clear homologies in other chromosomes between these Diptera [30,31], all orthologs of D. melanogaster Y-linked genes are autosomal in mosquitoes. Thus, the gene content of the Drosophila Y is younger than the other chromosomes.

Comparisons between gene gains and losses also yield interesting clues about the evolution of the Drosophila Y:
the rate of gene gain is ~11 times higher than the rate of gene loss ($P = 0.003$; 95% confidence interval: 2.3–52.5; [9]).

Thus, the Drosophila Y shows another unusual pattern (for a Y-chromosome): gene gains have a prominent role in its evolution; indeed, its gene content has increased in the last 63 Myr. This finding also suggests that the Drosophila Y is young because it is gaining genes and yet has few of them.

The D. pseudoobscura case: wholesale replacement of the Y chromosome

Given the phylogenetic proximity between D. melanogaster and D. pseudoobscura (they belong to the sister groups melanogaster and obscura), we expected their Y chromosomes to be very similar. Instead, they have nothing in common! The ancestral Drosophila Y chromosome became part of an autosome in the D. pseudoobscura lineage and was replaced by a new Y chromosome, possibly derived from a neo-Y [8]. This new Y chromosome is essential for male fertility [32], so it presumably acquired one or more fertility genes from other chromosomes, by the process outlined earlier for D. melanogaster (alternatively, some mechanical problem in meiosis might cause sterility in X0 males). We identified 15 genes and pseudogenes in the D. pseudoobscura Y chromosome and none are shared with the D. melanogaster Y [8]. Hence, despite their functional and cytogenetic similarities (both are required for male fertility, pair with the X, and are heterochromatic), these Y chromosomes are not homologous. This unexpected finding has obvious implications for the origin and evolution of Y chromosomes and raises the question of why it happened. A possible explanation follows. The incorporation of the ancestral Y into an autosome occurred only in species such as D. pseudoobscura and D. affinis which also have a known X–autosome fusion, whereas the more distantly related D. bifasciata and D. guanche (which do not have the X–autosome fusion) still carry the ancestral Y [8].

Figure 1. Origins of Y chromosomes. (a) Y-linked genes in (i) D. melanogaster and (ii) humans. Genes ancestrally shared with the X are shown in red and those acquired from autosomes are shown in blue. Genes with unknown origin or that are later additions to both the X and the Y are grouped in the 'other' class (green). Genes on the human Y chromosome encode 27 different proteins. The majority of these genes are ancestrally shared with the X chromosome, indicating that these chromosomes are homologous [6,7]. This class of genes is absent among the 12 known single-copy genes in the D. melanogaster Y chromosome, suggesting that X and Y are not homologous [9]. Several human Y-linked genes are multi-copy; we counted them only once. (b) Main paths for the origin of Y chromosomes. Only male karyotypes are shown. (i) The canonical path begins when an autosome acquires a strong male determining gene M, becoming a nascent Y (its homolog became a nascent X). Degeneration of the nascent Y and the evolution of dosage compensation in the X originate mature sex chromosomes [1–5]. (ii) The neo-Y path (shown here in a species with X0/XX sex-determination) begins with an X–autosome fusion, transforming the fused autosome into a neo-X. The free homolog became a neo-Y, which then degenerates [1]. (iii) The non-canonical path begins when a parasitic B chromosome (which usually does not pair) acquires the capacity to pair with the X. Improvement of B-X pairing and the acquisition of male-fertility genes (F) form a chromosome that will be termed "Y" [10,34–36]. However, in contrast with the two other paths, these non-canonical Y chromosomes do not share any homologous region with the X, and are not formed by degeneration. Note that only canonical Y chromosomes are expected to have male-determining function (imparted by the M gene), and that the neo-Y and non-canonical paths can be distinguished because the former reduces the number of autosomes [1]. Color code: blue, autosomes; red, mature sex chromosomes; yellow, extra chromosomes such as a B chromosome.
association provides a clue: X–autosome fusions cause meiotic problems in XX/XY species because three chromosomes (the ancestral Y, the X and the neo-Y) must pair (Box 1). Hence, the incorporation of the ancestral Y into an autosome might have been advantageous because it solved the meiotic problems caused by the X–autosome fusion [8].

For a long time, it was thought that the D. pseudoobscura Y chromosome was the ancestral Y, and that the neo-Y was lost or incorporated into the ancestral Y [1]. Instead, the genomic data showed that the Y became part of an autosome. The data also suggest a possible origin for the current Y: it might be a degenerated neo-Y. The finding that ten of the 15 known genes and pseudogenes have homologs on the neo-X (the autosome that became fused to the ancestral X) seems at first sight to confirm this hypothesis but, actually, the evidence is at best weak, for two reasons. First, the 15 genes do not represent independent events because most of them are adjacent to each other in their original locations. They came from five different locations and, among these five, only two are homologous to the neo-X [8]. Hence, the proper evidence in favor of a neo-Y origin of the D. pseudoobscura Y chromosome was the ancestral Y, and that the neo-Y was lost or incorporated into the ancestral Y [1]. Instead, the genomic data showed that the Y became part of an autosome. The data also suggest a possible origin for the current Y: it might be a degenerated neo-Y. The finding that ten of the 15 known genes and pseudogenes have homologs on the neo-X (the autosome that became fused to the ancestral X) seems at first sight to confirm this hypothesis but, actually, the evidence is at best weak, for two reasons. First, the 15 genes do not represent independent events because most of them are adjacent to each other in their original locations. They came from five different locations and, among these five, only two are homologous to the neo-X [8]. Hence, the proper evidence in favor of a neo-Y origin of the D. pseudoobscura Y is not ten out of 15, but rather two out of five. Second, and more importantly, if these 15 Y-linked genes are part of the neo-Y chromosome originated by the X-autosome fusion, at least some should be present in the Y chromosome of other species, such as D. affinis, that share the same X-autosome fusion. To our knowledge, this has been verified only in D. persimilis (by Carlos Machado’s laboratory), which is the closest species to D. pseudoobscura. Testing more distant species is necessary because the high nucleotide identity (typically >97%) between these 15 Y-linked genes and their autosomal paralogs might suggest that they are recent acquisitions that occurred after the X–autosome fusion. Additional support for the neo-Y hypothesis might come from the identification of more Y-linked genes and identification of the pairing regions between the X and Y in D. pseudoobscura. The bottom line is that the neo-Y hypothesis provides a simple explanation for the origin of the current Y, but the question remains unsolved. However, the data in hand already show that Drosophila Y chromosomes can be very labile; indeed, chromosomes that seem to be identical (e.g. those of D. melanogaster and D. pseudoobscura) can be non-homologous. These new results also hint that X-chromosome pairing and a determining role in male fertility, which are characteristics that define a Drosophila Y chromosome, can evolve rapidly (the current D. pseudoobscura Y originated between 2 and 18 Mya [8]) and provide little information about the origin of the chromosome itself.

How frequent is the phenomenon of wholesale replacement of the Y? Does it occur in every case of X–autosome fusion? Is it restricted to species with X–autosome fusion, as required by the ‘neo-Y origin’ hypothesis? These three questions are amenable to direct experimental investigation and the results described in the previous section have answered one of them. In particular, D. willistoni, which has undergone an independent X-autosome fusion, carries the ancestral Y chromosome [9]; therefore, Y–autosome fusions do not necessarily follow X–autosome fusions.
The remaining two questions are being addressed by an ongoing study of a large number of Drosophila species. Preliminary data suggest that, among the 310 tested species, 42 had their Y chromosomes replaced, owing to four independent events (the D. pseudoobscura lineage case and three additional lineages). None of the three additional events (amounting to 34 species) has an X-autosome fusion, so the current Y of these species cannot be a degenerated neo-Y. Instead, they must have a different origin.

An alternative hypothesis for the origin of the Drosophila Y

Given the many similarities between the Drosophila Y and other Y chromosomes (e.g. X-Y pairing, low gene density, heterochromatic state) and the seemingly universal validity of sex-chromosome evolution theory, it is easy to understand why the Drosophila Y is usually thought to have originated through the same canonical path. However, researchers have repeatedly uncovered contradictory evidence while studying this chromosome in detail. The Drosophila Y lacks a sex-determining gene [16], which, according to the canonical model, should be present. Furthermore, Brosseau [17] (see also Ref. [33]) commented that ‘the Y has no genetic regions homologous to most of the X(…). This view is diametrically opposed to the notion that the Y is a degenerate X.’ Finally, the recent genomic data [8,9,21–23] also do not fit well with the canonical model. Each of these discrepancies can be accommodated by an ad hoc assumption: the lack of X-Y shared genes can be explained by the assumption that all signs of X-Y homology were erased. The lack of a male sex-determining gene can be explained by the assumption that the degeneration erased even this gene (or the Drosophila Y being a neo-Y; but see later). The odd D. pseudoobscura data could be rationalized by the assumption that its current Y is a neo-Y (although this explanation ignores the unexpected wholesale replacement of the Y). To explain the prominent role of gene gains across the 12 species, one could assume that the Drosophila Y arose from the degeneration of the X chromosome and, hence, only more recently have gene gains become important. Although these arguments cannot be completely excluded as a possibility, it might be time to consider alternative hypotheses that generate new experimental questions rather than ad hoc explanations. In fact, these discrepancies can be explained parsimoniously by the hypothesis that the Drosophila Y chromosome is not a degenerated X, but rather that it originated from the addition of an extra chromosome (that evolved the ability to pair with the X) to an X0/XX sex-chromosome system [10,34] (Figure 1b). Although this path might seem odd, it happened independently in two Homoptera species [35,36] in which the Y chromosomes originated from ‘B chromosomes’ (supernumerary dispensable chromosomes that are present in many species [1,37]), which evolved accurate pairing with the X-chromosome (Box 2).

How common are these non-canonical Y chromosomes? It is common practice to term as Y any chromosome that pairs with the X and is present only in males; moreover, it is implicitly assumed to be a degenerated X, even if it does not have a role in sex-determination (e.g. Drosophila). Thus, almost by definition non-canonical Y chromosomes will be overlooked. The unconventional origin of the Y chromosome of the Homoptera Rhinocola aceris was identified owing to the combination of incomplete fixation and high confidence of the ancestral state [35], which probably reflects the fortuitous observation of a recent event. After the R. aceris study, a careful investigation of Cacopsylla peregrina revealed the same phenomenon [36]. It is likely that additional cases will be found as researchers become aware of the possibility of non-canonical origin of Y-chromosomes; tsetse flies, in which the Y chromosome has clear similarities with B chromosomes, could be an example (Box 3). Because the X0/XX sex-chromosome system is widespread among insects and is almost certainly the ancestral system in several orders [38], there might be many additional cases of Y chromosomes arising from B chromosomes. Furthermore, although it is easier to envisage the origin of non-canonical Y chromosomes in species with X0/XX sex-chromosomes, B chromosomes are also able to pair with the sex-chromosomes in XY/XX species [39].

Will direct testing of the ‘non-canonical origin’ hypothesis be possible in Drosophila? The Homoptera examples

### Box 2. Y chromosomes that originated from B chromosomes

Nokkala and co-workers showed that the Y chromosomes of two Homoptera species (Rhinocola aceris and Cacopsylla peregrina) originated from ‘B chromosomes’ [35,36], supernumerary dispensable chromosomes that are present in many species [1,37]. B chromosomes frequently cause detrimental fitness effects but, as in other cases of selfish genetic elements, their elimination from populations is prevented by meiotic drive. In some cases, they evolve another mechanism that ensures their maintenance in populations: the ability to pair with X chromosomes in X0/XX species [37].

B chromosomes typically are heterochromatic and occur in variable numbers, both within and among populations; in the same population, individuals might carry 0, 1, 2, 3 or more copies. This characteristic provides the strongest evidence that the R. aceris Y originated from a B chromosome: in one population, males and females carry a variable number of heterochromatic chromosomes which pair imperfectly with the X in males (making it clear that they are typical B chromosomes), whereas in other populations, a similar chromosome is present only in males, in a single copy, and pairs nearly perfectly with the X (in the same way as a bona fide Y chromosome). The foreknowledge that the basic sex-chromosome system is X0/XX in the Psylloidea family [50], together with the maintenance of the number of autosomes in R. aceris, ruled out the possibility of a neo-Y origin. The lack of chiasmata between the X and Y of the two species (which are present in other Psylloidea species with neo-Y) provided additional evidence that their Y chromosomes originated from B chromosomes [35,36].

Because B chromosomes are rare (although not absent) in Drosophila [51], perhaps a more likely source of a non-canonical Y chromosome is the small ‘dot’ chromosome (D. melanogaster chromosome 4; [34]). Indeed, trisomy for the dot chromosome is well tolerated [52] and it pairs with the X [53,54]. Of course, the suggestion of a non-canonical origin of the Drosophila Y does not imply that the canonical evolutionary mechanism could not operate in Drosophila; neo-Y chromosomes also are known to degenerate in these species (e.g. see Refs [12,48]). Therefore, the important question is whether or not the canonical model provides the most likely explanation, given the available evidence.
Box 3. The Y chromosome of tsetse flies

Tsetse flies (genus Glossina) are considered to have an XY/XX sex-chromosome system, but their Y chromosome is not involved in sex determination and shows irregular segregation with the X [55]. Indeed, aneuploidy is common in natural populations (e.g. X0, XY and XXY males co-exist [55]). Furthermore, the Y seems to cause meiotic drive in XXY females because they produce twice as many XY as X eggs [56]: Variable numbers and meiotic drive are hallmarks of B chromosomes [1,37] and, if not for the sterility of X0 males [56], the Glossina Y would perfectly fit the definition of a B chromosome. Indeed Glossina is known to have B chromosomes [57] and the close similarity between their B and Y chromosomes in meiotic behavior and C-band pattern led Amos and Dover [58] to propose that their B chromosomes originated from the Y (but note that the opposite relationship – Y originated from B – additionally explains the irregular X-Y segregation). The Glossina genome is currently being sequenced. The genomic data, together with studies aimed at uncovering the ancestral state of sex-chromosomes in related families, might show whether or not the Glossina Y chromosome is actually a specialized B chromosome that acquired male-fertility genes.

described in Box 2 might provide some guidance. These cases were uncovered using cytogenetics (optical microscopy of chromosomes), and analogous work in the Drosophilidae sister families (e.g. Ephydridae) is very much needed (Box 4). Interestingly, the only Ephydridae species investigated so far has X0/XX sex chromosomes [40]. As seen in R. aceris, uncovering recent events is crucial because the mechanisms of Y origination have not yet been blurred by additional chromosomal changes. This forms part of the rationale for studying Y-linked gene content in ~300 Drosophila species; confirmation of the suspected cases of non-canonical Y origin in several Drosophila lineages would support a similar origin for the ancestral Drosophila Y. Finally, as the cost of genome sequencing continues to fall, it is certain that many Diptera genome sequences will become available during the next decade, shedding light on the origin and evolution of their sex chromosomes. For example, species in which the Y chromosome originated from a neo-Y should have a large block of additional genes (derived from the autosomes) on the X chromosome when compared to proper outgroups, even if the fusion is ancient. Some glimpses of this approach are already possible.

Mosquito (Anopheles and Aedes) and Drosophila X chromosomes are homologous [30,31]; therefore, the genesis of the X (and presumably of a canonical Y) occurred >260 Mya, and X chromosome identity is conserved across very distant Diptera families. This ancestral X is the sole major contributor to the Drosophila X [30,31], with no evidence of X–autosome fusions, which strongly suggests that the ancestral Drosophila Y is not a neo-Y. By contrast, half of the Aedes X chromosome is homologous to the Drosophila and Anopheles X, but the other half is homologous to a single autosome in each of these species [30], presumably owing to an X–autosome fusion in the Aedes lineage. However, the Aedes Y is not a neo-Y: it is nearly identical to the X (except for the presence of the male-determining gene [41]), so additional changes must have occurred. In contrast to mosquitoes, the X chromosomes of the much more closely related families Tephritidae (Ceratitis capitata) and Muscidae (Musca domestica) are not homologous to the Drosophila X: they are completely heterochromatic, carry very few genes, and the genes orthologous to Drosophila X-linked genes that have been mapped are autosomal in these species [42,43]. Thus, in some Diptera families the X chromosome was probably replaced; such a possibility creates an interesting problem with the pre-existing dosage-compensation mechanism. Namely, the former X chromosome (now an autosome) is present in equal dose in males and females in these Diptera families and hence its hyper-transcription in males should have been turned off [44]. This prediction might also be tested in Aedes, which seems to have evolved into a system with very little differentiation between the X and the Y [41].

Concluding remarks

What is a Y chromosome? A good definition is that it is a chromosome that regularly segregates from the X chromosomes, irrespective of its origin. Many Y chromosomes will be degenerated Xs or neo-Ys, and a currently unknown number (particularly among insects) will be traced to a non-canonical origin. The canonical theory of sex-chromosome evolution, with its emphasis on X–Y homology, slow degeneration and gene loss, provides an incomplete conceptual framework to study the Drosophila Y chromosome, in which there is no sign of X–Y homology, several cases of wholesale replacement seem to have occurred, and gene gains have a prominent role. By considering the possibility of a non-canonical origin, the focus shifts to questions such as the origin of the Drosophila Y, its evolutionary lability,

Box 4. Cytogenetics and the origin of the Drosophila Y

Chromosome studies have provided important evidence for the origin of Y chromosomes in many species and they are especially effective when the ancestral state (i.e. before the origin of the Y) is known [1,35,36]. The majority of Drosophila species have an XY/XX sex-chromosome system [59], so we must infer the ancestral state by looking outside the genus. The best outgroups for this purpose are the Steganinae subfamily (which belongs to the same Drosophilidae family) and sister families such as Ephydridae and Curtonotididae. Little is known about their chromosomes and the limited data from more distant Acalyptrata families are contradictory, with one study suggesting that the Drosophilidae karyotype is highly derived [80] and another suggesting that it is ancestral [61]. An investigation of the karyotype of outgroups such as Ephydridae is clearly needed. This investigation should eliminate at least one mechanism from the list of possible origins of the Drosophila Y (i.e. X-degeneration, neo-Y and non-canonical). For example, an X0/XX ancestral state would rule out the X-degeneration hypothesis. Neo-Y formation by X–autosome fusions can be detected by the reduction in the number of autosomes and (in many cases) by the transformation of a single-armed into a two-armed X [1]. The application of these ideas to the study of the origin of the Drosophila Y is hampered by the lack of data from outgroups. The proposed cytogenetic approach also has some caveats. Neo-Y detection is straightforward in evolutionarily recent events with a known ancestral state [1,50,62], but the Drosophila Y originated >63 Mya [9]; pending the rate of chromosomal evolution, further changes that alter chromosome number and structure (such as chromosome fusions, fissions, and inversions) might have blurred the signs of the origin of the Y. Another limitation is that Y chromosomes that are non-homologous (e.g. D. melanogaster and D. pseudoobscura) [8]; can look nearly identical when using simple cytogenetics methods (e.g. orcein staining). However, the use of more detailed cytogenetic methods, such as fluorescence in situ hybridization (FISH) and chromosome painting (e.g. see Ref. [63]), can alleviate these concerns.
the sex chromosomes of Ephydridae and other Diptera families, and to the close relationship between Y and B chromosomes in some species. Throughout this review we have emphasized that these questions are amenable to experimental investigation and, therefore, will be answered eventually. As the Glossina case suggests (Box 3), such a shift in focus might illuminate Y-chromosome evolution far beyond Drosophila.

On the experimental side, we cannot emphasize enough the importance of an exhaustive identification of Y-linked genes. This is the main obstacle to the study of the Y chromosome because its richness of repetitive DNA precludes sequence assembly into large and easily studied scaffolds. Instead, short Y-linked scaffolds (usually between 1 kb and 100 kb) must be individually identified among thousands of unmapped scaffolds that came from other heterochromatic regions, using computational methods followed by experimental verification of Y-linkage [8,21–23]. The experimental verification is necessary owing to the high rate of false-positives and false-negatives that occur using the current computational methods; this step is labor intensive (when applied to hundreds of scaffolds) and in practice is done only in the scaffolds that seem to encode genes [8,21–23]. Two recent developments will make this individual experimental verification unnecessary and hence are likely to have a deep impact on the knowledge of Y chromosomes. We developed a method based on short-read sequences that enables massive identification of Y-linked sequences (http://www.drosophila-conf.org/2008/pdf/abstract_platform.pdf; #124). This method is suited for genomes that have already been sequenced using male–female mixed libraries (e.g. see Ref. [45]). However, as proposed by Krzywinski et al. [46], the simple separation of male and female libraries before sequencing provides the most straightforward and powerful method to identify Y-linked sequences, at nearly zero additional cost. Currently, several Anopheles genomes and the hemipteran Rhodnius prolixus are being sequenced in this way. We hope that this will become standard practice for future genome projects.

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