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Y not a dead end: epistatic interactions between Y-polymorphisms and genetic background affect global gene expression

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Abstract

Molecular understanding of the Y chromosome has been hindered by its heterochromatic nature and lack of recombination. However, recent work has suggested a dynamic role for the Y chromosome, and its relevance to spermatogenesis and male fitness. We studied Y chromosomes from two populations of *Drosophila melanogaster* that have previously been shown to have major effects on the thermal tolerance of spermatogenesis. We show that these Y chromosomes differentially modify the expression of hundreds of autosomal and X-linked genes. Genes showing Y-linked regulatory variation also show an association with immune response and pheromone detection. Indeed, genes located proximal to the X euchromatin-heterochromain boundary appear particularly responsive to Y-linked variation, including a substantial number of odorant-binding genes. Furthermore, the data shows significant interactions between Y-chromosome lineage and the remaining genetic background of autosomes and X-chromosome. Altogether, our findings support the view that inter-population Y-linked polymorphisms can differentially modulate the expression of many genes important to male fitness, and point to complex Y-chromosome-by-background interactions on global gene expression.

Introduction

The Y chromosome is transmitted without sexual recombination from father to son. In the Y-chromosome, as in other nonrecombining regions, complete linkage between genes reduces the efficacy of natural selection and makes the Y-chromosome conducive to the accumulation of deleterious mutations through genetic hitchhiking and Muller's ratchet (Bull, 1983; Rice, 1987, Charlesworth and Charlesworth, 2000; Bachtrog, et al. 2008). Consistent with theory, the Y chromosome of *Drosophila melanogaster* carries about 12 single copy protein coding genes (Vibranovski et al 2008), whereas over 5,000 genes were to be expected from typical gene densities at euchromatic segments.

Six of the twelve genes discovered on the Y chromosome are male fertility factors that encode for either structural components of spermatogenesis or regulate spermatogenesis-specific processes such as individualization (Carvalho 2000). Spermatogenesis in *D. melanogaster* males is extremely sensitive to heat, with males becoming sterile anywhere from 23°C in heat-sensitive species to 31°C in heat-tolerant species (David et al 2005). Rohmer et al (2004) found that polymorphism between Y chromosome lineages from tropical and temperate regions is responsible for much of the variation in thermal sensitivity of spermatogenesis. Since spermatogenesis is essential for male fitness, we expect a Y chromosome effect on the former to translate into effects on the latter. Indeed, Chippindale and Rice (2001) showed that polymorphisms on the Y-chromosome have a large effect on male fitness, with a limited contribution of additive genetic variance and yet a substantial contribution of epistatic genetic variance components to total variation. Accordingly, a Y-chromosome's contribution to fitness was highly dependent on the genetic background of autosomes and X-chromosomes; Y-

chromosomes conferred high fitness to males in one background and low fitness in others backgrounds.

While Y-linked protein coding genes show effectively zero nucleotide diversity (π) within *Drosophila melanogaster* (Zurovcova and Eanes, 1999), and very low levels of diversity in human populations, Y-linked heterochromatic and rDNA repeats in humans and flies can vary in repeat number or length (Karafet et al, 1998; Lyckegaard and Clark, 1989; Lyckegaard and Clark, 1991; Repping et al, 2003). Consistent with the idea that it is far from inert, recent studies showed that the Y chromosome has undergone rapid evolution and turnover of protein coding genes between humans and chimpanzee (Hughes et al, 2010) and among species of *Drosophila* (Koerich et al, 2008)

While no Y-linked transcription factors have been found in Drosophila, the Y chromosome is known to be a pervasive modulator of gene activity elsewhere in the genome. One phenomenon in which the Y chromosome affects expression of genes is position effect variegation (PEV) (Muller, 1930; Gatti and Pimpinelli, 1992; Talbert and Henikoff, 2006; Schulze and Wallrath, 2007). PEV occurs when genes are relocated next to a heterochromatin-euchromatin boundary. While these genes remain unchanged at the DNA level, they are transcriptionally repressed in some cells but not others. A classic example is the repositioning of the *w[m4]* allele from its normal location on distal X chromosome euchromatin to a new location close to an AT microsatellite-rich region in the X pericentromeric heterochromatin (Muller, 1930). The variegated expression of *w[m4]* results in a mosaic red-white eye phenotype. PEV-associated repression of gene transcription is thought to be a result of the spread of pericentromeric heterochromatin into neighboring genes, and the subsequent silencing of these genes (Schulze and

Wallrath, 2007). Y chromosomes are known to suppress PEV in XY males and XXY females (Dorer and Henikoff, 1994), with level of suppression relative to the amount of the Y-chromosome segment tested (Dimitri and Pisano, 1989). One model for these effects is the competitive sequestration of chromatin-associated proteins by Y-linked microsatellite repeats (Lloyd et al, 1997; Wallrath, 1998).

Recent work by Lemos et al (2008) has shown that the modulating effect of cryptic Y chromosome polymorphisms on gene expression is pervasive throughout the *D. melanogaster* genome. Accordingly, males differing only in the origin of the Y-chromosome showed differential expression at hundreds of non-Y-linked genes. Most interestingly, many of these genes have male-biased expression, and seem to be involved in species divergence and temperature adaptation. These results provided the first molecular framework for how the Y chromosome affects adaptive phenotypic variation including effects on fitness (Voelker and Kojima, 1971; Chippindale and Rice, 2001; Rohmer et al, 2004).

The role of genetic background on Y-regulatory variation (YRV) remains to be addressed. Previous experiments by Lemos et al (2008) placed Y-chromosomes in an inbred, homogeneous laboratory stock (Bloomington 4361). This stock was chosen to enforce homozygosity of background. However, autosomal and X-chromosome polymorphisms occurring in natural populations may lend themselves to subtle modifications by cryptic Y-polymorphisms. In accordance with this possibility, Chippindale and Rice (2001) found significant background-by-Y interaction effects on male fitness. In addition, no studies have yet investigated the physical clustering of these genes along chromosomes.

This study is aimed at addressing the following questions: (i) what genes show modulation by Y-by-background effects, (ii) what biological functional categories do these genes fall into, and (iii) do these genes show distinctive physical clustering patterns along autosomal and X-chromosomes. The Y-chromosomes chosen were sampled from a tropical (India) and temperate (France) population of *D. melanogaster*. Flies from these populations have previously been shown to have major differences on their ability to carry out spermatogenesis under heat-stress, in large part due to polymorphic variation between their Y-chromosomes (Rohmer et al 2004). Here we test the effect of the Y-chromosomes on gene expression not only in an inbred laboratory stock background, but also in the background of both the tropical and temperate populations from which the Y's were derived. This allowed us to address the extant by which the expression of polymorphic Y-linked variation depends on the subtleties of genomic background. A gene-density plotting algorithm was used to test for physical clustering of genes showing YRV. Finally, both naturally occurring Y-chromosomes were assayed for polymorphisms capable of regulating PEV.

Materials and Methods

Fly strains

Wild flies were collected from Draveil, France (temperate population) and Delhi, India (tropical population) by members of Jean R. David's research group, and generously provided to us for analysis. Through a series of crosses (Supplementary Figure 1), Y chromosomes from the French (F) population were introgressed into an

otherwise Indian (I) autosomal and X-chromosomal background, and vice versa. Y chromosomes from both populations were also introgressed into the same lab stock background (B4361) used by Lemos et al (2008). Hence, the six populations used for analysis were: the original lines with their native Y's, F:Y_F and I:Y_I, as well as four Y-substituted populations, I:Y_F, B4361:Y_F, F:Y_I and B4361:Y_I (listed here with background first and Y chromosome second). All crosses for each Y-substitution line were carried out with 15-20 vials with multiple mating pairs per vial. Flies were grown under 24h light, temperature and humidity controlled incubators. Males from these populations were collected for use in microarray dye-swap experiments. Newly emerged males were collected on the tenth day after egg laying and allowed to age for three days at 25°C, after which they were flash frozen in liquid nitrogen and stored at -80°C.

Microarray hybridizations and analysis

Microarrays were ~18,000-feature cDNA arrays spotted with *D. melanogaster* cDNA PCR products as described (Lemos et al, 2008). Total RNA was extracted from frozen males using TRIZOL (Gibco- BRL, Life Technologies, Gaithersburg, MD) according to the manufacturer's recommendations. Total RNA quality, as measured by A260/A280 ratios, was confirmed by spectrophotometric analysis to be close to 2. cDNA synthesis with fluorescent dyes (Cy3 and Cy5) and hybridization reactions were carried out using 3DNA protocols and reagents (Genisphere Inc., Hatfield, PA). Our experimental design consisted of 16 cDNA microarrays, 4-6 for each of the three backgrounds background (F, I, and B4361), involving 32 separate labeling reactions. We contrasted two Y-chromosomes (Y_I and Y_F) on each microarray. Upon hybridization,

slides were scanned using AXON 4000B scanner (Axon Instruments, Foster City, CA) and the GenePix Pro 6.0 software.

Stringent quality-control criteria were used to ensure reliability of foreground intensity reads for both Cy3 and Cy5 channels. Foreground fluorescence of dye intensities was normalized by the Loess method implemented in the library Limma of the statistical software R. Significance of variation in gene expression in each background due only to the Y chromosome was assessed using the Bayesian Analysis of Gene Expression Levels (BAGEL) model (Townsend and Hartl, 2002). False discovery rates (FDR) were estimated empirically based on the variation observed when randomized versions of the original dataset were analyzed.

Density along chromosomes of genes showing Y-linked regulatory variation as assessed by BAGEL was plotted using a sliding window algorithm with window size of 2 Mb, sliding along 1 Mb pair at a time. Confidence intervals were estimated empirically by running the density-plotting algorithm on 1000 sets of randomly sampled genes, with gene number equal to the number of differentially expressed genes. 95% densities were plotted and any clusters with observed densities beyond were called significant.

To test for Y-by-background interaction effects on gene expression, a linear model was fitted to normalized data: $\gamma_{ij} = \mu + B_i + Y_j + I (B \times Y)_{ij} + e_{ijk}$, where γ_{ij} is the normalized-transformed gene expression, μ is the population mean, B_i is the effect of the ith background, Y_j is the effect of the jth Y chromosome, $I (B \times Y)_{ij}$ is the effect of background by Y interaction, and e_{ijk} is the residual effect. The significance of effects from background, Y, and their interactions was tested by using the Fs-test, a modified F-

statistic incorporating shrinkage variance components (Cui et al, 2005). P-values were calculated by performing 1000 permutations of samples, then corrected for multiple hypothesis testing by the q-value false discovery rate method (Storey and Tibshirani, 2003). Significant changes were determined at the FDR threshold of 0.01. K-means analysis was used to identify groups of genes with similar expression patterns across Y-by-background groups. In the bootstrapped k-means algorithm, a gene was assigned to a group if it was identified in 80% of 1,000 iterations. This was repeated for different values of k to find the k needed to minimize the number of genes not identified in any group. All Y-by-background effects analyses were computed with the R/Maanova package (Wu et al, 2003).

Enrichment in gene ontology categories was assessed with GeneMerge (Castillo-Davis and Hartl, 2003), which uses a hypergeometric distribution to assess significance. Because GeneMerge tests for all categories, a modified Bonferonni correction is used to account for multiple testing.

Position effect variegation (PEV)

Males from all four populations were crossed to females from a stock carrying *w[m4]* (Figure 2). These females possess an inversion on the X that places *w* proximal to the X-centromere. Culture was performed at either 25°C or 18°C. Males from these crosses were collected, flash frozen in liquid nitrogen, aged for 3 days at either 25°C or 18°C, and stored at -80°C. Heads of males were moved with a blade, and homogenized 5 to a tube with 10uL of acidified ethanol (30% ethanol acidified to pH 2 with HCl). Eye pigment expression was assessed with spectrophotometric analysis at an optical density

of 480nm. 4-6 biological replicates were used per treatment, with two measurements

taken per replicate. The correlation between repeat measures was high (Pearson's r =

0.90), thus their means were used in subsequent analyses. Males displaying typical eye

pigmentation phenotypes were imaged using an auto-montage system (Snycroscopy,

Frederick, MD). A 3-way ANOVA analysis, using statistical software JMP, was

performed using male background (I, F, or B4361), Y chromosome (Y_I or Y_F), and

temperature (25°C or 18°C) as factors.

<div align="center">Results</div>

Global gene expression variation

Males differing only in their Y chromosomes (either Y_I or Y_F) showed differential

expression of a substantial number of genes, with the exact number depending on the

genetic background of the male and cut-off for significance used [12 to 1178 genes,

Bayesian posterior probability > 0.999 to 0.90; false discovery rate (FDR) < 1 to 35%;

Figure 1]. For every background, and at every significance cut-off value, the observed

number of genes differentially expressed among Y_I and Y_F males exceeded the number

expected by chance. Overlap of differentially expressed genes sets between the three

different backgrounds is shown in Figure 2. The results suggest that although Y-linked

polymorphisms have different modulating effects on genes depending on the genetic

background of the male, there is some agreement on the genes showing Y-regulatory

variation (YRV). All following analyses are for genes with Bayesian posterior

probability > 0.95.

Clustering of genes showing YRV into significant biological function categories is listed in Table 2. Of note, pheromone binding and immune response genes are heavily represented, as well as genes localized to extracellular regions across all three backgrounds. This again suggests that genes showing YRV show a consistent pattern with respect to function.

Physical clustering of genes along chromosomes was also examined. While no physical clustering is apparent in the autosomes, males possessing Y_F showed overexpression of genes near the X euchromatin-heterochromain boundary (at chromosome position 22 Mb) when compared to males possessing Y_I (Figure 3). This pattern holds true in both the I and F backgrounds, but not in the B4361 background. In the I background, 5 genes near the X chromosome euchromatin-heterochromatin boundary showed this pattern of overexpression in Y_F males compared to Y_I. Of these, one was odorant-binding protein 19a (Obp19a). This gene encodes for a protein used in sensory perception of chemical stimulus. In the F background, 3 genes near the X euchromatin-heterochromatin boundary showed this pattern. All three (Obp19a, Obp19b, and pheromone binding protein related protein 3) are involved in the sensory perception of chemical stimulus. The observed effect of the Y chromosome on modulating genes associated with pheromone binding and sensory perception has important implications for male fitness and sexual selection.

The relative expression levels of three representative genes showing YRV are plotted in Figure 4. One gene, odorant-binding protein 19b (Obp19b), was chosen because it is located near the X-euchromatin-heterochromatin boundary and was also identified in both the F and I backgrounds as being significantly overexpressed in Y_F

compared to Y_I males. Two other genes, Drosocin (Dro) and pheromone binding protein-related protein 3 (Pbprp3) were chosen because they belonged to biological clusters identified to be over-represented in the YRV-regulated gene sets (immune response and pheromone binding, respectively). Ratios represent relative expression levels of genes in Y_F males over Y_I males in the three genetic backgrounds. The results show, again, how epistatic interactions between Y-linked polymorphisms and background can modulate the expression of non-Y genes. Particularly striking is the differential relative expression of Dro, an immune response gene, in the three backgrounds: when comparing expression of Dro in Y_F males to Y_I males, it is underexpressed in the I background, overexpressed in the F background, and slightly underexpressed in the B4361 background.

Extensive Y-by-background interaction

Background-by-Y effects, as assessed by a mixed-effects model, influenced the expression of 346 genes (FDR < 0.01). Agreement between these results and the BAGEL results is strong: 252 (74%) of the 346 genes were also identified by BAGEL as differentially regulated by Y-polymorphisms in at least one of the genetic backgrounds (4361, I, or F) (Supplementary Table 1). 200 (57.8%) of the 346 genes can be grouped most parsimoniously into three clusters of gene expression (Figure 5). In each cluster, genes show similar patterns of high expression in some Y-background combinations, and low expression in others. Significant biological function GO categories within each cluster are listed in Table 2. In both methods of analysis (BAGEL and Maanova), immune response genes are heavily represented within significantly differentially expressed genes.

Y-only effects, as analyzed by Maanova, regulate the differential expression of 192 genes. A proper comparison with BAGEL results called for re-analysis of global gene expression patterns across all arrays (regardless of background) using BAGEL. When this was done, 484 genes showed differential gene expression (Bayesian Posterior Probability > 0.95). Of the 192 genes identified by Maanova, 106 (55%) of them were also identified in the BAGEL gene set, while 86 (45%) were not. These 86 novel targets of YRV are not too surprising in view of our results indicating strong epistatic Y-by-background effects on gene expression. This is because genes that are highly modulated by the Y-chromosome in one genetic background, and less so in others might not be identified as consistently different in expression between the temperate and tropical Y-chromosomes across all backgrounds.

Polymorphic Y chromosome effects on position effect variegation

Both the Y-by-background interaction effects on gene expression and as well as the large effect of the Y-chromosome on genes located next to X euchromatin-heterochromatin boundary observed from the microarray data were confirmed with a PEV assay. Males in the assay possessed either a Y_I or Y_F in a hybrid genetic background consisting of an X chromosome with *w[m4]*, a haploid autosomal genome sampled from the original stock containing the PEV marker, and a haploid autosomal genome sampled from the I, F, or B4361 laboratory stock populations. The results suggest that temperature does not affect suppression of PEV ($P = 0.26$). This is surprising, as previous studies have shown that high temperatures during development suppress PEV, while low

temperatures enhance PEV (Spofford, 1976; Zhang and Stankiewicz, 1998). There were also no significant temperature-interaction factors (temperature x Y chromosome, $P = 0.92$; temperature x background, $P = 0.41$; temperature x background x Y chromosome, $P = 0.73$). On the other hand, Y_I and Y_F differed dramatically in their effects on position effect variegation (Figure 6, $p < 0.0001$), with Y_I males showing broader expression of *w[m4]* than Y_F in all backgrounds; however the effect is least pronounced in the B4361 background.. Also importantly, background showed a significant effect on PEV ($P < 0.0001$), with a similarly significant effect for Y-by-background interaction ($P < 0.001$). Hence, this suggests that the modulation of PEV is sensitive to epistatic interactions between the Y and background, and is thus in agreement with our finding for genome-wide gene expression,

Discussion

The data presented here suggest that polymorphic variation in Y chromosomes from two geographically diverse *D. melanogaster* populations differentially regulate the expression of hundreds of autosomal and X-linked genes. However, the contribution of Y-chromosomes to global expression profiles depends on the genetic background of the bearer. Accordingly, we observed that the contribution of a temperate and a tropical Y-chromosome to global gene expression is most pronounced when assayed in two natural occurring backgrounds. This study also presents new data suggesting that physical clustering of genes exhibiting Y-regulatory variation (YRV). We found significant physical and functional clustering around the euchromatin-heterochromatin boundary of

the X-chromosome with X-linked olfaction-related genes showing higher transcription levels in males with Y_F than in males with Y_I. Finally, the Y-by-background interaction effects on autosomal and X-linked gene expression, as well as the existence of polymorphic variation between the two Y chromosomes in their affect on modulating genes proximal to the euchromatin-heterochromatin boundary in the X-chromosome were both confirmed with a position-effect variegation assay.

Y-linked genetic variance has been previously documented for sex ratio (Carvalho et al, 1997; Montchamp-Moreau et al, 2001), male courtship (Huttunen and Aspi, 2003); geotaxis (Stoltenberg and Hirsch, 1997), thermal sensitivity of spermatogenesis (Rohmer et al, 2004) and fitness (Chippindale and Rice, 2001). For many of these traits (male courtship, geotaxis, spermatogenesis, and fitness), significant Y-by-background interaction effects have also been detected. Thus, our observations regarding substantial Y-by-background interaction for gene expression traits are in good agreement with these previous findings regarding higher-level phenotypes.

These findings of Y-effects on male phenotypes contrasts with molecular analyses showing no polymorphism among 11 alleles of a 1738-bp region of a Y-linked gene in *D. melanogaster* (Zurovcova and Eanes, 1999). In humans, Y-linked genes also show decreased levels of molecular variation, with a large-scale analysis of four Y-linked genes finding that coding regions show between 0% and 20% of the polymorphism of a sample of autosomal genes (Shen et al, 1997; Rozen et al, 2009). Despite the lack of nucleotide diversity in coding sequences of Y-linked genes, considerable structural polymorphism has been detected in Y-heterochromatin repeat copy-numbers in humans and flies (Karafet et al, 1998; Lyckegaard and Clark, 1989; Lyckegaard and Clark, 1991;

Repping et al, 2003). Repeat sites have now been shown to act as nucleation sites for heterochromatin formation via the RNAi pathway (Dorer and Henikoff, 1994; Elgin and Grewal, 2003; Volpe et al, 2002; Pal-Bhadra et al, 2004).

Heterochromatin can influence transcription epigenetically, with the effect most easily observed in the modification of PEV by the Y chromosome (Dimitri and Pisano, 1989; Dorer and Henikoff 1994). Large heterochromatic blocks, such as the Y chromosome, are thought to sequester limited heterochromatin factors from other regions, thus impeding the spread of heterochromatin to nearby loci (Lloyd et al, 1996; Schulze and Wallrath, 2007). In this way, silencing of genes located near heterochromain-euchromain boundaries is suppressed. Balanced polymorphisms in Y chromosome heterochromatin repeats may provide the necessary molecular variation for differential competitive binding ability of chromatin proteins. The concomitant redistribution of chromatin proteins will most strongly influence the expression genes located next to other heterochromatic blocks, such as euchromatin-heterochromatin boundaries. An alternate explanation for the influence of the Y on PEV is via the Y chromosome's effect on RNA-interference pathways. The spread of heterochromatin is initiated through the transcription of repeat-DNA, and then propagated via the RNA-interference pathway. Lemos et al (2008) found Y-linked polymorphisms responsible for the differential expression of transposable elements, which are known to undergo RNAi-mediated silencing. Therefore, mechanistic similarities underlying Y-effects on gene expression and PEV may exist.

As our results suggest, many of the genes showing Y-linked regulatory variation near the euchromatin-heterochromatin boundary in the X-chromosome, are odorant-

binding proteins, which are components of the insect olfactory system (Wang et al, 2009). Odorant receptors are rapidly evolving molecules in the Drosophila proteome (Robertson et al 2003) and show regulation following mating (McGraw et al 2004). Interestingly, the genes affected by Y-linked regulatory elements exhibit significant functional coherence in showing association with pheromone detection. The influence of the Y chromosome on pheromone detection as well as odorant-binding proteins suggests a role for the Y in mating behavior, and may help to explain the cessation of rigorous courtship and the reduced mating success of XO *Drosophila* males (Cordts and Partridge, 1996; Kuijper et al, 2006). In *Anopholes* mosquitos, the Y chromosome has also been implicated for influencing mating behavior (Fraccaro et al, 1977). Because many mating-behavior related proteins are selected for in different ways in males and females, there may be selection for sex-limitation of modifiers of their expression. Since the Y chromosome is male-limited, it serves as the perfect platform for these modifiers. Lemos et al (2008) showed that genes showing Y-regulatory variation are more highly expressed in males than females, suggesting that the recruitment of modifiers of male-biased genes may have shaped the evolution of the Y-chromosome.

In addition to pheromone binding proteins, genes showing Y-linked regulatory variation are also associated with immune response and are more likely to be localized to the extracellular matrix than expected by chance. Although there are no previous studies of Y chromosome effects on immune response genes in *Drosophila*, studies in mice have found that Y-linked polymorphisms are capable of modifying autoimmune disease susceptibility (Teuscher et al, 2006; Spach et al, 2009). However, in mice several genes of immunologic significance are located on the Y and may serve as candidates for

explaining the effect. In *Drosophila*, no Y-linked immune-related genes are known. Therefore we suggest that our findings of *Drosophila* immune response genes being responsive to YRV are most likely explained by variation in non-genic components of the Y-chromosome, such as repeat copy number. *Drosophila melanogaster* populations from France and India are known to differ in the thermal sensitivity of spermatogenesis (Rohmer et al 2004, David et al 2005), with temperate and tropical Y-chromosomes contributing substantially for this difference. Among genes that show YRV in at least two of the three backgrounds we find candidates known to be structural constituents of cytoskeleton (*nod*, *CG9279*, and *tm2*) and lipid metabolism (*CG9914, CG17292, CG9458, CG11426, CG6295, CG6277, CG18815, CG31872*). In addition, fatty acid metabolism genes are overrepresented in one of the clusters detected by k-means analysis using Maanova. This suggests that while the heat sensitivity of spermatogenesis express itself sharply at higher temperatures, the modulating effects of the Y chromosome on sperm-related traits may be subtle at permissive or less stressful temperatures. Lastly, localization of genes showing YRV to extracellular regions is expected, as many pheromone binding proteins and proteins involved in immune response are receptor proteins with large extracellular domains.

In summary, our finding of cryptic Y-linked regulatory control of hundreds of genes across various genetic backgrounds suggests standing Y-linked balanced polymorphisms in natural populations. At a cursory glance, this result seems incongruent with previous theoretical and empirical work suggesting little Y-linked polymorphism can be supported on a nonrecombining chromosome. However, our findings together with other studies of the nontransitivity of sperm competition and Y-by-background

interactions for male fitness (Clark et al, 2000; Chippindale and Rice, 2001) bring to light some complex and previously under-appreciated dynamics for maintaining Y-linked polymorphisms.

Figure 1. Black bars represent number of genes differentially expressed by males possessing Y_I or Y_F in I (top panel), F (middle panel) or B4361 background (lower panel), as a function of the Bayesian posterior probability of differential expression. Gray bars indicate the estimated number of genes expected by chance.

Figure 3. Clustering of genes showing YRV along the X chromosome in I (top panel), F (middle panel) or B4361 background (lower panel). Black lines indicate observed density of genes around a 2 Mb sliding window (step size 1 Mb). Grey lines indicate 95% confidence intervals. A schematic X is drawn in the top left panal with light areas representing euchromatin and dark areas representing heterochromatin. The dark knob at the right represents the euchromatin-heterochromatin boundary. Columns represent genes for which males possessing Y_F showed enhanced expression over males possessing Y_I ($Y_F > Y_I$) and vice versa ($Y_I > Y_F$). An asterisk denotes a chromosomal segment housing significantly more genes showing Y-regulation than expected by chance.

Figure 6. Y chromosome effects on position effect variegation (PEV). Y_I suppresses PEV more, and thus allows more expression of *w[m4]*, than Y_F in all three backgrounds. Eye pigmentation was measured as absorption of light at 480 nm. b) Pictures of representative male flies.

Figure 4. Relative expression levels of three genes showing YRV in Y_F males versus Y_I males in three backgrounds. Expression levels are shown as the ratio of Y_F over Y_I expression ($\pm$S.E). *Obp19a* is an odorant binding protein near the X euchromatin-heterochromatin boundary. Drosocin (*Dro*) is an immune response gene. Pheromone-binding protein-related protein 3 (*Pbprp3*) is a pheromone binding protein.

Figure 5. Gene expression profiles generated by k-means clustering. Each line represents the expression of one gene across each background-by-Y group. Expression measures were standardized across groups, and analyzed using k-means cluster analysis. There were 19, 88, and 93 genes in clusters 1, 2, and 3, respectively.

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