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Context-dependent effects of Y chromosome and mitochondrial haplotype on male locomotive activity in *Drosophila melanogaster*

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Abstract

Some regions of the genome exhibit sexual asymmetries in inheritance and are thus subjected to sex-biased evolutionary forces. Maternal inheritance of mitochondrial DNA (mtDNA) enables mtDNA mutations harmful to males, but not females, to accumulate. In the face of male-harmful mtDNA mutation accumulation, selection will favour the evolution of compensatory modifiers in the nuclear genome that offset fitness losses to males. The Y chromosome is a candidate to host these modifiers, because it is paternally inherited, known to harbour an abundance of genetic variation for male fertility, and therefore likely to be under strong selection to uphold male viability. Here, we test for intergenomic interactions involving mtDNA and Y chromosomes in male *Drosophila melanogaster*. Specifically, we examine effects of each of these genomic regions, and their interaction, on locomotive activity, across different environmental contexts – both dietary and social. We found that both the mtDNA haplotype and Y chromosome haplotype affected activity in males assayed in an environment perceived as social. These effects, however, were not evident in males assayed in perceived solitary environments, and neither social nor solitary treatments revealed evidence for intergenomic interactions. Finally, the magnitude and direction of these genetic effects was further contingent on the diet treatment of the males. Thus, genes within the mtDNA and Y chromosome are involved in genotype-by-environment interactions. These interactions might contribute to the maintenance of genetic variation within these asymmetrically inherited gene regions and complicate the dynamics of genetic interactions between the mtDNA and the Y chromosome.

Introduction

Interactions between the mitochondrial and nuclear genomes represent one of the most prominent examples of genomic cooperation (Gillham, 1994). Genes originally present in the ancestral mitochondria have been translocated to the nuclear genome over the course of evolutionary history (Adams & Palmer, 2003; Rand *et al.*, 2004; Burt & Trivers, 2006), but have remained essential for mitochondrial function (Lotz *et al.*, 2013). Hundreds of nuclear encoded proteins require import into the mitochondria to ensure structural assembly and regulation of the mitochondrial electron transport chain (Taanman, 1999; Guarente, 2008). Coordination between mitochondrial and nuclear genomes is required for uncompromised metabolism and energy production, traits that ultimately underpin the expression of key life-history components and shape organismal fitness (Blier *et al.*, 2001; Ballard & Whitlock, 2004; Rand *et al.*, 2004; Dowling *et al.*, 2008; Montooth *et al.*, 2010; Levin *et al.*, 2014; Wolff *et al.*, 2014; Zhu *et al.*, 2014; Hill, 2015). However, in most eukaryotes, mitochondria are maternally inherited (Birky, 1978), creating a sex-specific environment for selection to act.

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Under strict maternal inheritance of mitochondria, males do not transmit mitochondrial DNA (mtDNA) to their offspring. The implication is that deleterious mtDNA mutations that are male-specific in their harmful effects will not be screened by selection because phenotypic variation in males attributable to their mitochondrial DNA is not heritable, and will thus be prone to accumulate within populations (Frank & Hurst, 1996; Gemmell *et al.*, 2004; Beekman *et al.*, 2014). Consequently, mitochondrial genomes are predicted to harbour male-biased mutation loads. Furthermore, evolutionary theory predicts that these mitochondrial mutation loads are likely to underpin sexually dimorphic traits (Friberg & Dowling, 2008; Innocenti *et al.*, 2011). Under sexual dimorphism, the genetic correlation between the sexes is decreased (Bonduriansky & Rowe, 2005; Poissant *et al.*, 2010; Griffin *et al.*, 2013), facilitating the accumulation of sex-specific allelic variance. When mitochondrial genetic variation affects the expression of sexually specific tissues, such as the testis for example, males will presumably be unable to rely on female-specific adaptation of the mtDNA sequence to optimize their mitochondrial requirements (Frank & Hurst, 1996; Friberg & Dowling, 2008; Innocenti *et al.*, 2011; Beekman *et al.*, 2014).

These evolutionary predictions recently received empirical support in the fruit fly, *Drosophila melanogaster*. Innocenti *et al.* (2011) reported that the expression patterns of thousands of nuclear genes were sensitive to the identity of the mitochondrial haplotype alongside which these nuclear genes sit in males, but not in females. This suggests that purifying selection has prevented functional (i.e. phenotype-modifying) allelic variance from accumulating within the mitochondrial genome when it affects females, but has enabled alleles that are male-biased in their expression to build up within the mitochondrial genome. Notably, the affected phenotypes were associated with sex-limited traits – genes enriched within the male reproductive tissues and that encode male reproductive function were those most sensitive to mitochondrial haplotypic variance (Innocenti *et al.*, 2011), and this also affected male fertility *in vivo* (Yee *et al.*, 2013). Similarly, Camus *et al.* (2012) showed that patterns of longevity and ageing were sensitive to mtDNA haplotypic variation in male, but not female, *D. melanogaster* (Camus *et al.*, 2012). The mitochondrial alleles underlying the male-biased effects in these studies are assumed to comprise of deleterious mutations that depress male fitness (and are either detrimental, neutral or even beneficial in females) (Frank & Hurst, 1996; Yee *et al.*, 2013; Beekman *et al.*, 2014). The accumulation of such mutations should place strong selection on the nuclear genome for compensatory mutations that restore mtDNA-mediated disruptions in function (Frank & Hurst, 1996; Yee *et al.*, 2013; Beekman *et al.*, 2014).

Traditionally, the nonrecombining and gene-poor Y chromosome was considered an unlikely candidate for involvement in adaptive evolutionary processes. However, recent insights suggest otherwise and implicate the Y as a possible host for compensatory modifiers that offset mtDNA-mediated male harm. First, in direct contrast to mitochondria, Y chromosomes are strictly paternally inherited and thus subject to male-specific selection that favours the accumulation of male-beneficial mutations (Bachtrog, 2013). Second, although they have few protein-coding genes, Y chromosomes harbour cryptic regulatory variation with broad-scale consequences on the expression patterns of nuclear genes across the rest of the genome (Lemos *et al.*, 2008). Third, it was recently noted that many of the nuclear genes that are sensitive to mitochondrial haplotypic variance are also sensitive to Y chromosome regulation (Rogell *et al.*, 2014). This suggests that the expression patterns of these nuclear genes might be at the centre of an evolutionary tug-of-war between the maternally inherited mitochondrial genome and paternally inherited Y chromosome over regulatory control of the genome (Rogell *et al.*, 2014).

If such a model of mitonuclear compensatory co-adaptation is relevant, then its genomic footprint is likely to be tractable to metabolically reliant sexually dimorphic traits (Hill & Johnson, 2013). Locomotive activity is a promising candidate (Long & Rice, 2007) because it is sexually dimorphic in expression, at least in fruit flies (*D. melanogaster*) (Long & Rice, 2007), and is a trait that is closely aligned to metabolic output (Rogowitz & Chappell, 2000; Speakman & Selman, 2003) and therefore to mitochondrial genetics (Arnqvist *et al.*, 2010). Here, we test whether mitochondrial genetic variation, Y chromosomal variation and their interaction affect locomotive activity in *Drosophila melanogaster*. Moreover, we investigate these effects under differing environmental (dietary and social) contexts, in the knowledge that cryptic genetic variation might manifest as phenotypic variation under certain contexts (McGuigan & Sgro, 2009). Indeed, a number of recent studies have suggested that the outcomes of mitonuclear epistatic interactions might be mediated by extrinsic environmental factors such as diet (Zhu *et al.*, 2014), temperature (Dowling *et al.*, 2007a; Arnqvist *et al.*, 2010) or social context (Dowling *et al.*, 2010).

Materials and methods

Creating mitochondrial and Y chromosome substitution lines

Clancy (2008) extracted mitochondrial haplotypes from isofemale lines descended from Dahomey (now Benin, Africa) and Madang (Papua New Guinea) populations, respectively, placing each haplotype alongside an isogenic line, w^{1118} (Bloomington Stock no. 5905). The

haplotypes differ by a solitary nonsynonymous SNP (G to A) in the protein-coding region, at site 4853 of the COX3 gene, which confers an amino acid transition from aspartic acid (Asp) in Madang (the consensus sequence – all other global mtDNA haplotypes sequenced by Clancy [pers. Comm.] harbour this amino acid at this point in the sequence), to asparagine (Asn) in Dahomey. Our preliminary work has identified 30 polymorphisms outside of the protein-coding regions, which further delineate the Madang and Dahomey haplotypes (J. Wolff, F. Camus, D. Dowling, unpublished data). The crossing scheme used to create these 'mitochondrial lines' (mt_{Dah} and mt_{Mad}) relies on balancer chromosomes that suppress recombination, and is outlined in Clancy (2008). These mitochondrial lines were duplicated in 2007 and have since been kept as independent replicates, backcrossed each generation (73 generations) to males of the w^{1118} line, which is itself maintained by a regime of full-sib mating each generation to ensure its isogenicity.

Similarly, Y chromosomes were extracted from respective Dahomey and Madang isofemale lines and placed alongside the isogenic w^{1118} nuclear background, in duplicate. This was achieved over a series of crosses in 2013. In the first generation, males from each isofemale line were crossed to females of an isogenic nuclear strain (Bloomington stock no. 4361), containing recessive markers on all four chromosomes: yellow [y^1; X chromosome], brown [bw^1; chromosome 2], ebony [e^4; chromosome 3], and cubitus interruptus and eyeless [ci^1, ey^R; chromosome 4] (Lemos *et al.*, 2008). Sons of this cross were themselves crossed to females of 4361. We ensured that all chromosomes associated with the original isofemale lines had been fully replaced by the chromosome of stock number 4361, by checking for the expression of each recessive phenotype (caused by alleles on each of the 4 chromosomes). At this point, Y chromosomes of Dahomey and Madang, which based on earlier findings are almost certain to harbour genetic variation in regulatory regions (Zurovcova & Eanes, 1999; Lemos *et al.*, 2008), sat alongside the 4361 strain. Males of each of these lines were then backcrossed to females of w^{1118} for four generations, to replace the 4361 chromosomes with those of the isogenic w^{1118} background, again relying on the lack of recombination in male fruit flies. We ensured that chromosomal replacement had been achieved in full, by crossing numerous males drawn from each of the resultant lines to females of 4361 and ensuring that no recessive phenotypes were observed. The production of the mitochondrial and Y chromosome lines is summarized in Fig. 1.

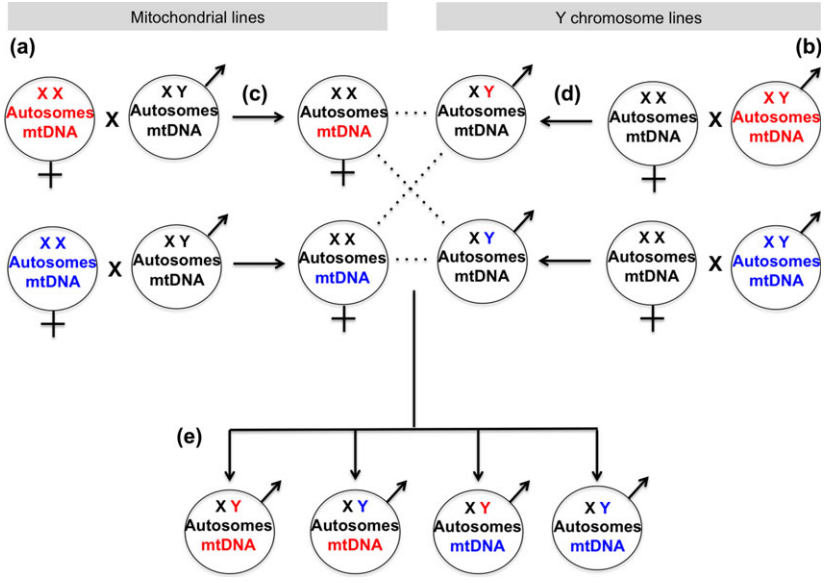

Fig. 1 The experimental breeding scheme illustrating how two base populations, Dahomey (in red) and Madang (in blue), were used to create the mitochondrial (a and c) and Y chromosome lines (b and d). (a) Virgin females from the base populations were mated to males from the w^{1118} line (shown in black). (c) The resulting female offspring had the mitochondrial genome from their maternal lineage. The remaining genetic background was from the paternal lineage (w^{1118}) and full replacement of chromosomes from the maternal line was ensured using genetic markers (crossing scheme outlined in Clancy, 2008). These lines were backcrossed each generation to males of the w^{1118} line. (b) The Y chromosome lines were created by mating males from the base populations with virgin females from the w^{1118} line. (d) The resulting male offspring had a Y chromosome from their paternal lineage. The remaining genetic background was from the maternal lineage, and full replacement of chromosomes from the base populations was ensured using recessive genetic markers (Lemos *et al.*, 2008). (e) Females from each mitochondrial line were crossed with males of the Y chromosome lines (shown by dashed lines) to create fully crossed combinations of mitochondrial DNA haplotype and Y chromosome genotype expressed alongside the w^{1118} background.

Lines were treated with substrate-laced tetracycline hydrochloride (0.3 mg mL^{-1}) many generations prior to the experiments, to eliminate potential *Wolbachia* infections. Duplication of each mito- and Y-line enables us to statistically partition effects attributable to mtDNA- and Y-linked genetic variation from confounding effects of any residual nuclear variation that might have accumulated in the w^{1118} background across the lines despite our careful efforts to maintain its isogenicity.

Creating focal males

Focal males were created by crossing virgin females from the mt_{Dah} and mt_{Mad} lines with males from the Y_{Dah} and Y_{Mad} lines. Newly emerged to 1-day-old parental flies were allowed to mate for 24 h before being transferred to fresh vials each day for 8 successive days. Eggs laid during the first 24 h were discarded. For each duplicate, crosses were performed such that males contained mitochondria and Y chromosomes from the two populations in a fully crossed 2 × 2 design, with each of the four different mito-Y genotypes independently duplicated.

Newly emerged focal males were collected in groups of eight males per vial. All flies, including parents and grandparents of the focal males, were reared under a standardized density of 80 eggs per vial, at 25 °C, under a 12-h:12-h light: dark cycle. The experiment was run in a series of replicated trials – which we denote 'Blocks', each separated in time, with the focal flies of each block descended from a different set of grandparents and parental mito-Y crosses. Grandparental age was standardized at 3–4 days post-eclosion. Within each block, parents were allowed to lay eggs over successive days to enable consecutive emergence of new focal males; thus, parental age varied between 2 and 8 days old, but the age of focal males remained constant at 4 days old (Fig. 2).

Environmental contexts

Within each experiment, focal males were randomly assigned to a diet treatment consisting of two levels – a standard yeast substrate (potato-dextrose-yeast agar) with *ad libitum* live yeast added to the surface of the medium (nutrient-rich), or an agar substrate which contained no nutrients and no live yeast, but prevented flies from desiccating (nutrient-deprived). Flies were put on this diet treatment within 6 h after their eclosion into adulthood, and maintained in single-sex groups of eight males on these substrates for 89–98 h (approximately 4 days) until assayed. w^{1118} is a particularly starvation-resistant strain (Baldal *et al.*, 2006).

Focal flies were also assayed under two 'social' environmental contexts, with separate experiments run for each social context. The first experiment measured the locomotory activity of focal flies in the presence of cuticular hydrocarbon signatures of other males in the assay chamber. These assays were therefore conducted within an environment in which the focal males perceived the pheromones of other males (Inoshita *et al.*, 2011). We denote this as the perceived 'social context '. In the remaining four blocks, males were assayed individually in chambers that had not previously held flies. We denote this as the perceived 'solitary context'.

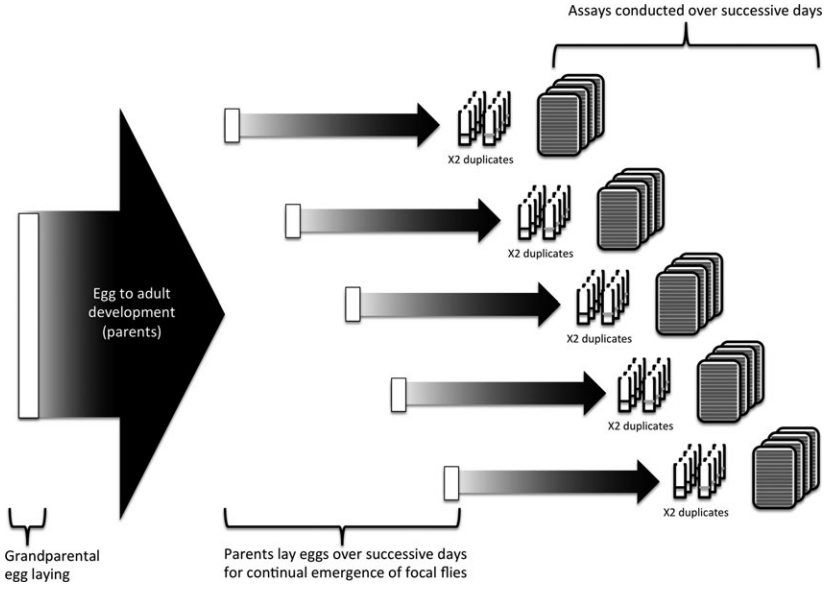

Fig. 2 Experimental plan for a single block, illustrating how focal flies are tested at the same age by varying the age at egg laying of parental flies. Between each block, focal flies are descended from a different set of grandparental flies; thus, blocks were conducted using different generations of flies descended from different sets of grandparents. Arrows represent egg to adult development for parents (thick arrow) and of focal flies (thin arrows). Egg laying over successive days shown as white blocks. Multiple assays (plates) were run per day. This design was repeated for both social contexts.

Locomotory assay

Four-day-old focal males were assayed for total distance travelled over a 30-min assay, using a ViewPoint ZebraBox (Viewpoint Life Sciences, Lissieu, France) automated behavioural tracking system. This setup allowed the simultaneous assay of 16 flies, individually, per assay. Several assays were run sequentially. To reduce variation attributed to circadian rhythm, assays began 120 min after the commencement of the daily light cycle, and were run for the next 240 min. Immediately prior to each assay, focal males were individually transferred into tubes (60 × 5 mm), in the absence of anaesthesia, and these tubes plugged with yarn at each end. The length of available space for flies to roam was standardized at 45 mm. Flies were randomly assigned to their positions on the assay plate, and the experiment was balanced, with each experimental unit (mtDNA haplotype × Y chromosome × Duplicate × Dietary treatment) represented once per assay. Assays containing flies tested in the social and solitary environmental contexts were run separately, hence ultimately analysed separately, because we were limited to a maximum of 16 positions per assay plate, and inclusion of this extra treatment would increase the number of experimental units to 32. Thus, for the first two blocks, focal males were assayed individually in vials in which other single focal males from the w^{1118} strain had been previously housed for at least 30 min, and thus, these assays were conducted in the presence of cuticular hydrocarbon signatures of other males in the assay chamber ('social context'). In the remaining four blocks, males were assayed individually in chambers that had not previously held flies ('solitary context').

Assays were run at 25 °C and in complete dark to remove any opportunity for visual interactions between flies in neighbouring tubes to influence the results.

Statistical analysis

For each social context, data were analysed using a general linear mixed model (GLMM), with the total distance travelled per male over a 30-min period as the response variable, and parental age at the time of egg laying, time of day, mitochondrial haplotype, Y chromosome haplotype and diet as fixed factors. All interactions between mitochondrial haplotype, Y chromosome haplotype and fixed effects were included in the model. Block, block nested within parental age and mitochondrial/Y chromosome duplicate were random factors. As the two social contexts were performed across different experimental blocks and grandparental generations, it was not possible to analyse the two different social contexts within one statistical model. For this reason, comparisons between the two social contexts were not possible. Deletion of nonsignificant, higher order interactions ($P > 0.05$) was conducted by testing the effect of removal of each term on the change in model deviance, using likelihood ratio tests and maximum likelihood (ML) for fixed effects, leaving the minimum adequate (final) models (Tables 1 and 2). AIC model selection with Multi-Model Inference (MuMIn package in R; Burnham & Anderson, 2002) for the GLMMs using lmer produced qualitatively similar results (Tables S1 and S2).

In total, the number of flies assayed in each social context, across the two different diets, was as follows: $N_{solitary} = 509$, $N_{social} = 320$. All analyses were conducted in R 2.13.0 (Burnham & Anderson, 2002; Bates *et al.*, 2012; R Development Core Team, 2013).

Results

Overall, males were slightly more active when they experienced an environment previously inhabited by

Table 1 Sources of variance explaining locomotive activity in males assayed in the perceived solitary context. mtDNA and Y chromosome lines were kept as independent replicates (Duplicates).

Factor	Chi-sq	d.f.	*P*
Intercept	200	1	< 0.0001
Diet	86.0	1	< 0.0001
mtDNA	2.12	1	0.145
Y	0.52	1	0.471
Random			**SD**
Block			69
Parental age (Block)			63
Duplicate			0

Table 2 Sources of variance explaining locomotive activity in males assayed in the perceived social context. Diet describes a nutrient-rich environment vs. a nutrient-deprived environment. mtDNA and Y chromosome lines were kept as independent replicates (Duplicates).

Factor	Chi-sq	d.f.	*P*
Intercept	279	1	< 0.0001
Time of day	21.1	1	< 0.0001
mtDNA	4.38	1	0.036
Y	3.08	1	0.079
Diet	25.8	1	< 0.0001
Y:Diet	7.46	1	0.006
mtDNA: Diet	6.93	1	0.008
Random			**SD**
Block			0
Parental age (Block)			50
Duplicate			0

other males (social = 609 mm, solitary = 549 mm, one-way test: $F_{1,618} = 18.2$ $P < 0.0001$; Kolmogorov–Smirnov test, $D = 0.27$, $P < 0.0001$, Fig. S2).

Solitary assays

In the perceived solitary context, males exposed to the nutrient-deprived diet were more active than males exposed to the nutrient-rich diet (Mean ± SE; Agar = 610 ± 8; Yeast = 489 ± 13; Table 1). There were no significant effects of mtDNA and Y haplotypes, nor mtDNA × Y interaction, nor interactions between the genotypes and the diet treatment, when male activity was assayed in the perceived solitary context (Fig. 3a,c; Tables 1 and 3). Flies tend to be less active in the dark (Martin *et al.*, 1999), but males of each mitochondrial haplotype and Y chromosome genotype responded to the assay conditions in similar ways over time (Fig. 4a,c).

Social assays

When assays were conducted in the perceived social context, we detected both mtDNA- and Y-linked effects, each of which was contingent on the diet treatment (Table 2). For males kept on a nutrient-rich diet, males with the Madang mtDNA haplotype were more active than Dahomey mtDNA (Mean ± SE; Mt_{Dah} = 495 ± 26; Mt_{Mad} = 552 ± 22), but there was a reversal in the activity associated with each mtDNA haplotype for males kept on the nutrient-deprived diet (Mean ± SE; Mt_{Dah} = 717 ± 14; Mt_{Mad} = 672 ± 19; Fig. 3b, Table 2). For males kept on a nutrient-rich diet, males with

Table 3 Activity levels (total distance travelled in mm over 30 min) for matched and mismatched mito-Y combinations under the different environmental contexts.

Mito-Y genotype	Mito-Y coevolution	Diet	Mean	SE
Solitary environment				
Dah-Dah	Matched	Yeast	495	25
Dah-Mad	Mismatched	Yeast	483	28
Mad-Dah	Mismatched	Yeast	478	25
Mad-Mad	Matched	Yeast	500	23
Dah-Dah	Matched	Agar	576	19
Dah-Mad	Mismatched	Agar	604	15
Mad-Dah	Mismatched	Agar	629	16
Mad-Mad	Matched	Agar	630	14
Social environment				
Dah-Dah	Matched	Yeast	510	34
Dah-Mad	Mismatched	Yeast	479	38
Mad-Dah	Mismatched	Yeast	584	28
Mad-Mad	Matched	Yeast	520	33
Dah-Dah	Matched	Agar	701	22
Dah-Mad	Mismatched	Agar	733	15
Mad-Dah	Mismatched	Agar	631	35
Mad-Mad	Matched	Agar	714	14

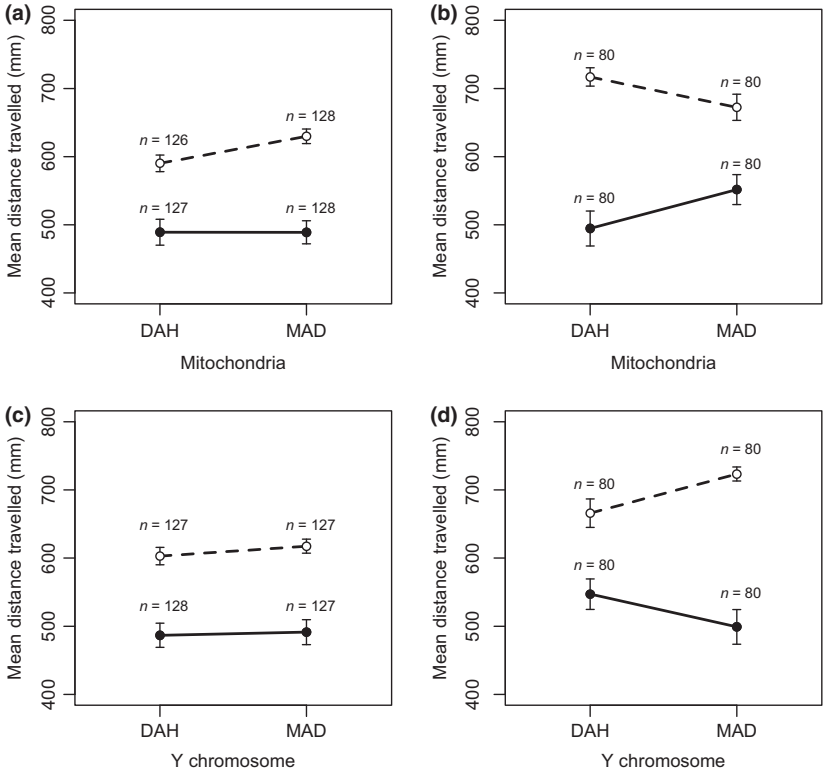

Fig. 3 Mean ± 1 SE male locomotive activity (total distance travelled over 30 min, in mm) per (top panels) mitochondrial haplotype, and (lower panels) Y chromosome haplotype under (a, c) solitary and (b, d) social conditions. Data points connected by solid lines (and solid circles) denote locomotive activity for males kept on nutrient-rich diets, whereas dashed lines (and open circles) indicate flies kept on nutrient-deprived diets.

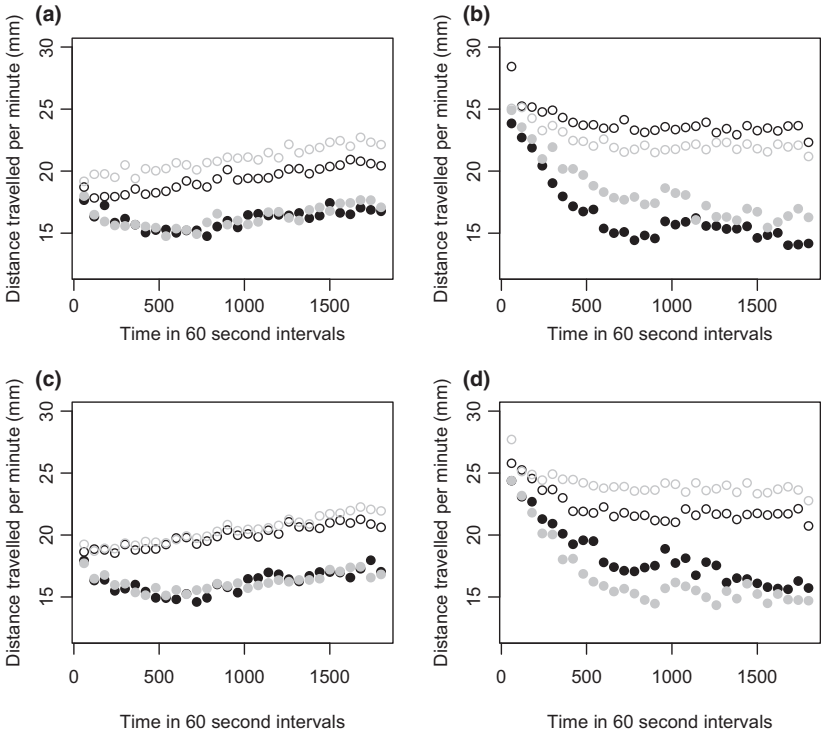

Fig. 4 Distance travelled (mm) at 1-min intervals throughout the 30-min assay (time shown in seconds), which was conducted in the dark, per (top panels) mitochondrial haplotype, and (lower panels) Y chromosome genotype, under solitary (a, c) and social (b, d) conditions. Mean values for Dahomey are shown in black and Madang in grey. Males kept on the nutrient-rich diet are shown in solid circles and males kept on nutrient-deprived diets in open circles.

the Dahomey Y chromosome were more active than males with the Madang Y chromosome (Mean ± SE; $Y_{\mathrm{Dah}} = 547 \pm 22$; $Y_{\mathrm{Mad}} = 499 \pm 25$). However, this pattern was also reversed on the nutrient-deprived diet, with males carrying the Madang Y chromosome more active than males carrying the Dahomey Y (Mean ± SE; $Y_{\mathrm{Dah}} = 666 \pm 21$; $Y_{\mathrm{Mad}} = 723 \pm 10$; Fig. 3d, Table 2). There were no significant interactions between mtDNA and Y haplotypes on activity (Table 2) and matched and mismatched mito-Y combinations performed in similar ways (Table 3). Males with the different mitochondrial haplotypes and Y chromosome genotypes responded to the assay conditions in similar ways (Fig. 4b,d). Male activity per minute declined during the initial stages of the assay, and this decline was particularly rapid for males on the nutrient-rich diet (Fig. 4b,d).

Discussion

We screened for allelic interactions between mitochondrial and Y haplotypes, as a step towards assessing the hypothesis that Y chromosomes harbour regulatory variation that offsets the effects of mtDNA-mediated male harm. Maternal inheritance of mitochondria means that mutations in the mtDNA that are relatively benign or even beneficial to females, but detrimental to males, can accumulate (Frank & Hurst, 1996; Gemmell *et al.*, 2004; Innocenti *et al.*, 2011). The accumulation of these mutations could, in theory, disrupt male function, and even result in the male-biased expression of mitochondrial diseases (Dowling, 2014). The nuclear genome, particularly when being carried by males, will be under strong selection for compensatory modifiers that ameliorate male-specific disruption (Frank & Hurst, 1996; Yee *et al.*, 2013; Beekman *et al.*, 2014). Differences in the rate of co-transmission of mitochondria with different chromosomes may predispose certain genomic locations as more likely to harbour modifiers that offset mitochondrial-mediated male harm (Drown *et al.*, 2012; Dean *et al.*, 2014; Hough *et al.*, 2014; Rogell *et al.*, 2014). The Y chromosome represents one intriguing candidate region. Given that the Y is paternally inherited and thus never co-transmitted with mitochondria (Hill, 2014; Rogell *et al.*, 2014), it will be under strong selection for alleles that maximize male fitness. Moreover, because the Y chromosome is found only (and therefore expressed only) in males, the presence of Y-linked modifier alleles that offset mtDNA-mediated male harm will not negatively interfere with female function, and thus, compensatory mito-Y evolution might ultimately resolve the sexual conflict that is inherent to the maternal transmission of mitochondria.

Our study, however, finds no support for the hypothesis that mitochondrial genomes and Y chromosomes are evolving in compensatory fashion, given we did not detect any interaction between these gene regions on the trait measured – male locomotive activity. Furthermore, our results indicate that the mismatched

combinations of mtDNA and Y chromosome performed no worse than did the coevolved combinations. That said, there are two caveats worth noting. First, in our study, we used just two different genotypes of each mtDNA and Y chromosomal haplotype. Additional tests of this hypothesis, using mitochondrial haplotypes and Y chromosome genotypes from different, or a greater number of populations, would prove valuable in further probing the capacity for compensatory mito-Y evolution. Second, in our study, the nuclear backgrounds associated with the mitochondrial and Y chromosome haplotypes were completely standardized, with an isogenic complement of autosomes and X chromosome sourced from the isogenic line, w^{1118}. The strength of this design was that it enabled us to directly attribute phenotypic variation in male activity to the mitochondrial haplotype or Y chromosome genotype. However, one interesting consequence is that the autosomes and X chromosomes were therefore technically evolutionarily 'mismatched' to the mtDNA and Y chromosome haplotypes (i.e. had not coevolved to the mitochondrial and Y chromosomal haplotypes used in our study). Although it seems unlikely, it is indeed possible, that the w^{1118} nuclear background used here occluded the detection of compensatory mito-Y interactions. Although it would increase the workload threefold, it would be possible and worthwhile to conduct this experiment across three different nuclear backgrounds that have coevolved with each of the mtDNA and Y chromosome haplotypes, to determine whether the nuclear background impeded our capacity to detect mito-Y epistasis for male activity.

Male locomotory activity appeared to be an excellent candidate with which to probe for mitochondrial-Y interactions; given it is sexually dimorphic (Long & Rice, 2007), metabolically reliant (i.e. tightly dependent on products of OXPHOS for its expression), and harbouring sexually antagonistic allelic variation (Long & Rice, 2007). However, it remains to be established whether the expression of locomotive activity, or other core physiological parameters such as metabolic rate, is directly susceptible to male-biased mitochondrial mutational variation. It will therefore be worthwhile to further explore the scope for mitochondrial-Y interactions to affect the expression of sexually dimorphic traits in males; for instance, by screening traits that have previously been shown to be sensitive to male-biased mtDNA variation – fertility (Innocenti *et al.*, 2011; Yee *et al.*, 2013) and ageing (Camus *et al.*, 2012).

Although we did not detect mitochondrial-by-Y genotype interactions, we did find independent effects of both mtDNA and Y chromosome on male activity. These genetic effects were themselves contingent on both the dietary and social environments in which the flies were assayed. From a historical perspective, mtDNA- and Y-linked effects on traits such as locomotory activity might themselves be considered surprising, given that the mitochondrial genome and Y chromosome each contain few protein-coding genes (Anderson *et al.*, 1981; Carvalho *et al.*, 2000, 2001), lack recombination and have low effective population sizes (Avise *et al.*, 1987; Ballard & Kreitman, 1995); all of which should curtail their capacity to evolve adaptive regulatory variation under selection (Lynch, 1997; Bachtrog, 2013). Yet, despite traditional predictions, experimental studies have recently shown that each of these nonrecombining genomic regions can harbour allelic variance that affects male functions (Lemos *et al.*, 2008; Innocenti *et al.*, 2011), and in particular the outcomes of male reproductive processes (Chippindale & Rice, 2001; Dowling *et al.*, 2007b; Yee *et al.*, 2013). Furthermore, although it seems reasonable to predict a link between the mtDNA haplotype and activity phenotype (Løvlie *et al.*, 2014), given the high metabolic reliance of the trait involved, the mechanistic basis for why the Y haplotype sequence would affect the male activity phenotype is less clear. The few protein-coding genes on the Y chromosome are thought to be highly specialized in encoding male-specific functions in *D. melanogaster*, such as components of sperm formation and male fertility in adult life (Carvalho *et al.*, 2000, 2001; Bachtrog, 2013). That said, recent transcriptomic insights reveal an abundance of cryptic regulatory variation on the Y chromosome that exerts widespread consequences on the male transcriptome, affecting functions such as microtubule stability, thermal sensitivity and components of mitochondrial metabolism (Lemos *et al.*, 2008), thus providing the putative link to the activity phenotype. Furthermore, Y haplotype modulates the expression of genes localized to the mitochondria (Lemos *et al.*, 2010).

The environmental sensitivity of our mtDNA- and Y-linked effects was striking, suggesting a previously unrealized capacity for phenotypic plasticity within the mitochondrial genome and Y chromosome. Furthermore, the mitochondrial genetic effects can putatively be tied to just one solitary nonsynonymous SNP in the mtDNA, which separates the Dahomey and Madang haplotypes. That said, we believe it is likely that the effects on activity were influenced by other cryptic polymorphisms with regulatory influence that lay outside of the protein-coding genes, of which 30 separated the two haplotypes (J. N. Wolff, F.M. Camus, D.K. Dowling, pers. comm). Although males were always assayed in solitude, their perceived social context varied according to whether the assay tubes were fresh (containing no CHC pheromone profile) or recycled (possessing a pheromone profile previously laid down by numerous other males). *Drosophila* use cuticular hydrocarbons (CHCs) during mate choice, and males can detect whether a potential rival has previously occupied the environment through the CHC signal (Inoshita *et al.*, 2011). We detected mitochondrial and Y chromosome-linked genetic variation for male activity, but only

when males were assayed in the perceived social environment, not in the solitary environment. Furthermore, within the social environment, we found further context dependency of the genetic effects, dependent on the pre-assay diet treatment of the flies. Males harbouring the Dahomey mtDNA haplotype were less active than males harbouring the Madang haplotype on the nutrient-rich diet, but more active on the nutrient-deprived diet. Similar reversals in the diet-dependent activity were evident across the two Y haplotypes. These results are robust, because each of our genetic combinations was independently duplicated, enabling us to partition out the genetic from other environmental sources of variance, and from cryptic residual nuclear allelic variation in the w^{1118} background. Thus, mitochondrial genomes and Y chromosomes are seemingly entwined in complex genotype × environment interactions.

Although it is unclear why the mtDNA- and Y-linked genetic effects were manifested only in the social context, there are plausible explanations. Firstly, males in the social environment would perceive the risk of sperm competition as high (Friberg, 2006; Bretman *et al.*, 2009), and this would likely result in increased expression of activity-dependent sexual behaviours, such as mate searching or scramble competition for access to females (Kelly *et al.*, 2009), which increase male reproductive success under sexual selection. Male activity is positively associated with male reproductive fitness in *D. melanogaster* (Long & Rice, 2007). Thus, a perceived increase in sexual competition within the social environment would likely manifest as a general increase in male activity levels, which we indeed observed, and possibly facilitate the detection of mtDNA- and Y-haplotypic effects on this trait through stress-induced release of cryptic genetic variation (McGuigan & Sgro, 2009).

The plasticity of the mtDNA and Y haplotype effects across diets, when assayed in the social context, is also noteworthy given the relative performance of each mtDNA and Y haplotype changed across diets. Generally, males were more active when nutrients were restricted during adulthood, prior to the assay, than when provided with a nutrient-rich diet. These diet-dependent effects on activity are consistent with recent neurological evidence showing that both feeding and locomotion are controlled by a small cluster of neurons in *Drosophila* larval brains (Schoofs *et al.*, 2014), which may act in a similar manner in adult flies. Activation of these neurons simultaneously stops feeding and activates locomotion (Schoofs *et al.*, 2014). Although nutrient restriction is likely to impose stress on males, and presumably induced males to increase their exploratory behaviours in the search for food, we note that this effect was only manifested in the social environment. Gene × environment effects may help explain why genetic variation in the mtDNA and Y chromosomes can be maintained within populations living in spatially heterogeneous environments. Some mtDNA- and Y- chromosome haplotypes might confer superior phenotypic performance in one set of environmental conditions (e.g. nutrient-abundant environments with high adult densities and force of sexual selection), but worse performance in others (e.g. nutrient scarce environments with relaxed sexual selection).

To conclude, our study found high environmental sensitivity of mtDNA- and Y-linked haplotypic variance for male locomotory activity. However, we found no evidence for intergenomic interactions involving alleles spanning mtDNA and Y haplotypes. Whether or not allelic variance harboured within the mitochondrial genome and Y chromosome might interact to affect the expression of other male life-history traits, or under different genomic backgrounds, and whether the outcomes of any such mitochondrial-Y interactions are themselves environmentally sensitive remain questions deserving of further experimental attention.

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Data accessibility

Data deposited at Dryad: doi: 10.5061/dryad.q86q0

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Mean ± 1 SE male locomotive activity (total distance travelled over 30 min, in mm) as a function of parental age at egg laying.
Figure S2 Distribution of male activity levels in the two social contexts.
Table S1 Model-averaged coefficients of variables for GLMM in the perceived solitary environment.
Table S2 Model-averaged coefficients of variables for GLMM in the perceived social environment.

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