Evidence for a sexual dimorphism in gene expression noise in metazoan species

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Many biological processes depend on very few copies of intervening elements, which makes such processes particularly susceptible to stochastic fluctuations of these elements. The intrinsic stochasticity of certain processes is propagated across biological levels causing genotype- and environment-independent biological variation, which might permit populations better coping with variable environments. Biological variation of stochastic nature might also allow the accumulation of variation at the genetic level hidden from natural selection, which might have a great potential for population diversification. The study of any mechanism that resulted in the modulation of stochastic variation is, therefore, of potential wide interest. Here, I propose that sex might be an important modulator of the stochastic variation in gene expression, i.e., gene expression noise. Based on known associations between different patterns of gene expression variation, I hypothesize that in metazoans gene expression noise might be generally larger in heterogametic than in homogametic individuals. I directly test this hypothesis by comparing putative genotype- and environment-independent variation in gene expression between females and males of Drosophila melanogaster strains. Also, considering the potential effect of the propagation of gene expression noise across biological levels, I test indirectly the existence of a metazoan sexual dimorphism in gene expression noise by analyzing putative genotype- and environment-independent variation in phenotypes related with the interaction with the environment in D. melanogaster strains and metazoan species. As hypothesized, the results of these analyses are consistent with gene expression being generally noisier in heterogametic than in homogametic individuals. Further analyses and discussion of existing literature permits speculating the sexual dimorphism in gene expression noise is ultimately based on the nuclear dynamics in gametogenesis and very early embryogenesis of sex-specific chromosomes, i.e., Y and W chromosomes.

2 **Evidence for a sexual dimorphism in gene expression noise in metazoan species**

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INTRODUCTION

24 Biological systems are prone to vary even in the absence of genetic modifications or environmental

25 changes (Burga & Lehner 2012; Feinberg & Irizarry 2010; Kaern et al. 2005; Kilfoil et al. 2009;

26 Lehner 2013; Raj & van Oudenaarden 2008; Raser & O'Shea 2005). Although not yet completely

27 understood, biological variation in the absence of genetic or environmental cues ultimately depends on

28 stochastic transitions and interactions of the elements that contribute to biological processes (Burga &

29 Lehner 2012; Feinberg & Irizarry 2010; Kaern et al. 2005; Kilfoil et al. 2009; Lehner 2013; Raj & van

30 Oudenaarden 2008; Raser & O'Shea 2005). One of the biological processes known to vary even in the

31 absence of genetic or environmental cues is gene expression (Burga & Lehner 2012; Feinberg &

32 Irizarry 2010; Kaern et al. 2005; Kilfoil et al. 2009; Lehner 2013; Raj & van Oudenaarden 2008; Raser

33 & O'Shea 2005). Many of the multiple steps encompassed by gene expression depend on very small

34 numbers for some of the intervening elements, making gene expression particularly susceptible to the

35 intrinsic stochasticity of these elements transitions and interactions (Burga & Lehner 2012; Feinberg &

36 Irizarry 2010; Kaern et al. 2005; Kilfoil et al. 2009; Lehner 2013; Raj & van Oudenaarden 2008; Raser

37 & O'Shea 2005).

39 Biological variation ultimately caused by stochastic events at the molecular level can be an important

40 evolutionary driving force (Burga & Lehner 2012; Feinberg & Irizarry 2010; Kaern et al. 2005; Kilfoil

41 et al. 2009; Lehner 2013; Raj & van Oudenaarden 2008; Raser & O'Shea 2005). On one side,

42 stochastic biological variation represents on its own a cheap phenotypic diversification that might

43 permit populations coping with variable environments (Burga & Lehner 2012; Feinberg & Irizarry

44 2010; Kaern et al. 2005; Kilfoil et al. 2009; Lehner 2013; Raj & van Oudenaarden 2008; Raser &

45 O'Shea 2005). On the other side, stochastic biological variation might act as genetic capacitor

46 (Chalancon et al. 2012). Genetic capacitance refers to the accumulation of genetic variation with no

47   phenotypic effect, i.e., cryptic genetic variation, and its release upon capacitance attenuation (Masel

48   2013; Masel & Trotter 2010; Paaby & Rockman 2014). Genetic variation with phenotypes

49   indistinguishable from the spectrum of stochastic phenotypes will be allowed to accumulate in a

50   cryptic state until stochastic capacitance is somehow attenuated, and cryptic genetic variation becomes

51   phenotypically relevant (Chalancon et al. 2012; Paaby & Rockman 2014). Thus, stochastic biological

52   variation could serve natural populations short-term endurance to variable environments, while

53   permitting the accumulation of cryptic genetic variation with great potential for their diversification.

54   Although stochastic variation is an intrinsic property of biological systems, any factor that modulated

55   stochastic biological variation would have direct and capacitance-driven indirect effects on short-term

56   response to environmental changes and long-term diversification of natural populations. The

57   identification of factors that resulted in the modulation of stochastic variation is, therefore, of broad

58   interest.

59

60   The integration of literature on gene expression variation suggests sex can be an important modulator

61   of stochastic biological variation in metazoan species. On one hand, as it would be expected from the

62   contribution of any source of stochastic biological variation to genetic capacitance, stochastic variation

63   in gene expression, or gene expression noise, has been shown to positively correlate with gene

64   expression variation in response to conditional changes and divergence (Dong et al. 2011). Genetic

65   variation for loci with noisier gene expression would be prone to accumulate in a cryptic state until

66   capacitance is attenuated, whereas genetic variation for loci with less noisy gene expression would be

67   more often phenotypically noticeable, and removed from populations if detrimental. On the other hand,

68   in dipterans and mammals, male-biased expression responds to conditional changes and diverges faster

69   than homogametic female-biased or unbiased gene expression, whereas in birds, it is the female-biased

70   expression the one with faster conditional response and divergence (Assis et al. 2012; Ellegren &

71   Parsch 2007; Gallach et al. 2011; Jiang et al. 2010; Mank 2009; Mank et al. 2007; Meisel 2011; Parsch

72   & Ellegren 2013; Singh & Artieri 2011; Wyman et al. 2010; Wyman et al. 2011). In dipterans and

73   mammals, females are homogametic and males are heterogametic, and, in birds, females are

74   heterogametic and males homogametic. Thus, it could be generalized that, in metazoan species,

75   heterogametic sex-biased gene expression responds to conditional changes and diverges faster than

76   homogametic sex-biased or unbiased gene expression (Assis et al. 2012; Ellegren & Parsch 2007;

77   Gallach et al. 2011; Jiang et al. 2010; Mank 2009; Mank et al. 2007; Meisel 2011; Parsch & Ellegren

78   2013; Singh & Artieri 2011; Wyman et al. 2010; Wyman et al. 2011).

79

80   Considering the contribution of gene expression noise to genetic capacitance, the differences in sex-

81   biased gene expression variation noticed in metazoan species could be explained if gene expression

82   was generally noisier in the heterogametic sex (Figure 1). Phenotypes due to genetic variation in loci

83   that are expressed in heterogametic individuals would often be indistinguishable from the broad

84   spectrum of phenotypes dependent on the generally noisier gene expression of the heterogametic sex.

85   Phenotypes due to genetic variation in loci that are expressed in homogametic individuals would be

86   often distinguishable from the narrower spectrum of phenotypes dependent on the less noisy gene

87   expression of the homogametic sex, and purged from populations if detrimental. Therefore, genetic

88   variation for loci that are specifically expressed or overexpressed in the heterogametic sex would be

89   prone to accumulate in a cryptic state, and become phenotypically relevant when capacitance is

90   somehow attenuated by conditional changes in single and/or divergent populations.

91

92   Here, I present evidence for the existence of a sexual dimorphism in gene expression noise in metazoan

93   species by comparing female and male putative genotype- and environment-independent variation for

94   transcript abundance and other phenotypic traits in *Drosophila melanogaster*, and analyzing sex-biased

95   dispersal in metazoan species. These analyses are consistent with gene expression being generally

96   noisier in the heterogametic sex, and point to the possibility that this sexual dimorphism in gene

97   expression noise might be ultimately dependent on sex-specific chromosomes, i.e., *Y* and *W*.

98   Mechanistic details on the effect of sex-specific chromosomes on gene expression noise are briefly

99   discussed.

100

101   **MATERIALS AND METHODS**

102   The original source of datasets used in this article is: *D. melanogaster* transcript abundance in adult

103   females and males (Diaz-Castillo et al. 2012), DGRP strains phenotypic data (Huang et al. 2014;

104   Mackay et al. 2012), coordinates of regions with distinctive chromatin features in *D. melanogaster*

105   somatic cells (Filion et al. 2010), and, sex-biased dispersal in metazoan species (Petit & Excoffier

106   2009). Coordinates of genes coding for transcripts in Diaz-Castillo and coworkers dataset were

107   obtained from FlyBase (Diaz-Castillo et al. 2012; St Pierre et al. 2014). Microsoft® Excel® for Mac

108   2011 (Microsoft Corporation) was used to perform Monte Carlo-Wilcoxon matched-pairs signed-ranks

109   tests, and Monte Carlo simulations. Prism 5 for Mac OS X (GraphPad Software, Inc) was used to

110   analyze polynomial regressions between transcript abundance mean and CV for *D. melanogaster*

111   strains and genotypes (Diaz-Castillo et al. 2012). Data processing and statistical analyses are described

112   in detail on the Results and Discussion section, and Table footnotes.

113

114   **RESULTS AND DISCUSSION**

115   **Direct evidence for the existence of a sexual dimorphism in gene expression noise in *Drosophila*.**

116   The existence of the hypothesized sexual dimorphism in gene expression noise should be easy to test.

117   When directly comparing gene expression between sexes, putative genotype- and environment-

118   independent variation should be generally larger in heterogametic than in homogametic individuals. In

119   2012, Diaz-Castillo and coworkers performed genome-wide microarray-based transcript abundance

120   analyses of adult naive females and males in six *D. melanogaster* strains (Diaz-Castillo et al. 2012).

121   These analyses resulted in abundance data of 16,637 transcripts for three biological replicates per strain

122   and sex (36 samples: 6 strains x 2 sexes x 3 biological replicates). All analyzed samples were

123   maintained under the same environmental conditions, and RNA was extracted simultaneously to

124   minimize environmental and technical variation between biological replicates. With the only exception

125   of the dosage of sex chromosomes, i.e., *X* and *Y*, genotype and environment were identical for

126   biological replicates of each strain. Thus, any transcript abundance variation detected for biological

127   replicates of a single strain might only be of sexual and/or stochastic nature, making it possible to test

128   the potential existence of a generalized difference in gene expression noise between sexes.

129

130   Interestingly, five of the analyzed strains shared the same exact genotype with at least one another

131   strain. INV1 and INV2 genotype is $w^{1118}/y^+ Y$; $In(2R)$ $[P\{FRT\}^{CB-0236-3}, P\{FRT,w^+\}^{5-HA-1995}]/SM6a$,

132   whereas SIM1, REV1 and REV2 genotype is $w^{1118}/y^+ Y$; $P\{FRT,w^-\}^{CB-0236-3}, P\{FRT, w^-\}^{5-HA-1995}/SM6a$.

133   Since all samples were reared in the same conditions, transcript abundance data of strains with the

134   same genotype could be pooled together permitting performing analyses with increasing analytical

135   power (6 and 9 biological replicates per sex for INV and SIM/REV genotypes, respectively).

136

137   Since in *Drosophila*, females are homogametic and males are heterogametic, it would be expected, that

138   in the dataset under study, measures for putative stochastic variation in transcript abundance were

139   generally larger in males than in females. To test this prediction, I retrieved Diaz-Castillo and

140   coworkers dataset from Gene Expression Omnibus database (GSE31120)(Diaz-Castillo et al. 2012).

141   Transcript abundance data in this dataset had been already normalized to permit comparisons between

142   samples (Diaz-Castillo et al. 2012). To compare transcript abundance variation putatively independent

143   of genetic and environmental cues, I calculated transcript abundance coefficients of variation for

144   females and males in each strain and after pooling data of strains with the same genotype (CV_F and

145   CV_M, respectively). The existence of a general trend for the difference of putative stochastic variation

146   in transcript abundance between sexes was tested by performing Monte Carlo-Wilcoxon matched-pairs

147   signed-ranks tests for each strain and genotype. For each transcript in the dataset, I subtracted CV_M

148   from CV_F. CV differences were ranked upon the absolute value of their difference from lower to

149   higher, and signs were assigned to each rank upon the sign of the difference between CV_F and CV_M.

150   Wilcoxon sum of signed ranks (W) were calculated by adding signed ranks for all the transcripts in the

151   dataset, for each strain and genotype. W is perfectly suited for the identification of general biases in

152   collections of paired data, because it is sensitive both to the number or elements in the dataset with

153   biased measures and the extent of such biases, eliminating the need to set arbitrary thresholds to infer

154   trends of putative biologically significance. To estimate the significance of observed W, I recalculated

155   W after randomly rearranging all CV data for each strain and genotype 10,000 times. Simulated W

156   would represent the value W can adopt for a dataset with the same number of elements and the same

157   value range without existing significant differences between sexes.

158

159   Considering W ultimately depends on the subtraction of CV_M from CV_F for each transcript, if gene

160   expression was indeed generally noisier in males than in females, it would be expected that observed

161   W were negative and commonly lower than simulated ones. In fact, except for strain INV2, all

162   observed W were negative and lower than any simulated W (Table 1 and Figure 2). Strains INV1 and

163   INV2 are genotypically identical and yet they differ with regard to the significance of the hypothesized

164   sexual dimorphism in gene expression noise. It is worth noting that observed W are considerably more

165   variable for single-strain analyses than for analyses of data from multiple strains sharing the same

166   genotype. Since there is a difference in the extent of biological replication between single- and

167   multiple-strain analyses (Table 1), it is possible that non-significant observed W, as it is the case of

168   strain INV2, might be related with power limitations imposed by lower levels of biological replication.

169   In fact, non-significant trends exclusively associated to data encompassing lower levels of biological

170   replication are also noticeable in forthcoming analyses, which stresses the importance of larger

171   biological replication for the study of biological variation of putative stochastic nature.

172

173   The study of the association between transcript abundance mean and CV for each sex in the dataset

174   under study also supports the existence of a sexual dimorphism in gene expression noise in *D.*

175   *melanogaster*, and hints about its potential cause. It is commonly accepted that the variation in gene

176   expression noise is mostly associated to changes in the mean level of gene expression (Bar-Even et al.

177   2006; Newman et al. 2006; Taniguchi et al. 2010). In those cases in which gene expression noise was

178   modulated by factors other than the variation in the mean level of expression, it should be expected a

179   weak association between measures for the mean level and stochastic variation in gene expression. In

180   Diaz-Castillo and coworkers dataset, with the exception of INV1 and REV2, the coefficient of

181   determination for the quadratic regression between transcript abundance mean and CV is considerably

182   larger in females than in males (Table 2). These results suggest that the association between the mean

183   level and stochastic variation in gene expression is weaker in males than in females. The male

184   reduction in gene expression noise variation that could be explained solely through changes in the

185   mean level of gene expression is consistent with the possibility that factors specific to males,

186   heterogametic in *Drosophila*, promoted gene expression noise.

187

188   **Indirect evidence for the existence of a sexual dimorphism in gene expression noise in *Drosophila*.**

189   It could be argued that the genome-wide difference in gene expression noise between *D. melanogaster*

190   females and males here reported is an aberration of a particular genomic background, and/or the use of

191   a particular gene expression quantification methodology. For example, the experimental setup that

192   resulted in Diaz-Castillo and coworkers dataset did not include technical replicates (Diaz-Castillo et al.

193   2012). Although it seems unlikely that consistent differences in the processing of female and male

194   samples were to fully account for the generalized difference in transcript abundance variation detected

195   in the dataset in question, it is currently not possible to remove from the measures of putative gene

196   expression noise the variation due exclusively to technical issues. Other analyses using different *D.*

197   *melanogaster* strains, other metazoan species, and/or other quantitative methods to measure gene

198   expression are needed to confirm the existence and characterize further the hypothesized sexual

199   dimorphism in gene expression noise in metazoan species.

200

201   Notwithstanding these limitations, the existence of a sexual dimorphism in gene expression noise in

202   metazoan species should also be indirectly tested by studying sexual differences for the intrinsic

203   variation of other phenotypic traits. Since the propagation of gene expression noise across biological

204   levels would result in genotype- and environment-independent variation of other phenotypic traits that

205   might permit populations coping with environmental changes (Burga & Lehner 2012; Feinberg &

206   Irizarry 2010; Kaern et al. 2005; Kilfoil et al. 2009; Lehner 2013; Raj & van Oudenaarden 2008; Raser

207   & O'Shea 2005), the existence of a sexual dimorphism in gene expression noise could be indirectly

208   supported by clear sexual differences for the variation of phenotypic traits related with the interaction

209   with the environment.

210

211   The *D. melanogaster* Genetic Reference Panel (DGRP) is a collection of over 200 inbred lines derived

212   from a single *D. melanogaster* population in Raleigh (North Carolina, USA)(Huang et al. 2014;

213   Mackay et al. 2012). The generation of these lines aimed to encompass standing genetic variation

214   present in the founder population, so their intensive genotypic and phenotypic characterization helped

215   in addressing the relationship between the variation in genes and phenotypes. Among other assays,

216   females and males from DGRP lines were used to measure their response to three environmental

217   stressors, namely startle, starvation, and cold (Mackay et al. 2012). If the sexual dimorphism in gene

218   expression noise detected in Diaz-Castillo and coworkers dataset was extensive to all *D. melanogaster*

219   populations, and a generalized difference in gene expression noise between sexes resulted in a sexual

220   difference in phenotypic variation, it would be expected that, regardless of genotypic and

221   environmental differences, the response to these stressors were more variable in males than in females

222   for DGRP lines.

223

224   To test this prediction, I performed Monte Carlo-Wilcoxon matched-pairs signed-ranks tests with

225   DGRP startle response, starvation resistance and chill comma recovery data

226   (http://dgrp2.gnets.ncsu.edu)(Mackay et al. 2012). For groups of females and males from the same

227   strain assayed in the same conditions, I calculated CV_F and CV_M. Although these three traits were

228   originally measured individually, the assortment of assayed individuals was different for each trait.

229   Data arrangement to best accommodate it in pairs of same genotype females and males assayed in the

230   same conditions resulted in differences in the level of biological replication and the number of

231   elements under study for each trait (Table 3). Startle response was assayed using groups of individuals

232   of the same sex per vial in two different dates (Mackay et al. 2012). Since environmental conditions

233   between dates might be slightly different, I considered assays in different days separately. I considered

234   individuals within each group independent biological replicates, and calculated startle response CV_F

235   and CV_M using measures for individuals of the same strain and sex assayed simultaneously. Starvation

236   resistance was assayed using groups of individuals of the same sex per vial simultaneously (Mackay et

237   al. 2012). I considered groups of individuals of the same strain and sex independent biological

238   replicates, and averaged the starvation response measures for individuals in the same group. I

239   calculated starvation resistance CV_F and CV_M using averaged measures for each biological replicate of

240   the same strain and sex. Chill comma recovery was assayed using same strain and sex groups of single

241   individual vials assayed simultaneously (Mackay et al. 2012). I considered each group of individuals of

242   the same strain and sex independent biological replicates, and averaged the chill comma recovery

243   measures for individuals in the same group. I calculated chill comma recovery CV_F and CV_M using

244   averaged measures for each biological replicate of the same strain and sex.

245

246   For each pair of CV_F and CV_M, I subtracted the latter from the former. CV differences were ranked

247   according to their absolute value from lower to higher, and signs were assigned to each rank upon the

248   sign of the difference between CV_F and CV_M. W was calculated for each trait using the observed

249   assortment of CV, and after randomly rearranging all CV data for each trait 10,000 times. As expected

250   if gene expression noise-dependent phenotypic variation was generally larger in males than in females,

251   observed W were negative for all three traits (Table 3). Moreover, for startle response and starvation

252   resistance, the fraction of simulated W lower than observed W was below the common threshold of

253   significance ($P<0.05$)(Table 3). Although for chill comma recovery observed W was not significantly

254   different from simulated W ($P>0.05$), it is worth noting that this is the trait here considered with the

255   lowest level of biological replication, and, still, observed W is lower than the majority of simulated W

256   (8,527 out of 10,000 simulated W are larger than the observed one)(Table 3). Further analyses of

257   sexual differences in the intrinsic variation of chill comma recovery with larger biological replication

258   would be needed to decide if this trait shows a similar trend to the other two.

259

260   **Indirect evidence for the existence of a sexual dimorphism in gene expression noise in Metazoa.**

261   Sexual differences in gene expression noise with effect on phenotypic traits related with the interaction

262   with the environment could influence each sex capacity to endure environmental changes, and,

263   therefore, their spatiotemporal dynamics. For instance, if larger gene expression noise resulted in a

264   phenotypic variation that facilitated enduring environmental changes, it would be expected that noisier

265   heterogametic individuals were able to better survive drastic environmental changes or occupy broader

266   and/or more diverse geographies than less noisy homogametic individuals.

267

268   The movement of individuals away from their birth place or dispersal is constrained both by

269   environmental conditions and factors intrinsic to dispersing individuals (Clobert et al. 2012). It is

270   known that dispersal is sexually dimorphic in many species (Clobert et al. 2012; Dobson 2013;

271   Greenwood 1980). For example, in mammals males tend to disperse more than females, whereas in

272   birds females are the ones dispersing more (Clobert et al. 2012; Dobson 2013; Greenwood 1980). Since

273   mammal males and bird females are heterogametic, their ability to disperse more than their

274   homogametic relatives would fit with an enhanced ability to endure environmental changes possibly

275   granted by a generalized larger gene expression noise in heterogametic individuals. The study of

276   karyotypic constraints to sex-biased dispersal lends indirect support to the existence of the sexual

277   dimorphism in gene expression noise in metazoan species promoted by heterogametic-specific factors.

278

279   In 2010, Petit and Excoffier studied the contribution of different factors to interspecific gene flow,

280   using a dataset that included 36 widely distributed metazoan species for which sex-biased dispersal

281   trends and chromosome systems were known (Petit & Excoffier 2009). In the dataset in question, birds,

282   insects, and mammals were represented by 11, 11, and 14 cases, respectively (Supplemental Table S1).

283   To confirm sex-biased dispersal could indeed reflect a generalized larger gene expression noise in

284   heterogametic metazoans, I calculated the fraction of cases within Petit and Excoffier dataset where the

285   heterogametic sex was the one dispersing the most, i.e., the fraction of heterogamety-biased dispersal.

286   To test the significance of observed heterogamety-biased dispersal, I also calculated the fraction of

287   heterogamety-biased dispersal after randomly rearranging chromosome system tags 10,000 times. As

288   expected if metazoan heterogametic individuals tended to disperse more than homogametic

289   individuals, the observed fraction of heterogamety-biased dispersal is significantly larger than expected

290   by chance (Table 4).

291

292   Interestingly, Petit and Excoffier dataset includes five cases in which species commonly or completely

293   lack sex-specific chromosomes, *W/Y* chromosomes hereinafter (Supplemental Table S1)(Ardila-Garcia

294   & Gregory 2009; Kandul et al. 2007; Morgan-Richards 1997; Narita et al. 2011; Petit & Excoffier

295   2009; Spence 1990). When the fraction of heterogamety-biased dispersal was calculated separately for

296   species where heterogametic individuals carried or lack *W/Y* chromosomes, heterogametic individuals

297   significantly disperse the most only if they carried *W/Y* chromosomes (Table 4). These results suggest

298   the presence of *W/Y* chromosomes is an even better predictor for dispersal than heterogamety is.

299   Considering I argued differences in sex-biased dispersal could be consequence of sexual differences for

300   the variation in phenotypic traits that influence the interaction with the environment, ultimately caused

301   by a generalized difference in gene expression noise between sexes, *W/Y* chromosomes-driven

302   dispersal could hint about a potential connection between *W/Y* chromosomes and an increase in

303   putative gene expression noise-based phenotypic variation. This inference agrees with the possibility

304   that in *D. melanogaster* gene expression noise is promoted by male-specific factors suggested by the

305   study of Diaz-Castillo and coworkers dataset. The connection between *W/Y* chromosomes and gene

306   expression noise requires further consideration.

307

308   **The sexual dimorphism in gene expression noise might depend on *W/Y* chromosomes acting as**

309   **genomic tuning knobs.** In *D. melanogaster*, the *Y* chromosome has been shown to have a modulator

310   effect on phenotypic variation of stochastic nature, and gene expression variation across the genome.

311   On one side, *D. melanogaster Y* chromosome acts as a suppressor of the phenomenon known as

312   position effect variegation (PEV), namely the stochastic inactivation of genes when they relocate into

313   or juxtaposed to regions with highly compacted chromatin, i.e., heterochromatin (Elgin & Reuter 2013;

314   Gowen & Gay 1934). On the other side, variation in *D. melanogaster Y* chromosome has been shown

315   to have an indirect effect on the expression of multiple genes across the genome, a phenomenon

316   referred to as *Y*-linked regulatory variation (YRV)(Lemos et al. 2010; Paredes et al. 2011; Sackton &

317   Hartl 2013; Zhou et al. 2012). Although YRV affects genes widespread in the genome, it tends to be

318   more accentuated for loci located in environments where gene expression is actively repressed, i.e.,

319   heterochromatin and nuclear periphery (Sackton & Hartl 2013). Since *D. melanogaster Y* chromosome

320   indirect effects seem to be heterochromatin-centric, and *D. melanogaster Y* chromosome encodes for

321   very few genes with functionalities not obviously connected with the regulation of gene expression, it

322   has been argued that *D. melanogaster Y* chromosome indirect effects can depend on it acting as a sink

323   for heterochromatin-forming elements (Berloco et al. 2014; Sackton & Hartl 2013; Zuckerkandl 1974).

324   The highly heterochromatic *D. melanogaster Y* chromosome requires such a large amount of elements

325   needed for heterochromatin formation that these would become depleted in heterochromatic loci

326   located in other chromosomes, i.e., non-*Y* heterochromatic loci. *Y* chromosome-mediated depletion in

327   heterochromatin-forming elements in non-*Y* loci will affect their level of chromatin compaction, and,

328   therefore, their accessibility for the transcription machinery. Since some of the elements required for

329   heterochromatin formation play also roles in non-heterochromatic loci (Cryderman et al. 2005; Fanti et

330   al. 2008), *D. melanogaster Y* chromosome sink effect would be heterochromatin-centric, but not

331   heterochromatin-exclusive.

332

333   *W/Y* chromosomes have originated from autosomes several independent times, and yet, they all seem to

334   proceed through a progressive loss of coding elements and enrichment in repetitive DNA (Bachtrog

335   2013; Ellegren 2011; Mank 2012; O'Meally et al. 2010). Except for chromosomes in early stages of

336   this process, *W/Y* chromosomes tend to be the largest repositories in repetitive DNA in the genome,

337   making of them the largest heterochromatic bodies in the nucleus (Ellegren 2011; Mank 2012;

338   O'Meally et al. 2010). To test if the putative dependence on *W/Y* chromosomes of the sexual

339   dimorphism in gene expression noise here documented was based on these chromosomes acting as a

340   sink for heterochromatic-forming elements, I used again Diaz-Castillo and coworkers dataset (Diaz-

341   Castillo et al. 2012).

342

343   In 2010, Filion and coworkers used genome-wide binding patterns of 53 chromatin elements to define

344   five components in the *D. melanogaster* genome, which they symbolized with different colors (Filion

345   et al. 2010). Four of these components are related with stable or transitory gene repression. BLUE and

346   GREEN represent known heterochromatin repositories. BLACK and RED are enriched in loci with

347   tissue-restricted gene expression and lamin-binding targets, suggesting they might be located towards

348   the repressive environment of the nuclear periphery. YELLOW is the only component associated with

349   broadly expressed loci. Transcripts in the Diaz-Castillo and coworkers dataset were assigned colors if

350   the genomic region where they are encoded according to *D. melanogater* genome annotation were

351   spanned in their entirety by a single-color tract according to Filion and coworkers (Filion et al. 2010; St

352   Pierre et al. 2014). As a measure of sexual dimorphism in gene expression noise, I calculated W for

353   transcripts in each color per strain and genotype, following the afore explained process. The

354   significance of the sexual dimorphism in gene expression noise for loci in each color was evaluated by

355   recalculating W after randomly rearranging 10,000 times all CV for each strain and genotype.

356

357   A common pattern to strains and genotypes in the dataset is that observed W are negative and

358   considerably smaller for YELLOW transcripts than for transcripts of any other color (Figure 3, and

359   Table 5). Such pattern suggests male-biased gene expression noise is less extreme for loci within

360   repressive areas of the genome, which is consistent with what it would be expected if *D. melanogaster*

361   *Y* chromosome sink effect altered chromatin compaction across the genome and, consequently, gene

362   expression noise. Since in males much of the material required for heterochromatin formation would be

363   diverted towards the *Y* chromosome, non-*Y* heterochromatic loci would be expected to be more

364   compacted in females than in males (Figure 4). Higher chromatin compaction structures have been

365   associated with an increase in gene expression noise due to slower gene expression dynamics (Kaern et

366   al. 2005; Raj & van Oudenaarden 2008; Raser & O'Shea 2005). Thus, non-*Y* heterochromatic loci

367   would be expected to be considerably noisy in females, and, therefore, show less extreme sexual

368   dimorphism in gene expression noise.

369

370   The analysis of the sexual dimorphism in gene expression noise for genes in different compartments

371   also showed that, with the exception of INV2 and REV2, observed W were negative and smaller than

372   any simulated W for each chromatin color (Table 5). These results suggest gene expression is generally

373   noisier in males than in females regardless of chromatin compaction and subnuclear localization. Thus,

374   although the deployment of heterochromatin-forming elements in the presence/absence of *W/Y*

375   chromosomes might cause differences in chromatin compaction and gene expression noise across

376   genomes, on its own, a heterochromatin sink effect common to all *W/Y* chromosomes could not fully

377   explain the existence of the putative sexual dimorphism in gene expression noise in metazoan species.

378

379   In 1997, David King and coworkers proposed the concept "genetic tuning knobs" to refer to the

380   indirect effect the variation in repetitive DNA has on genetic elements they belong to or are intimately

381   associated with (King et al. 1997). Small, reversible, and very frequent variation in repetitive DNA

382   copy number would have a fine-tuning modifier effect for coding units and/or non-coding motives with

383   regulatory attributes (Gemayel et al. 2010; Kashi & King 2006; King et al. 1997). If repetitive DNA

384   motives in *W/Y* chromosome were susceptible to frequent variation in copy number, it could be

385   expected that the fraction of repetitive DNA in *W/Y* chromosomes was a very variable trait. Since the

386   heterochromatic nature of *W/Y* chromosomes depends on their content in repetitive DNA, it could be

387   inferred that *W/Y* chromosomes with slightly different fractions of repetitive DNA would withdraw

388   slightly different amounts from the limiting pool of heterochromatin-forming elements (Figure 4). The

389   variation in the amount of heterochromatin-forming elements deployed in *W/Y* chromosomes with

390   slightly different fractions of repetitive DNA would be translated in a variation in chromatin

391   compaction in genes across the genome, and, therefore in their accessibility to the transcription

392   machinery (Figure 4). Thus, a small variation in the fraction of repetitive DNA in *W/Y* chromosomes

393   could ultimately cause an increase in gene expression noise across the genome in heterogametic

394   individuals. In other words, the sexual dimorphism in gene expression noise in metazoan species could

395   be consequence of *W/Y* chromosomes acting as tuning knobs at a genomic scale. Following with the

396   musical theme, *W/Y* chromosomes could be compared to the pulley-and-lever system of single-stringed

397   whamolas. Handling of a whamola lever modifies the tension of the string causing a variation in noise

398   when played. Similarly, variation in *W/Y* chromosomes repetitive DNA would modify chromatin

399   compaction across the genome, and, with it, the accessibility for the transcription machinery.

400

401   The characterization of genome regions enriched in repetitive DNA is one of the unresolved challenges

402   in genomics (Chain et al. 2009; Treangen & Salzberg 2012). In fact, genomic regions enriched in

403   repetitive DNA, such as *W/Y* chromosomes, are remarkably misrepresented in genome assemblies. The

404   confirmation of *W/Y* chromosomes role as genomic tuning knobs, and its importance for the sexual

405   dimorphism in gene expression noise here documented are currently hard to address empirically.

406   Despite current technical limitations, in *D. melanogaster* indirect evidence exists consistent with the

407   possibility that the *Y* chromosome might act as a genomic tuning knob. On one side, large and subtler

408   induced changes in the fraction of repetitive DNA in *D. melanogaster Y* chromosome have effects

409   similar to PEV modulation and YRV (Berloco et al. 2014; Dimitri & Pisano 1989; Paredes et al. 2011).

410   Also, the fraction of repetitive DNA in *W/Y* chromosomes has been shown to be very variable between

411   closely related species and natural populations of a single species (Halfer 1981; Hughes & Rozen 2012;

412   Lyckegaard & Clark 1989; Lyckegaard & Clark 1991; Nova et al. 2002; Paredes et al. 2011; Repping

413   et al. 2006; Sahara et al. 2012; Singh et al. 1980). In *D. melanogaster*, repetitive DNA derivatives

414   suggested to mediate its copy number plasticity have been detected for the *Y* chromosome in

415   individuals of a single strain (Cohen et al. 2005; Cohen & Segal 2009), consistent with the possibility

416   that *D. melanogaster Y* chromosome repetitive DNA variation can be spontaneous and frequent. Thus,

417   at least for *D. melanogaster Y* chromosome, independent lines of evidence exist consistent with a

418   frequent variation in its repetitive DNA and for this type of variation to have an effect on gene

419   expression across the genome, as it would be expected if this chromosome acted as a genomic tuning

420   knob. Further research is required to confirm *W/Y* chromosomes acting as genomic tuning knobs

421   caused a sexual dimorphism in gene expression noise in *D. melanogaster* and other metazoan species.

422

423   **The sexual dimorphism in gene expression noise might depend on nuclear dynamics in**

424   **gametogenesis and early embryogenesis.** The *W/Y* chromosomes genomic tuning knob-sink effect

425   model to explain sexual differences in gene expression noise in metazoan species is contingent not only

426   upon the spontaneous and frequent variation in *W/Y* chromosomes repetitive DNA, but also upon

427   heterochromatin-forming elements being in roughly the same limiting amounts in nuclei of both sexes

428   (Figure 4). There is only one moment common to all metazoans in which the pool of heterochromatin-

429   forming elements might be in comparable limiting amounts regardless of chromosome composition.

430   From oocyte fertilization until the zygotic genome gets fully activated, all new chromatin is formed at

431   the expense of maternally-deposited material (Banaszynski et al. 2010; Baroux et al. 2008; Tadros &

432   Lipshitz 2009). Furthermore, sperm chromosomes enter the oocyte in a state of extreme compaction

433   thanks to the almost complete substitution of histones by protamine-like proteins along

434   spermatogenesis (Banaszynski et al. 2010; Tadros & Lipshitz 2009). During the transformation of

435   sperm nuclei into paternal pronuclei, protamine-like proteins are substituted by histones, a process so

436   intensive that will take a very large amount of the already limiting maternally-deposited material

437   (Banaszynski et al. 2010; Tadros & Lipshitz 2009). In this context, the deployment of heterochromatin-

438   forming elements during the first zygotic nuclear divisions might not run the same course in the

439   presence or absence of *W/Y* chromosomes, or when the amount of repetitive DNA in these

440   chromosomes is different (Figure 4). It could be speculated that the sexual dimorphism in gene

441   expression noise in metazoan species ultimately depended on *W/Y* chromosomes acting as genomic

442   tuning knobs-sinks very early in embryogenesis.

443

444   Interestingly, in *Drosophila* and Lepidoptera, evidence exists for the preferential use of DNA break

445   repair strategies that can cause repetitive DNA variation in the germ line of heterogametic individuals

446   (Cohen & Segal 2009; Díaz-Castillo 2013; Diaz-Castillo & Ranz 2012; Lieber 2010; Peng & Karpen

447   2007; Preston et al. 2006; Suzuki et al. 2009). Also, in *Drosophila*, silencing patterns of PEV reporters

448   when maternally- or paternally-inherited are consistent with heterochromatin in later stages of

449   development being dependent on the assortment of heterochromatic-forming elements very early in

450   embryogenesis (Golic et al. 1998; Maggert & Golic 2002). Both the intrinsic spontaneous variation in

451   repetitive DNA for *W/Y* chromosomes occurred along gametogenesis, and the preservation along

452   development of early embryogenesis chromatin compaction genomic patterns are required for *W/Y*

453   chromosomes acting as genomic tuning knobs-sinks in very early embryogenesis to be the base for the

454   sexual dimorphism in gene expression noise in metazoan species. Further research is required to

455   confirm that nuclear dynamics along gametogenesis and early embryogenesis can indeed have such

456   effect on the intrinsic variability in gene expression.

457

458   **CONCLUSIONS**

459   In the present article, I hypothesized gene expression might be generally noisier for heterogametic than

460   for homogametic individuals in metazoan species. I present direct evidence of the hypothesized sexual

461   dimorphism by studying a *D. melanogaster* dataset where transcript variation might be mostly of

462   stochastic and sexual nature. Also, taking into consideration the direct and capacitance-mediated

463   indirect contribution of gene expression noise to phenotypic variation, I predicted phenotypic traits

464   related with the interaction with the environment should be more variable for heterogametic than

465   homogametic individuals. Evidence of the putative gene expression noise-mediated dimorphism in

466   phenotypic traits were found studying genotype- and environment-independent variation of the

467   response to three stressors in *D. melanogaster*, and sex-biased dispersal in metazoan species. Further

468   analyses permit speculating the sexual dimorphism in gene expression noise might be dependent on

469   sex-specific chromosomes acting as genomic tuning knobs very early in embryogenesis. The

470   intrinsically frequent variation in repetitive DNA for *W/Y* chromosomes might cause variation in

471   chromatin compaction across the genome, which ultimately is transformed in an increase in gene

472   expression noise. The genomic tuning knob-sink model for the origin of the sexual dimorphism in gene

473   expression noise, which subsequently contributes to sexual differences for the variation of other

474   phenotypic traits, illustrates the difficulty for the understanding of the connection between genotypes

475   and phenotypes. *W/Y* chromosomes genomic tuning knob-sink effect could be described as epigenetic,

476   i.e., it depends on chromatin compaction variation, but also as stochastic and genetic, i.e., it depends on

477   spontaneous variation in repetitive DNA. Notwithstanding semantic tribulations, the possibility that

478   chromosomes with very limited coding potential, i.e., *W/Y* chromosomes, can have genome-wide

479 effects such as PEV modifier, YRV or gene expression noise modulation talks about the potential

480 importance nuclear dynamics might have for the phenotypic expression of genotypes.

481

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486

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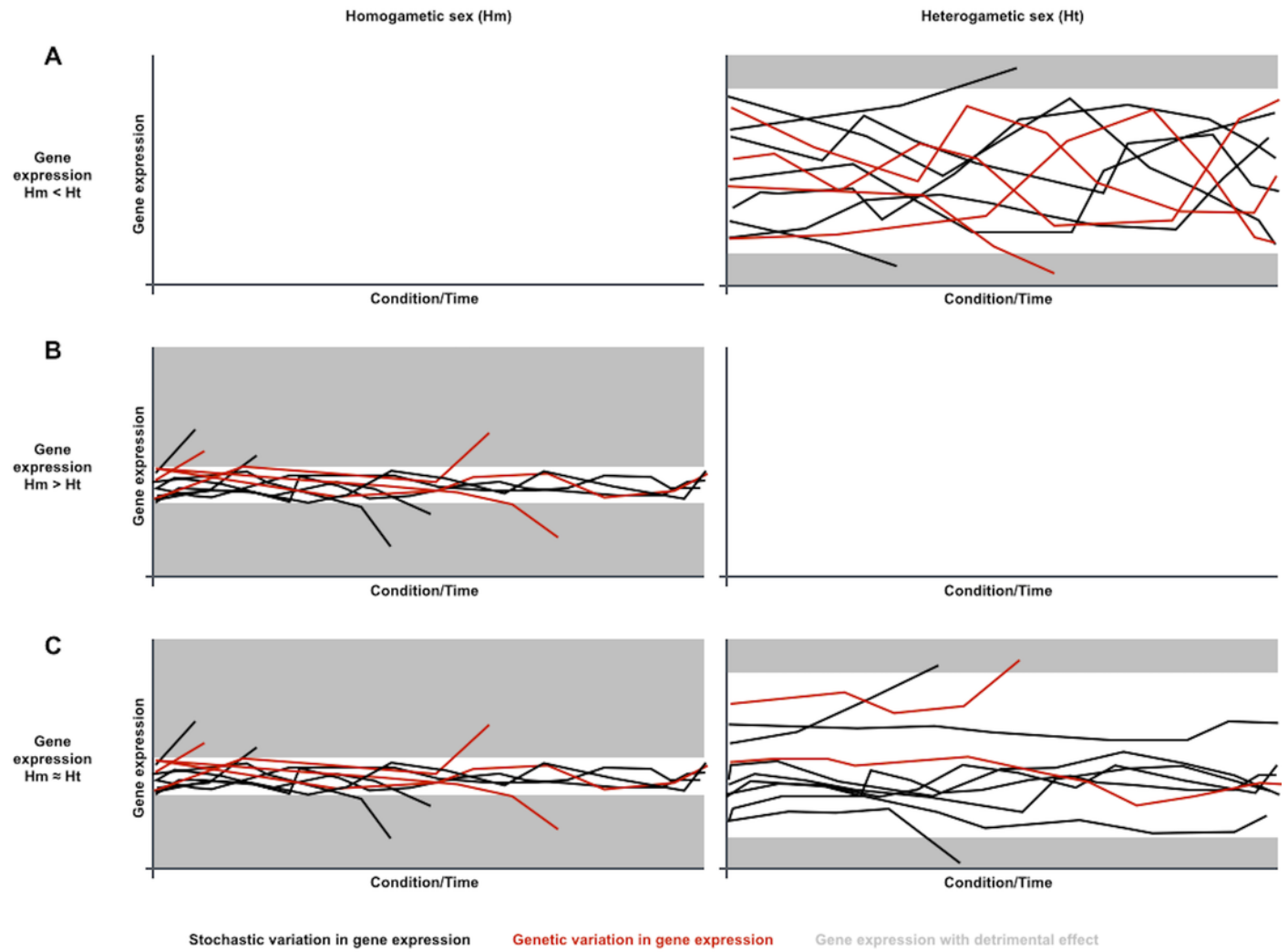
1

A sexual dimorphism in gene expression noise could explain differences in conditional response and divergence for sex-biased gene expression.

Charts symbolize gene expression dynamics for three transcripts in a population under different conditions or along time, under the assumption that gene expression is generally noisier in the heterogametic sex than in the homogametic sex. (A) Dynamics for a transcript that is overexpressed in the generally noisier heterogametic sex (Hm < Ht). (B) Dynamics for a transcript that is overexpressed in the generally less noisy homogametic sex (Hm > Ht). (C) Dynamics for a transcript equally expressed in both sexes (Hm ≈ Ht). Each line represents transcript abundance variation for a single individual in the population. Black and red lines represent variation in gene expression of stochastic or genetic nature, respectively. Grey areas represent gene expression levels with detrimental effects. In this case, both overexpression and underexpression beyond certain levels are detrimental. Individuals with detrimental expression stop contributing to the population. Noisier gene expression permits a better endurance for environmental changes, symbolized with narrower ranges for detrimental expression. Genetic variation in gene expression is prone to accumulate for transcripts that are overexpressed in the noisiest sex, because their phenotypes are often indistinguishable from the noise-driven phenotypic spectrum (A). Genetic variation in gene expression is less prone to accumulate for transcripts that are overexpressed in the less noisy sex or equally expressed in both sexes, because their phenotypes are often distinguishable from the noise-driven phenotypic spectrum, and removed from the population if detrimental (B and C).

Homogametic sex (Hm)   Heterogametic sex (Ht)

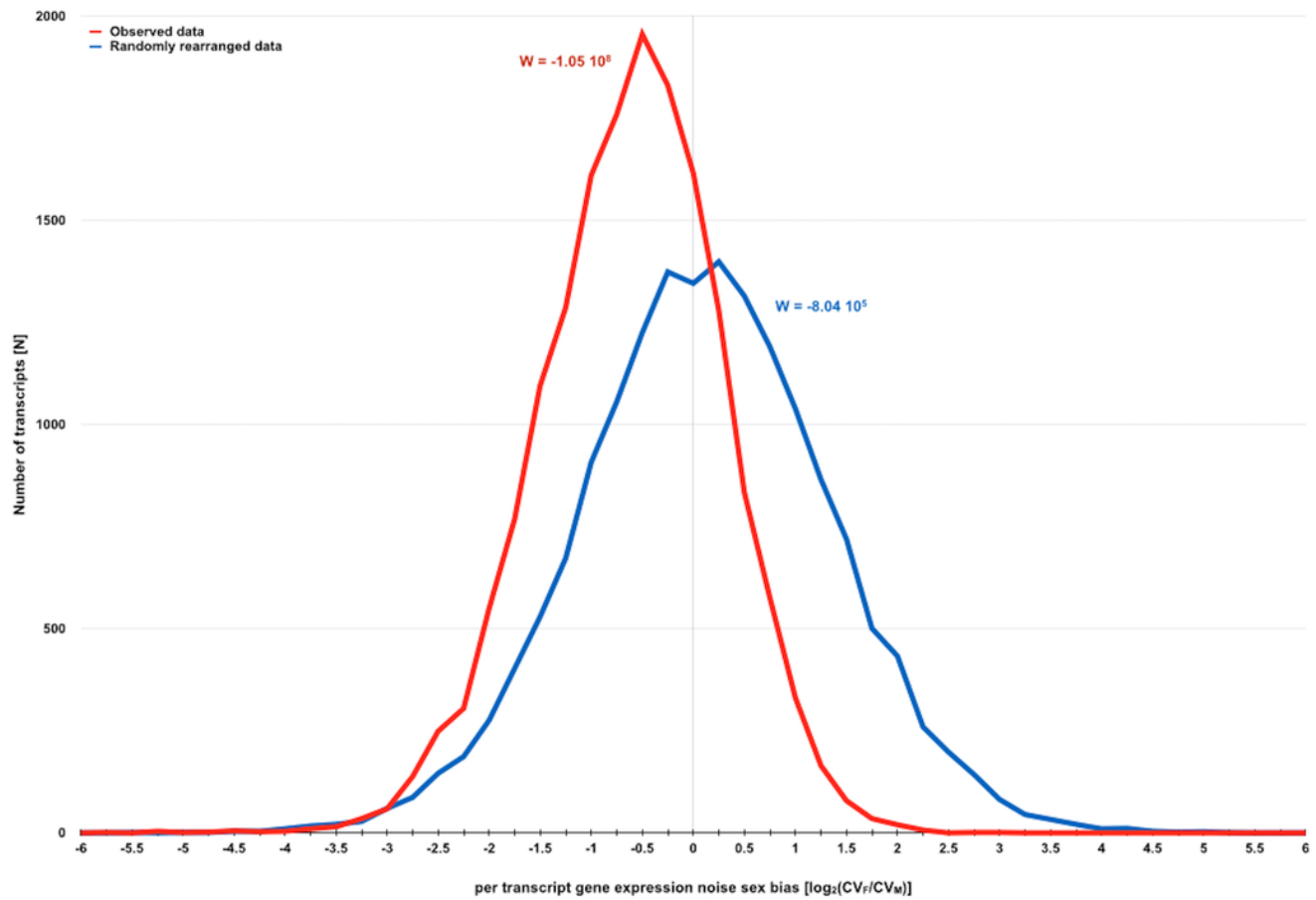
A Gene expression Hm < Ht

B Gene expression Hm > Ht

C Gene expression Hm ≈ Ht

Stochastic variation in gene expression Genetic variation in gene expression Gene expression with detrimental effect

Direct evidence for the existence of a sexual dimorphism in gene expression noise in *D. melanogaster*.

Distribution of measures for per transcript gene expression noise sex bias before and after randomly rearranging observed gene expression noise measures for *D. melanogaster strains* with SIM/REV genotype according to Diaz-Castillo and coworkers dataset. Gene expression noise is measured as transcript abundance coefficient of variation in females and males (CV_F and CV_M). Sexual dimorphism in gene expression noise for the whole transcriptome before and after randomly rearranging the observed data was measured using W (see main text for further details). The negative skew of the distribution of observed data is consistent with gene expression being generally noisier in males than in females.

3

Sexual dimorphism in gene expression noise for genes in five different compartments of *D. melanogaster* genome.

Graph representing measures of sexual dimorphism in gene expression noise for loci with different chromatin structure and/or subnuclear localization according to Filion and coworkers (Filion et al. 2010), in *D. melanogaster* strains and genotypes represented in Diaz-Castillo and coworkers dataset (Diaz-Castillo et al. 2012). BLUE and GREEN components represent known heterochromatin repositories (Filion et al. 2010). BLACK and RED are enriched in loci with tissue-restricted gene expression and lamin-binding targets, suggesting they might be located towards the repressive environment of the nuclear periphery (Filion et al. 2010). YELLOW is the only component associated with broadly expressed loci (Filion et al. 2010). Sexual dimorphism in gene expression noise was measured using W (see main text for further details). The area of the graph representing W for BLUE, RED, and GREEN loci is amplified in the inner box

4

Genomic tuning knob-sink effect of *Y* chromosomes.

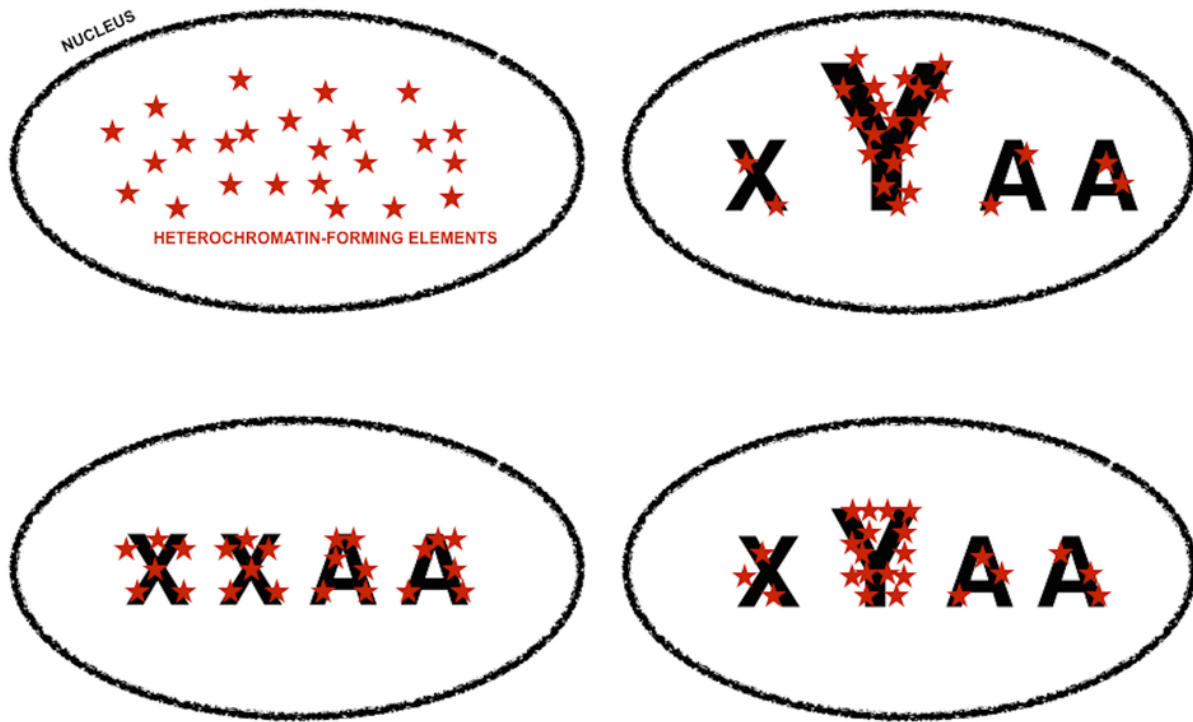
Model for the assortment of heterochromatin-forming elements in homogametic and heterogametic nuclei, under the assumption that heterochromatic-forming elements are found in similar and limiting amounts in all nuclei. Font size is used to symbolize differences in repetitive DNA content of sexual chromosomes, i.e., *X* and *Y*, and autosomes (*A*). The fraction of heterochromatin-forming elements deployed in *Y* chromosomes can vary depending on their content in repetitive DNA. The assortment of heterochromatin-forming elements in non-*Y* loci would be very different if nuclei carry or lack *Y* chromosomes, i.e., *XYAA* or *XXAA*, and slightly different if Y chromosomes have different amounts of repetitive DNA. Slight differences in chromatin compaction across the genome ultimately based in small differences in *Y* chromosome repetitive DNA will cause an increase in gene expression noise across the genome, whereas higher level of chromatin compaction in non-*Y* heterochromatic loci in *XXAA* nuclei will be translated in less extreme male-biased gene expression noise in these loci (Figure 3, and main text).

Monte Carlo-Wilcoxon matched-pairs signed-ranks tests for putative stochastic variation in transcript abundance in *D. melanogaster* females and males.

Table 1. Monte Carlo-Wilcoxon matched-pairs signed-ranks tests for putative stochastic variation in transcript abundance in *D. melanogaster* females and males.

Strains	N replicates	N data pairs	Observed W	Simulated W [5th/95th percentiles]	P_{upper}/P_{lower}
REC	3	16,637	$-1.21 \cdot 10^8$	$-2.06 \cdot 10^6/2.07 \cdot 10^6$	1.0000/<0.0001
INV1	3	16,637	$-1.24 \cdot 10^8$	$-2.04 \cdot 10^6/2.03 \cdot 10^6$	1.0000/<0.0001
INV2	3	16,637	$-1.88 \cdot 10^6$	$-2.03 \cdot 10^6/2.04 \cdot 10^6$	0.9354/0.0646
SIM1	3	16,637	$-1.09 \cdot 10^8$	$-1.97 \cdot 10^6/2.05 \cdot 10^6$	1.0000/<0.0001
REV1	3	16,637	$-5.96 \cdot 10^7$	$-2.05 \cdot 10^6/2.08 \cdot 10^6$	1.0000/<0.0001
REV2	3	16,637	$-1.49 \cdot 10^7$	$-2.03 \cdot 10^6/2.02 \cdot 10^6$	1.0000/<0.0001
INV	6	16,637	$-1.18 \cdot 10^8$	$-2.00 \cdot 10^6/2.03 \cdot 10^6$	1.0000/<0.0001
SIM/REV	9	16,637	$-1.05 \cdot 10^8$	$-2.00 \cdot 10^6/2.04 \cdot 10^6$	1.0000/<0.0001

Transcript abundance data was obtained from (Diaz-Castillo, et al. 2012). Observed Wilcoxon W were obtained by subtracting CV_M from CV_F for each element in the dataset for each strain or genotype. CV differences were ranked from lower to higher according to their absolute value. Signs were assigned to ranks according to the sign of CV differences. Finally, W were obtained by adding signed ranks for all the elements in the dataset for each strain or genotype. Simulated W were obtained by repeating the same process after randomly rearranging all CV values for each strain or genotype 10,000 times. P_{upper} and P_{lower} values represent the fraction of random simulations with measures larger or equal, and lower or equal than the observed ones, respectively.

Coefficients of determination (R^2) for quadratic regressions between transcript abundance mean and CV for *D. melanogaster* females and males.

2 **Table 2. Coefficients of determination (R^2) for quadratic regressions between transcript abundance mean**
3 **and CV for *D. melanogaster* females and males.**

Strains	Females	Males	Females / Males
REC	0.0496	0.0294	1.6834
INV1	0.0444	0.0750	0.5923
INV2	0.1218	0.0619	1.9677
SIM1	0.0918	0.0475	1.9333
REV1	0.0467	0.0123	3.7853
REV2	0.0639	0.0719	0.8885
INV	0.1563	0.0608	2.5707
SIM/REV	0.1617	0.0281	5.7585

6 Transcript abundance data was obtained from (Diaz-Castillo, et al. 2012).

Monte Carlo-Wilcoxon matched-pairs signed-ranks tests for putative stochastic variation in phenotypic traits for *D. melanogaster* females and males.

Table 3. Monte Carlo-Wilcoxon matched-pairs signed-ranks tests for putative stochastic variation in phenotypic traits for *D. melanogaster* females and males.

Trait	N replicates	N data pairs	Observed W	Simulated W [5th/95th percentiles]	P_{upper}/P_{lower}
Startle response	18-40	405	-13,773	-7,788/7,639	0.9986/0.0014
Starvation resistance	2-11	203	-3,176	-2,724/2,780	0.9722/0.0280
Chill coma recovery	2-4	174	-1,395	-2,201/2,151	0.8527/0.1476

Phenotypic trait data was obtained from http://dgrp2.gnets.ncsu.edu (Mackay, et al. 2012). Observed Wilcoxon W were obtained by subtracting CV_M from CV_F for each element in the dataset for each strain or genotype. CV differences were ranked from lower to higher according to their absolute value. Signs were assigned to ranks according to the sign of CV differences. Finally, W were obtained by adding signed ranks for all the elements in the dataset for each strain or genotype. Simulated W were obtained by repeating the same process after randomly rearranging all CV values for each strain or genotype 10,000 times. P_{upper} and P_{lower} values represent the fraction of random simulations with measures larger or equal, and lower or equal than the observed ones, respectively.

Monte Carlo simulations for heterogamety-biased dispersal in metazoan species with and without sex-specific heterochromatic chromosomes.

2 **Table 4. Monte Carlo simulations for heterogamety-biased dispersal in metazoan species with and without**
3 **sex-specific heterochromatic chromosomes.**

Species	Observed fraction of heterogamety-biased dispersal	Simulated fraction of heterogamety-biased dispersal [5th-95th percentiles]	P_{upper}/P_{lower}
All	0.81	0.36/0.64	0.0005/1.0000
XY+ZW	0.90	0.36/0.65	0.0001/1.0000
XO+ZO	0.20	0.20/0.80	0.9731/0.1788

6 Sex-biased dispersal and chromosome system data were obtained from (Spence 1990; Morgan-Richards 1997; Kandul, et al. 2007; Ardila-Garcia and
7 Gregory 2009; Petit and Excoffier 2009; Narita, et al. 2011). *XO* and *ZO* represent species in which heterogametic individuals commonly or completely
8 lack *W* and *Y*. The fraction of heterogamety-biased dispersal represents the number of cases in the dataset in which heterogametic individuals tend to
9 disperse more than homogametic individuals. Simulated fractions of heterogamety-biased dispersal were calculated after randomly rearranging
10 chromosome system tags 10,000 times. P_{upper} and P_{lower} values represent the fraction of random simulations with measures larger or equal, and lower or
11 equal than the observed ones, respectively.

Monte Carlo-Wilcoxon matched-pairs signed-ranks tests for putative stochastic variation in transcript abundance in five different chromatin compartments in *D. melanogaster* females and males.

Table 5. Monte Carlo-Wilcoxon matched-pairs signed-ranks tests for putative stochastic variation in transcript abundance in five different chromatin compartments in *D. melanogaster* females and males.

Strains	Chromatin color	N replicates	N data pairs	Observed W	Simulated W [5th/95th percentiles]	P_{upper}/P_{lower}
REC	YELLOW	3	4,907	$-1.05\ 10^7$	$-3.30\ 10^5/3.20\ 10^5$	1.0000/<0.0001
REC	BLACK	3	2,721	$-3.26\ 10^6$	$-1.36\ 10^5/1.34\ 10^5$	1.0000/<0.0001
REC	BLUE	3	775	$-2.53\ 10^5$	$-2.06\ 10^4/2.01\ 10^4$	1.0000/<0.0001
REC	RED	3	266	$-3.00\ 10^4$	$-4.19\ 10^3/4.06\ 10^3$	1.0000/<0.0001
REC	GREEN	3	147	$-1.00\ 10^4$	$-1.70\ 10^3/1.67\ 10^3$	1.0000/<0.0001
INV1	YELLOW	3	4,907	$-1.08\ 10^7$	$-3.32\ 10^5/3.22\ 10^5$	1.0000/<0.0001
INV1	BLACK	3	2,721	$-3.34\ 10^6$	$-1.36\ 10^5/1.36\ 10^5$	1.0000/<0.0001
INV1	BLUE	3	775	$-2.71\ 10^5$	$-2.06\ 10^4/2.10\ 10^4$	1.0000/<0.0001
INV1	RED	3	266	$-3.44\ 10^4$	$-4.14\ 10^3/4.17\ 10^3$	1.0000/<0.0001
INV1	GREEN	3	147	$-9.28\ 10^3$	$-1.71\ 10^3/1.68\ 10^3$	1.0000/<0.0001
INV2	YELLOW	3	4,907	$2.11\ 10^6$	$-3.29\ 10^5/3.27\ 10^5$	<0.0001/1.0000
INV2	BLACK	3	2,721	$-9.20\ 10^5$	$-1.37\ 10^5/1.36\ 10^5$	1.0000/<0.0001
INV2	BLUE	3	775	$-4.46\ 10^4$	$-2.11\ 10^4/2.03\ 10^4$	0.9999/0.0001
INV2	RED	3	266	$2.56\ 10^3$	$-4.19\ 10^3/4.15\ 10^3$	0.1497/0.8505
INV2	GREEN	3	147	$2.17\ 10^3$	$-1.70\ 10^3/1.66\ 10^3$	0.0149/0.9851
SIM1	YELLOW	3	4,907	$-9.21\ 10^6$	$-3.33\ 10^5/3.28\ 10^5$	1.0000/<0.0001
SIM1	BLACK	3	2,721	$-3.12\ 10^6$	$-1.34\ 10^5/1.37\ 10^5$	1.0000/<0.0001
SIM1	BLUE	3	775	$-2.48\ 10^5$	$-2.02\ 10^4/2.07\ 10^4$	1.0000/<0.0001
SIM1	RED	3	266	$-3.11\ 10^4$	$-4.17\ 10^3/4.12\ 10^3$	1.0000/<0.0001
SIM1	GREEN	3	147	$-9.26\ 10^3$	$-1.70\ 10^3/1.72\ 10^3$	1.0000/<0.0001
REV1	YELLOW	3	4,907	$-5.77\ 10^6$	$-3.25\ 10^5/3.21\ 10^5$	1.0000/<0.0001
REV1	BLACK	3	2,721	$-1.42\ 10^6$	$-1.34\ 10^5/1.37\ 10^5$	1.0000/<0.0001
REV1	BLUE	3	775	$-1.04\ 10^5$	$-2.05\ 10^4/2.03\ 10^4$	1.0000/<0.0001
REV1	RED	3	266	$-1.37\ 10^4$	$-4.11\ 10^3/4.09\ 10^3$	1.0000/<0.0001
REV1	GREEN	3	147	$-5.61\ 10^3$	$-1.69\ 10^3/1.71\ 10^3$	1.0000/<0.0001
REV2	YELLOW	3	4,907	$-4.94\ 10^5$	$-3.27\ 10^5/3.29\ 10^5$	0.9930/0.0070
REV2	BLACK	3	2,721	$-8.79\ 10^5$	$-1.34\ 10^5/1.33\ 10^5$	1.0000/<0.0001
REV2	BLUE	3	775	$-8.35\ 10^4$	$-2.06\ 10^4/2.02\ 10^4$	1.0000/<0.0001
REV2	RED	3	266	$-9.38\ 10^3$	$-4.04\ 10^3/4.15\ 10^3$	0.9998/0.0002
REV2	GREEN	3	147	$3.15\ 10^3$	$-1.66\ 10^3/1.68\ 10^3$	0.0010/0.9990
INV	YELLOW	6	4,907	$-1.00\ 10^7$	$-3.29\ 10^5/3.22\ 10^5$	1.0000/<0.0001
INV	BLACK	6	2,721	$-3.28\ 10^6$	$-1.35\ 10^5/1.38\ 10^5$	1.0000/<0.0001
INV	BLUE	6	775	$-2.63\ 10^5$	$-2.06\ 10^4/2.07\ 10^4$	1.0000/<0.0001
INV	RED	6	266	$-3.35\ 10^4$	$-4.11\ 10^3/4.09\ 10^3$	1.0000/<0.0001

INV	GREEN	6	147	-8.39×10^3	$-1.68 \times 10^3 / 1.70 \times 10^3$	$1.0000 / <0.0001$
SIM/REV	YELLOW	9	4,907	-9.13×10^6	$-3.21 \times 10^5 / 3.30 \times 10^5$	$1.0000 / <0.0001$
SIM/REV	BLACK	9	2,721	-3.04×10^6	$-1.33 \times 10^5 / 1.33 \times 10^5$	$1.0000 / <0.0001$
SIM/REV	BLUE	9	775	-2.52×10^5	$-2.07 \times 10^4 / 2.07 \times 10^4$	$1.0000 / <0.0001$
SIM/REV	RED	9	266	-2.94×10^4	$-4.16 \times 10^3 / 4.15 \times 10^3$	$1.0000 / <0.0001$
SIM/REV	GREEN	9	147	-6.51×10^3	$-1.69 \times 10^3 / 1.73 \times 10^3$	$1.0000 / <0.0001$

Transcript abundance data was obtained from (Diaz-Castillo, et al. 2012). Coordinates of chromatin components symbolized with colors were obtained from (Filion, et al. 2010). Observed Wilcoxon W were obtained by subtracting CV_M from CV_F for each element in every chromatin compartment for each strain or genotype. CV differences were ranked from lower to higher according to their absolute value. Signs were assigned to ranks according to the sign of CV differences. Finally, W were obtained by adding signed ranks for all the elements with the same color for each strain or genotype. Simulated W were obtained by repeating the same process after randomly rearranging all CV values for each strain or genotype 10,000 times. P_{upper} and P_{lower} values represent the fraction of random simulations with measures larger or equal, and lower or equal than the observed ones, respectively.