UDC 575.1

Original articles

CHANGES IN DIFFERENTIAL GENE EXPRESSION PATTERN IN FLY HEADS AS THE RESPONSE TO AN EXPERIMENTALLY EVOLVED X CHROMOSOME

Manat Y.[1,*], Issabekova A.S.[1], Kaukabayeva G.K.[1]

[1] *National center for biotechnology, 13/5, Korgalzhyn road, Kazakhstan, Astana, 010000.*
[*] *manat@biocenter.kz*

ABSTRACT

Sexually antagonistic (SA) mutations that increase fitness in one sex and decrease it in the other sex (also known as intralocus sexual conflict) are central to the sexual antagonism hypothesis of sex chromosome evolution. It was suggested long ago (by Fisher, in 1931) that tight linkage to a sex-determining locus facilitates the accumulation of SA mutations even when their detrimental effect in one sex exceeds its benefit in the other. Due to the difficulties in detecting SA alleles directly, we know very little about the nature of X-linked SA mutations and their role in the evolution of differential gene expression between sexes. To investigate how X-linked SA mutations affect the transcriptome profile in both sexes, we analyzed changes in genome-wide gene expression pattern in *Drosophila melanogaster* head tissue that had undergone 95 generations of a female-limited X-chromosome experimental evolution. We found an evidence of change in gene expression towards to the female optimum, which we interpret as the result of resolution of sexual conflict over X-linked SA mutations. In addition, we also found a potential unknown gene expression effect of the balancer chromosome on gene expression (that was necessary to use to limit the inheritance of target X chromosome to the female line). All these findings indicate that a highly polymorphic nature of the X chromosome with an important role in genome-wide gene expression profile.

Keywords: X chromosome, sexually antagonistic mutation, differential gene expression, experimental evolution, feminization, inheritance.

INTRODUCTION

Differences in variance in reproductive success between males and females put them in evolutionary conflict, and generate different selection pressures on many traits that maximize the fitness of one sex at the expense of the other sex [1-3]. The result of divergent selection pressures in sexually differentiated species depends on there being two different sexes with females producing large macrogametes: eggs, and males making microgametes: sperm. This anisogamy, gametes of two different sizes, ultimately underlies the evolution of sex differences in behavior and morphology. It is an almost universal phenomenon in sexually reproducing organisms, occurring whenever traits shared by males and females have sex-specific optima that cannot be attained simultaneously, generating the evolutionary conflict of interest between the sexes [4]. Intra-locus sexual conflict (or a sexually antagonistic (SA) mutation) is common in a wide variety of traits in many taxa and has been found in both natural and laboratory populations [5, 6]. These shared traits have a common genetic basis, which means there is a strong, positive inter-sexual genetic correlation of these traits between two sexes [7, 8], and the conflict may be mitigated or fully resolved by mechanisms leading to the evolution of sexual dimorphisms, such as sex-specific gene expression, genomic imprinting, or reduced opposite-sex heritability [9].

Many multicellular eukaryotes with genetic sex determination systems have specialized sex chromosomes that carry sex determining region [10-12]. Sex chromosomes present several unique characteristics that distinguish them from other parts of the genome, including inheritance pattern, hemizygosity, and reduced recombination, which influence their response to evolutionary factors (e.g., mutation rates, drift, selection, effective population size, recombination rates, dosage compensation, and diverse forms of genetic conflict) [3, 10, 13-16]. Asymmetric inheritance of sex chromosomes is one of the intriguing features of heteromorphic sex chromosomes that has attracted many researchers' attention and made the sex chromosome evolution as a dynamic field [10, 11, 13, 17]. In male heterogametic system (X/Y), under equal sex ratio and equal variance in male and female reproductive success the effective population size of the X chromosome is equal to three-fourths that of the autosomes [18, 19]. Thus, the mutation accumulation rate can be different from autosomes [16, 18]. Earlier theoretical literature (mainly theoretical) showed that the more flexible dynamics of X-linked mutations than autosomes [3, 16, 20-22], and the potential evolutionary importance of sex chromosomes in the evolution of sexual dimorphism and speciation [3, 16]. In addition, sex-linkage may contribute to the resolution of intra-locus sexual conflict resolution [3], and sex chromosomes can either increase or reduce this conflict, depending on dominance and pleiotropy. For example, at the adult stage in a laboratory-adapted *D. melanogaster* the X chromosome was estimated to harbour 97% of the genome-wide sexually antagonistic variation [23]. However, most of this type of conflict remains unresolved in the genome, and maintained additive genetic variance [24], resulting in reduced population mean fitness (figure. 2) [25]. However, gaps in our understanding the nature of X-linked mutations especially X-linked sexually antagonistic (SA) mutations still remain.

Therefore, the X-chromosome a particularly interesting part of the genome for investigating the nature of sexually antagonistic mutations and their role in the evolution of differential gene expression in male and female.

In this study, we wanted to investigate how X-linked mutations affect genome-wide patterns of gene expression. If the X chromosome is highly polymorphic and enriched for SA mutations, and subject to intra-locus sexual conflict, then removing male selective constraints and generating long-term female-specific selection on the X chromosome should lead

to more feminized X chromosomes. Observed as an increase in female-biased gene expression and a decrease male-biased gene expression.

To test this idea, and to provide a better understanding of the evolutionary dynamics of X-linked polymorphic loci, we performed a female limited X-chromosome (FLX) evolution experiment in *D. melanogaster* where the X-chromosome was passed from mother to daughter without passing through males, thereby limiting selection on the X-chromosome to females only. We expected that expressing the evolved X chromosome would change genome-wide gene expression pattern between male and female, with females and males expressing the FLX-chromosome showing a more 'feminised' expression profile. After limiting the expression of the X chromosome to females for 95 generations, we analysed the effect of the evolved X chromosome on genome-wide gene expression pattern in head tissue. *We chose fly heads because changes in expression are unlikely to be confounded by allometric differences in organ sizes.* To detect sex-specific responses in gene expression to the evolved X chromosome, we carried out pairwise comparisons between the selection regimes separately in males and females. Analysing the sexes separately also allowed us to disentangle the long-term effect of the FM balancer in FLX regime from the FLX selection effect, which was detected as in previous studies [26, 27]. We therefore further categorized the differentially expressed genes into three classes: FLX effect, CFM effect and CFM vs. FLX effect. Consistent with our expectations, we did identify a more 'feminized' gene expression profile as the result of the FLX evolution. *We also found potential confounding effects of using the FM balancer chromosome, some of which were consistent with a reduction in the level of conflict over mating rate and fertilisation success [26].* These results presented here highlight the importance of X-linked mutations in gene expression pattern between sexes and may be shape the genetic architecture of many shared traits between male and female.

MATERIALS AND METHODS

Experimental design

The most compelling empirical evidence for intra-locus sexual conflict (IASC) comes from *D. melanogaster* [9]. In prior experimental evolutionary studies with *D. melanogaster* researchers have been able to evaluate the evolutionary impact of IASC, by using a sex-biased selection method that removes the opportunity for selection in one sex, which results in increased fitness for the selected sex [28-31]. So far, most of these sex-limited experiments have focused on the response to male-specific selection.

In *D. melanogaster* the X chromosome is estimated to account for 45% of the genome-wide fitness variation [23]. The large size of the X, approximately 20% of the euchromatic genome, in relation to the rest of the genome means that X-linked loci are likely to make a large contribution to the variance in the many polygenic traits [32]. So, even though it is still unclear how much SA variation is on the X chromosome, the X in itself has a large genomic importance in *D. melanogaster.*

As no female-specific genomic selection evolution experiment had been done before and because of the importance of the X chromosome in IASC, a female-limited X chromosome (FLX) evolution experiment was established.

To control the inheritance of the selected X chromosomes we used an FM (First Multiple: FM7a) balancer chromosome, which is an X chromosome with a series of inversions so that it cannot recombine with its homolog but should still act like a normal X chromosome. The FM balancer carries phenotypic markers, so the flies' genotypes could distinguish by eye (Figure 1).

The FLX evolution experiment consists of three experimental regimes in four replicate populations. The FLX selection regime and the two control regimes: control wild type (Cwt), and control FM (CFM). CFM is a methodological control to control for the confounding effect of the FM balancer chromosome in the FLX populations. The CFM regime is handled in the same way as the FLX regime, except that the X chromosome goes through repeating cycles of two generations in females followed by one generation in males. This eliminated the sex-specific selection done in the FLX regime since the 2:1 ratio of time spent in each sex is the same as the average wild type X chromosome. The FLX and CFM regimes also have a "recombination box" to prevent clonal evolution of the selected X chromosomes [28, 29, 31]. The Cwt regime is a group of wild type flies, which are maintained under the same experimental conditions as the FLX and CFM regimes (virgin collection, smaller population size), but without sex-limited selection or the FM balancer. I am thereby able to control for the experimental protocol itself and for any effects that may be caused by a reduction in effective population size.

The base population used to start the evolution experiment, LH_M, has been maintained as a large, outbred population generated from 400 inseminated females collected from central California in 1991 [33].

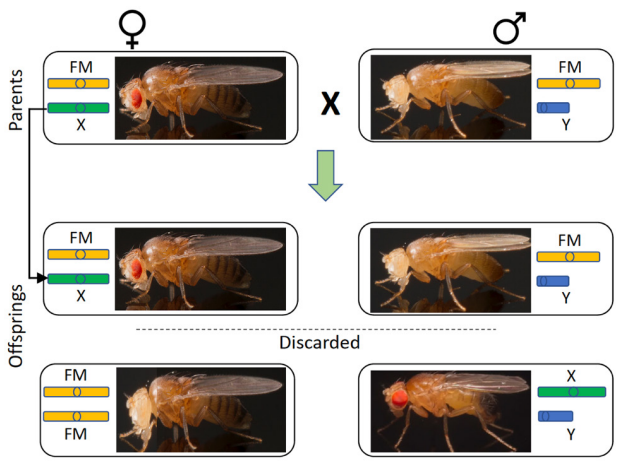

Figure 1 – Protocol for the female-limited X-chromosome (FLX) evolution experiment and graphical interpretation of the selection regimes' effects on the fly performance. The evolving X-chromosome (green bar) is passed from mother to daughter with the help of an FM balancer chromosome (yellow bar). The parental cross produces four genotypes, of which the offspring above the dashed line are crossed to produce the next generation, and the offspring under the dashed line are discarded. The FM balancer carries several phenotypic markers, which can be used to phenotype offspring, as illustrated by the pictures next to the genotypes.

RNA extraction, gene expression profiling and analysis

After 95 generations of experimental evolution, in total 102 samples were collected from the three treatment groups

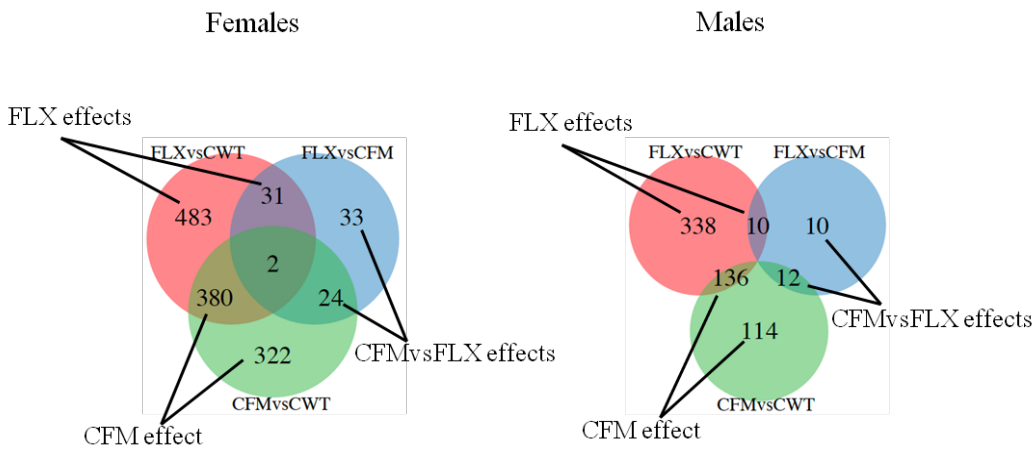

Figure 2 – Number of significantly differentially expressed genes between selection regimes and effects in females (left) and males (right).

with their four replicate populations. There were five female genotypes (FLXho, FLXhe, CFMho, CFMhe, and CWT), three male genotypes (FLX, CFM, and CWT) and the ancestral LHm population. We collected three biological replicate pools of 25 flies per genotype treatment per replicate population, as well as three biological replicate pools of each sex from the original LHM population. The virgin males and females from all populations were collected and held in same sex groups of 25 individuals for another 10 days. On day 11 after oviposition, flies were quickly anaesthetized and heads were dissected into liquid nitrogen, then stored at -80°C until extraction. Total RNA was extracted from these heads using RNeasy Mini Kit (Qiagen) following the manufacture's instruction.

The samples were sent to the SNP&SEQ Technology Platform in Uppsala (Sweden), where the RNA quality and quantity (QC control) were assessed using the Agilent 2200 TapeStation system and Agilent 2100 Bioanalyzer system (Agilent Technologies) prior to library preparation. From the QC control 9 samples did not pass, therefore 93 samples were sequenced (with at least two biological replicates per sample). Libraries were generated using TruSeq stranded mRNA library preparation kit with poly-A selection (Illumina Inc.). Then cluster generation and 125 cycles of paired-end sequencing of the 93 libraries in 18 lanes using the HiSeq2500 system and v4 sequencing chemistry were performed. The result was 180 million read pairs per lane, which achieved average 65M reads per sample.

After removing adaptors and low-quality sequences from the data using the *trimmomatic* command line tool [34], reads were mapped to the *D. melanogaster* Release 6 plus ISO1 MT reference genome, using the HISAT2 (hierarchical indexing for spliced alignment of transcripts) alignment program [35], resulting in a 94.5% overall alignment rate. Preliminary analysis of the differential gene expression in response to the experimental evolution experiment was carried out in RStudio using the Ballgown package, following the Nature protocol pipeline [36]. Ballgown is a software package designed to do mainly two-group comparisons at a time, and because data analysis is still ongoing, I have not done any multiple pairwise comparisons yet. Therefore, we subset our dataset into each combination of genotype/treatment (for example) and ran the Ballgown pipeline on them pair by pair. The statistical model of Ballgown is a standard linear model comparison [36]. Multiple testing was therefore controlled for within each analysis, but not across pairwise analyses, and although the results presented in this project should be considered preliminary, they should accurately reflect general patterns since the most significant results are likely to be consistent regardless of method of analysis.

Genes with an adjusted *p*-value <0.05 (*qval,* the estimated false discovery rate) were considered to be differentially expressed between groups. Up- and dow-nregulation of these significant genes was analyzed by the confounder-adjusted fold change [36]. Fold change (FC) refers to the ratio between expression in two groups, if the ratio (FC) between male.FLX versus male.CWT was below 1 means that the transcript was expressed at a lower level in male.FLX. Analysing the sexes separately also allowed us to disentangle the long-term effect of the FM balancer in FLX regime from the FLX selection effect. We therefore further categorized the differentially expressed genes into three classes: FLX effect, CFM effect and CFM vs. FLX effect *(Figure 2).*

RESULTS

Female gene expression

The first step of the differential expression from the full dataset showed 17773 genes that were differentially expressed between the sexes. After removing the low abundance genes with less than one count, this number reduced to 7586 genes, and further subsets were made from the filtered dataset.

From the comparisons of five female genotypes' whole genome expression pattern with each other, We investigated the dominance interactions and predicted similar expression profiles for significant genes between homozygote and heterozygote genotypes [3]. We found almost exactly the same expression pattern between FLX homo and FLX hetero genotype groups (no significant genes by q<0.05) and between CFM genotypes (only two genes expression were significantly different). Since the heterozygote (He) females have one evolving and one wild type X-chromosome we expected their gene expression pattern to be intermediate to that of Cwt and homozygote females (Ho) under additivity, which also was true in both FLX and CFM genotypes against CWT (Table 1). We also expected that the CFM treatment would be more similar compared to the Cwt treatment than the FLX treatment compared to the Cwt treatment, since the CFM treatment is

a methodological control. This prediction was also supported (Table 1).

Then we analyzed differential expression of X-chromosome genes specifically, since they are the ones that should respond directly to the experimental evolution. X-linked genes showed the same differential expression patterns between genotypes and treatments as the whole-genome data (figure 2, Table 1). There were more FLX homozygote X chromosome genes that were differentially expressed (111) compared to Cwt, than in CFM homozygotes (56) compared to Cwt. However, there were fewer genes in the heterozygote FLX X chromosome comparison (8) than in the heterozygote CFM comparison (12).

All these significantly differentially expressed genes were classified into two categories: up-regulated and down-regulated, both listed in Table 1. The proportions of up-regulated genes were slightly higher than the down-regulated genes; however in homozygote FLX flies down-regulated genes were more numerous in both the whole genome and X chromosome gene data (Table 1). We also found a large number of genes that expressions were effect as the result of the adaptation to the FM balancer which was classified as the CFM effect in both sexes (Figure 2).

Table 1 – The gene expression levels of significantly differentially expressed genes between groups.

Between female genotype treatments, **Whole genome expression**			
group	Total number	Regulation	
(groups)	of Sig. genes	Up-regulated	Down-regulated
CFM.Ho (CFM.Ho-CFM.He)	2	1	1
FLX.Ho (FLX.Ho-CWT)	461	227	237
FLX.He (FLX.He-CWT)	47	27	20
CFM.Ho (CFM.Ho-CWT)	267	140	127
CFM.He (CFM.He-CWT)	92	53	39
Between female treatments. **X chromosome expression**			
FLX.chrXX (FLX.chrXX-CWT)	111	49	62
FLX.chrXx (FLX.chrXx-CWT)	8	6	2
CFM.chrXX (CFM.chrXX-CWT)	56	28	28
CFM.chrXx (CFM.chrXx-CWT)	12	7	5

Male gene expression

For the male model, after filtering the low abundance genes there were 7586 genes differentially expressed between treatments. Then we compared differential expression patterns between treatments group by group: FLX vs CWT, FLX vs CFM and CFM vs CWT. As shown in *table 2*, FLX male flies have more significant genes (172) than CFM (15). And there was only one gene significantly differentially expressed between FLX and CFM treatments. All of these significantly different genes were again classified into two categories by fold change: up-regulated and down-regulated, and results are presented in table 4. Most of these significant genes are down-regulated in the FLX and CFM treatments, and the same is also true for X chromosome gene expression pattern. This suggests that there is an effect of adaptation to the FM balancer, but that most of the response in FLX males is due to female-specific selection on the X.

Table 2 – *The gene expression levels of significant genes between male groups.*

Between male treatments. **Whole genome expression**			
group (groups)	Total number of Sig. genes	Regulation	
		Up-regulated	Down-regulated
FLX (FLXvsCWT)	172	47	125
CFM (CFMvsCWT)	15	5	10
FLX (FLXvsCFM)	1	1	0
Between male treatments. **X chromosome expression**			
Groups	Total number of Sig. genes	Regulation	
		Up-regulated	Down-regulated
FLX (FLXvsCWT)	21	5	16
CFM (CFMvsCWT)	7	2	5
FLX (FLXvsCFM)	0	0	0

DISCUSSION

The X chromosome spends most of its time (2/3) in a female body, therefore X-linked polymorphic genes for traits experiencing sexually antagonistic selection should tend to evolve toward the female optimum. Furthermore, theory predicts that female-benefit X chromosome genes are dominant [3]. By completely limiting the X chromosome expression to females, we expected to see more altered regulation of the X chromosome and X-linked genes in the FLX treatment flies compared to in other treatments. For the CFM genotypes, every third generation the target X chromosome in CFM had chance to crossover with unevolved homologs and be expressed in a male body. Therefore, we expected a more similar gene expression pattern in the CFM treatment compared to Cwt than FLX compared to Cwt. All of these expectations were supported by our DE analysis results so far.

To investigate the whole genome and genes on X chromosome's expression pattern in response to the FLX evolution experiment, differential gene expression (DE) analysis was performed at generation 95. Because of the limitations of the program we used (Ballgown), we could not compare the DE pattern between the sexes, however, despite the complex design of the protocol we have completed some other pairwise comparisons and got many more results consistent with our expectations.

From the homozygote FLX and heterozygote FLX (female) comparison, they showed the same expression pattern for significant genes. This is consistent with similar effects in homozygotes and heterozygotes because of dominance on the X chromosome. The same was also true for CFM treatment. However there were more significant genes in the homozygote FLX versus Cwt comparison than in the heterozygote FLX versus Cwt comparison, which suggests incomplete dominance. That we found more significant genes between FLX and Cwt than between CFM and Cwt (for both whole genome and X chromosome expression) is consistent with a more "feminized" pattern of expression in FLX flies. This pattern of DE was repeated in male groups.

However, despite the high average coverage in our data (more than 55x), after filtering the data for low abundance genes, there were only 7586 genes left. This means that a considerable portion of the genome could not be investigated. It might therefore be interesting to look for changes in all X-linked genes, including genes with low levels of expression. Also, since the total amount of significantly differentially expressed genes was low in most cases, this may be an indication that the X chromosome is already close to the female optimum expression. Categorization the differentially expressed genes into three classes allowed us to disentangle the FLX effect from the long-term effect of carrying the FM balancer, and examined the possible signatures of these differences at the gene expression level.

CONCLUSION

One general reason that makes the sex chromosomes interesting is their unusual inheritance pattern. Because of their unequal transition between sexes, any biological differences between sexes force them to experience a distinct evolutionary environment. As a consequence, sex chromosomes are often central to the various types of sexual conflict. By forcing the X chromosome to only be expressed in one sex, we expected to see a mitigation of intralocus sexual conflict. Overall results

of these experiments support our expectations. Our results indicate evidence of feminization in genome-wide gene expression pattern in both sexes. The changes in expression due to FLX evolution were more than just to adaptation to the FM balancer. But the large amount of genes which were found in the CFM effect class indicated the potential confounding effects of using a balancer chromosome (FM7a). Overall, our results showed the X chromosome is highly polymorphic, enriched for SA mutations and the highly dynamic nature of intralocus sexual conflict on the X chromosome, which can be partly resolved experimentally.

ACKNOWLEDGMENTS

We would like to thank to Jessica Abbott for designing the project.

FUNDING

This work was supported a grant from the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan (AP13068273).

LITERATURE

1 Andersson, M.B., Sexual selection. 1994. - Princeton University Press.

2 Ranz, J.M., et al., Sex-dependent gene expression and evolution of the Drosophila transcriptome. Science. - 2003. - №300(5626). - P. 1742-1745.

3 Rice, W.R., Sex Chromosomes and the Evolution of Sexual Dimorphism. Evolution. - 1984. - №38(4). - P. 735-742.

4 Burke, N.W. and R. Bonduriansky, Sexual Conflict, Facultative Asexuality, and the True Paradox of Sex. Trends Ecol Evol. - 2017. - №32(9). - P. 646-652.

5 Abbott, J.K., Intra-locus sexual conflict and sexually antagonistic genetic variation in hermaphroditic animals. Proc Biol Sci. - 2011. - №278(1703). - P. 161-9.

6 Stewart, A.D., A. Pischedda, and W.R. Rice, Resolving intralocus sexual conflict: genetic mechanisms and time frame. Journal of Heredity. - 2010. - №101(suppl_1). - P. S94-S99.

7 Bonduriansky, R. and S.F. Chenoweth, Intralocus sexual conflict. Trends in ecology & evolution. - 2009. - №24(5). - P. 280-288.

8 Lande, R., Sexual dimorphism, sexual selection, and adaptation in polygenic characters. Evolution. - 1980. - №34(2). - P. 292-305.

9 Pischedda, A. and A.K. Chippindale, Intralocus sexual conflict diminishes the benefits of sexual selection. PLoS biology. - 2006. - №4(11). - P. e356.

10 Beukeboom, L.W. and N. Perrin, The Evolution of Sex Determination. - 2014. - Oxford University Press.

11 Bachtrog, D., et al., Sex determination: why so many ways of doing it? PLoS Biol. -2014. - №12(7). - P. e1001899.

12 Rice, W.R., Evolution of the Y sex chromosome in animals. Bioscience. - 1996. - №46(5). - P. 331-343.

13 Ellegren, H., Sex-chromosome evolution: recent progress and the influence of male and female heterogamety. Nat Rev Genet. - 2011. - №12(3). - P. 157-66.

14 Bachtrog, D., et al., Are all sex chromosomes created equal? Trends Genet. - 2011. -№27(9). - P. 350-7.

15 Charlesworth, B. and N.D. Dempsey, A model of the evolution of the unusual sex chromosome system of *Microtus oregoni*. Heredity (Edinb). - 2001. - №86(Pt 4). - P. 387-94.

16 Charlesworth, B., J.A. Coyne, and N.H. Barton, The Relative Rates of Evolution of Sex Chromosomes and Autosomes. The American Naturalist. - 1987. - №130(1). - P. 113-146.

17 Abbott, J.K., A.K. Norden, and B. Hansson, Sex chromosome evolution: historical insights and future perspectives. Proc Biol Sci. - 2017. - №284(1854).

18. Vicoso, B. and B. Charlesworth, Effective population size and the faster-X effect: an extended model. Evolution. - 2009. - №63(9). - P. 2413-26.

19 Caballero, A., On the effective size of populations with separate sexes, with particular reference to sex-linked genes. Genetics. - 1995. -№139(2). - P. 1007-11.

20 Lester, L.J. and R.K. Selander, Population genetics of haplodiploid insects. Genetics. - 1979. - №92(4). - P. 1329-45.

21 Hartl, D.L., A Fundamental Theorem of Natural Selection for Sex Linkage or Arrhenotoky. The American Naturalist. - 1972. - №106(950). - P. 516-524.

22 HALDANE, J.B.S., A mathematical theory of natural and artificial selection. part ii the influence of partial self-fertilisation, inbreeding, assortative mating, and selective fertilisation on the composition of mendelian populations, and on natural selection. Biological Reviews. - 1924. - №1(3). - P. 158-163.

23 Gibson, J.R., A.K. Chippindale, and W.R. Rice, The X chromosome is a hot spot for sexually antagonistic fitness variation. Proc Biol Sci. - 2002. - №269(1490). - P. 499-505.

24 Bonduriansky, R. and S.F. Chenoweth, Intralocus sexual conflict. Trends Ecol Evol. - 2009. - №24(5). - P. 280-8.

25 Cox, R.M. and R. Calsbeek, Sexually antagonistic selection, sexual dimorphism, and the resolution of intralocus sexual conflict. The American Naturalist. - 2009. - №173(2). - P. 176-187.

26 Manat, Y., et al., Female-limited X-chromosome evolution effects on male pre- and post-copulatory success. Biology Letters. - 2021. - №17(3)., P. 20200915.

27 Lund-Hansen, K.K., J.K. Abbott, and E.H. Morrow, Feminization of complex traits in *Drosophila melanogaster* via female-limited X chromosome evolution. Evolution. - 2020. - №74(12). - P. 2703-2713.

28 Abbott, J.K., et al., Epigenetics and sex-specific fitness: an experimental test using male-limited evolution in *Drosophila melanogaster*. PLoS One. - 2013. - №8(7)., P. e70493.

29 Prasad, N.G., et al., An evolutionary cost of separate genders revealed by male-limited evolution. Am Nat, 2007. - №169(1). - P. 29-37.

30 Rice, W.R., Male fitness increases when females are eliminated from gene pool: Implications for the Y chromosome. Proceedings of the National Academy of Sciences. -

1998. -№95(11). - P. 6217-6221.

31 Rice, W.R., Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. Nature. - 1996. - №381(6579). - P. 232-4.

32 Adams, M.D., et al., The genome sequence of *Drosophila melanogaster*. Science. - 2000. - №287(5461). - P. 2185-95.

33 Chippindale, A.K. and W.R. Rice, Y chromosome polymorphism is a strong determinant of male fitness in Drosophila melanogaster. Proceedings of the National Academy of Sciences. - 2001. - №98(10). - P. 5677-5682.

34 Bolger, A.M., M. Lohse, and B. Usadel, Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. - 2014. - №30(15). - P. 2114-20.

35 Kim, D., B. Langmead, and S.L. Salzberg, HISAT: a fast spliced aligner with low memory requirements. Nature Methods. - 2015. - №12. - P. 357.

36 Pertea, M., et al., Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. Nature protocols. - 2016. - №11(9). - P. 1650-1667.

REFERENCES

1 Andersson, M.B., Sexual selection. 1994. - Princeton University Press.

2 Ranz, J.M., et al., Sex-dependent gene expression and evolution of the Drosophila transcriptome. Science. - 2003. - №300(5626). - P. 1742-1745.

3 Rice, W.R., Sex Chromosomes and the Evolution of Sexual Dimorphism. Evolution. - 1984. - №38(4). - P. 735-742.

4 Burke, N.W. and R. Bonduriansky, Sexual Conflict, Facultative Asexuality, and the True Paradox of Sex. Trends Ecol Evol. - 2017. - №32(9). - P. 646-652.

5 Abbott, J.K., Intra-locus sexual conflict and sexually antagonistic genetic variation in hermaphroditic animals. Proc Biol Sci. - 2011. - №278(1703). - P. 161-9.

6 Stewart, A.D., A. Pischedda, and W.R. Rice, Resolving intralocus sexual conflict: genetic mechanisms and time frame. Journal of Heredity. - 2010. - №101(suppl_1). - P. S94-S99.

7 Bonduriansky, R. and S.F. Chenoweth, Intralocus sexual conflict. Trends in ecology & evolution. - 2009. - №24(5). - P. 280-288.

8 Lande, R., Sexual dimorphism, sexual selection, and adaptation in polygenic characters. Evolution. - 1980. - №34(2). - P. 292-305.

9 Pischedda, A. and A.K. Chippindale, Intralocus sexual conflict diminishes the benefits of sexual selection. PLoS biology. - 2006. - №4(11). - P. e356.

10 Beukeboom, L.W. and N. Perrin, The Evolution of Sex Determination. - 2014. - Oxford University Press.

11 Bachtrog, D., et al., Sex determination: why so many ways of doing it? PLoS Biol. -2014. - №12(7). - P. e1001899.

12 Rice, W.R., Evolution of the Y sex chromosome in animals. Bioscience. - 1996. - №46(5). - P. 331-343.

13 Ellegren, H., Sex-chromosome evolution: recent progress and the influence of male and female heterogamety. Nat Rev Genet. - 2011. - №12(3). - P. 157-66.

14 Bachtrog, D., et al., Are all sex chromosomes created equal? Trends Genet. - 2011. -№27(9). - P. 350-7.

15 Charlesworth, B. and N.D. Dempsey, A model of the evolution of the unusual sex chromosome system of *Microtus oregoni*. Heredity (Edinb). - 2001. - №86(Pt 4). - P. 387-94.

16 Charlesworth, B., J.A. Coyne, and N.H. Barton, The Relative Rates of Evolution of Sex Chromosomes and Autosomes. The American Naturalist. - 1987. - №130(1). - P. 113-146.

17 Abbott, J.K., A.K. Norden, and B. Hansson, Sex chromosome evolution: historical insights and future perspectives. Proc Biol Sci. - 2017. - №284(1854).

18. Vicoso, B. and B. Charlesworth, Effective population size and the faster-X effect: an extended model. Evolution. - 2009. - №63(9). - P. 2413-26.

19 Caballero, A., On the effective size of populations with separate sexes, with particular reference to sex-linked genes. Genetics. - 1995. -№139(2). - P. 1007-11.

20 Lester, L.J. and R.K. Selander, Population genetics of haplodiploid insects. Genetics. - 1979. - №92(4). - P. 1329-45.

21 Hartl, D.L., A Fundamental Theorem of Natural Selection for Sex Linkage or Arrhenotoky. The American Naturalist. - 1972. - №106(950). - P. 516-524.

22 HALDANE, J.B.S., A mathematical theory of natural and artificial selection. part ii the influence of partial self-fertilisation, inbreeding, assortative mating, and selective fertilisation on the composition of mendelian populations, and on natural selection. Biological Reviews. - 1924. - №1(3). - P. 158-163.

23 Gibson, J.R., A.K. Chippindale, and W.R. Rice, The X chromosome is a hot spot for sexually antagonistic fitness variation. Proc Biol Sci. - 2002. - №269(1490). - P. 499-505.

24 Bonduriansky, R. and S.F. Chenoweth, Intralocus sexual conflict. Trends Ecol Evol. - 2009. - №24(5). - P. 280-8.

25 Cox, R.M. and R. Calsbeek, Sexually antagonistic selection, sexual dimorphism, and the resolution of intralocus sexual conflict. The American Naturalist. - 2009. - №173(2). - P. 176-187.

26 Manat, Y., et al., Female-limited X-chromosome evolution effects on male pre- and post-copulatory success. Biology Letters. - 2021. - №17(3)., P. 20200915.

27 Lund-Hansen, K.K., J.K. Abbott, and E.H. Morrow, Feminization of complex traits in *Drosophila melanogaster* via female-limited X chromosome evolution. Evolution. - 2020. - №74(12). - P. 2703-2713.

28 Abbott, J.K., et al., Epigenetics and sex-specific fitness: an experimental test using male-limited evolution in *Drosophila melanogaster*. PLoS One. - 2013. - №8(7)., P. e70493.

29 Prasad, N.G., et al., An evolutionary cost of separate genders revealed by male-limited evolution. Am Nat, 2007. - №169(1). - P. 29-37.

30 Rice, W.R., Male fitness increases when females are eliminated from gene pool: Implications for the Y chromosome. Proceedings of the National Academy of Sciences. - 1998. -№95(11). - P. 6217-6221.

31 Rice, W.R., Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. Nature. - 1996. - №381(6579). - P. 232-4.

32 Adams, M.D., et al., The genome sequence of *Drosophila melanogaster*. Science. - 2000. - №287(5461). - P. 2185-95.

33 Chippindale, A.K. and W.R. Rice, Y chromosome polymorphism is a strong determinant of male fitness in Drosophila melanogaster. Proceedings of the National Academy of Sciences. - 2001. - №98(10). - P. 5677-5682.

34 Bolger, A.M., M. Lohse, and B. Usadel, Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. - 2014. - №30(15). - P. 2114-20.

35 Kim, D., B. Langmead, and S.L. Salzberg, HISAT: a fast spliced aligner with low memory requirements. Nature Methods. - 2015. - №12. - P. 357.

36 Pertea, M., et al., Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. Nature protocols. - 2016. - №11(9). - P. 1650-1667.

ИЗМЕНЕНИЯ ДИФФЕРЕНЦИАЛЬНОЙ ЭКСПРЕССИИ ГЕН В ГОЛОВАХ МУХ КАК ОТВЕТ НА ЭКСПЕРИМЕНТАЛЬНО ЭВОЛЮЦИОННУЮ Х-ХРОМОСОМУ

Манат Е.[1,*], Исабекова А.С.[1], Каукабаева Г.К.[1]

[1] Национальный центр биотехнологии, Кургальжинское шоссе, 13/5, Казахстан, Астана, 010000
**manat@biocenter.kz*

АННОТАЦИЯ

Сексуально-антагонистические (SA) мутации, которые повышают приспособленность у одного пола и снижают ее у другого пола (также известные как внутрилокусный половой конфликт), занимают центральное место в гипотезе полового антагонизма в эволюции половых хромосом. Давно предполагалось (Фишер, в 1931 г.), что тесное сцепление с локусом, определяющим пол, способствует накоплению СА-мутаций даже тогда, когда их вредное воздействие на один пол превышает пользу на другой. Из-за трудностей прямого обнаружения аллелей SA мы очень мало знаем о природе Х-сцепленных мутаций SA и их роли в эволюции дифференциальной экспрессии генов между полами. Чтобы исследовать, как Х-сцепленные мутации SA влияют на профиль транскриптома у обоих полов, мы проанализировали изменения в геномном паттерне экспрессии генов в ткани головы *Drosophila melanogaster*, которая претерпела 95 поколений экспериментальной эволюции Х-хромосомы, ограниченной самками. Мы обнаружили доказательства изменения экспрессии генов в сторону оптимума самки, что мы интерпретируем как результат разрешения полового конфликта из-за Х-сцепленных мутаций SA. Кроме того, мы также обнаружили потенциальный неизвестный эффект генной экспрессии балансирной хромосомы на генную экспрессию (это необходимо было использовать для ограничения наследования Х-хромосомы-мишени женской линии). Все эти данные указывают на то, что высокополиморфная природа Х-хромосомы играет важную роль в профиле экспрессии генов в масштабах всего генома.

Ключевые слова: Х-хромосома, половоантагонистическая мутация, дифференциальная экспрессия генов, экспериментальная эволюция, феминизация, наследование.

ТӘЖІРИБЕЛІК ЭВОЛЮЦИЯЛАНҒАН Х ХРОМОСОМАСЫНА ЖАУАП РЕТІНДЕГІ ДИФФЕРЕНЦИАЛДЫ ГЕН ЭКСПРЕССИЯСЫНДАҒЫ ӨЗГЕРІСТЕР

Манат Е.[1,*], Исабекова А.С.[1], Каукабаева Г.Қ.[1]

[1] Ұлттық биотехнология орталығы, Қорғалжынтасжолы, 13/5, Қазақстан, Астана, 010000.
**manat@biocenter.kz*

ТҮЙІН

Бір жыныстағы фитнесті арттыратын және екінші жыныста оны төмендететін жыныстық антагонистік (ЖА) мутациялар (сонымен қатар локус ішкілік жыныстық қақтығыс деп те белгілі) жыныстық хромосома эволюциясының жыныстық антагонизм гипотезасының негізгі мәні болып табылады. Быраз бұрын (Фишер, 1931 ж.) жынысты анықтайтын локуспен тығыз байланыс SA мутацияларының жинақталуын жеңілдетеді, тіпті олардың бір жыныстағы зиянды әсері басқа жыныстағы пайдасынан асып кетсе де, ұсынылды. ЖА аллельдерін тікелей анықтаудағы қиындықтарға байланысты біз Х-байланысты ЖА мутацияларының табиғаты және олардың жыныстар арасындағы дифференциалды ген экспрессиясының эволюциясындағы рөлі туралы өте аз білеміз. Х-байланысты ЖА мутацияларының екі жыныстағы транскриптом профиліне қалай әсер ететінін зерттеу үшін біз әйелдермен шектелген Х-хромосомасының эксперименттік эволюциясының 95 ұрпағын бастан өткерген *Drosophila melanogaster* бас тініндегі геномдық ген экспрессиясының үлгісіндегі өзгерістерді талдадық. Біз ген экспрессиясының әйелдік оптимумға қарай өзгеруінің дәлелін таптық, оны Х-байланысты SA мутациялары бойынша жыныстық қақтығысты шешудің нәтижесі ретінде түсіндіреміз. Сонымен қатар, біз баланстық хромосоманың ген экспрессиясына әлеуетті белгісіз ген экспрессиясының әсерін таптық (бұл мақсатты Х хромосомасының әйел сызығына тұқым қуалауын шектеу үшін қажет болды). Барлық осы тұжырымдар геномдық ген экспрессия профилінде маңызды рөл атқаратын Х хромосомасының жоғары полиморфты табиғатын көрсетеді.

Кілттік сөздер: Х хромосома, жыныстық антагонистік мутация, дифференциалды ген экспрессиясы, эксперименттік эволюция, феминизация, тұқым қуалаушылық.