Variability of polyteny of giant chromosomes in Drosophila melanogaster salivary glands

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Research Article

Keywords: polytene chromosomes, endoreduplication, genotype differences, sex differences, fitness.

Posted Date: July 22nd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1877346/v1

Abstract

Polyteny is an effective mechanism for accelerating growth and enhancing gene expression in eukaryotes. The purpose of investigation was to study the genetic variability of polyteny degree of giant chromosomes in the salivary glands of *Drosophila melanogaster* Meig. in relation to the differential fitness of different genotypes. 16 strains, lines and hybrids of fruit flies were studied. This study demonstrates the significant influence of hereditary factors on the level of polytenization of giant chromosomes in *Drosophila*. This is manifested in the differences between strains and lines, the effect of inbreeding, chromosome isogenization, hybridization, adaptively significant selection, sexual differences, and varying degrees of individual variability of a trait in different strains, lines, and hybrids. The effect size of the genotype on the degree of chromosome polyteny in *Drosophila* salivary glands was 45.3%, the effect size of sex was 9.5%. The data obtained allow us to conclude that the variations in the level of polyteny should be considered as a manifestation of the structural and functional polymorphism of giant chromosomes, which is an essential factor in the differential fitness of flies and has a selective value.

Introduction

Recently, there has been growing interest in studying the genetic effects of polyteny as an effective mechanism for accelerating growth and enhancing gene expression in eukaryotes (Bandura and Zielke 2017; Øvrebø and Edgar 2018; Ren et al. 2020; Costa et al. 2021; Peterson and Fox 2021).

Polyteny is a variety of endopolyploidy that is widespread in animals and plants (Brodskiy and Uryvayeva 1981; Stormo and Fox 2017). The basis of this phenomenon is endoreduplication, a mechanism that provides the appearance of many identical copies of DNA in the cell nucleus in the absence of chromatin condensation, chromosome segregation, and cytokinesis. In endoreduplication, the cell cycle differs from the mitotic cycle. Polytene chromosomes are formed as a result of endocycles in which mitosis is completely lost. It all comes down to alternating G- and S-phases, which follow each other. Another variant of the cell cycle that leads to endopolyploidy is called endomitosis. In this case, incomplete mitosis occurs (Bandura and Zielke 2017; Peterson and Fox 2021). Endoreduplication underlies postmitotic tissue growth in plants and animals and is an alternative to the proliferative growth type. According to experts (Sugimoto-Shirasu and Roberts 2003; Zielke et al. 2011), this produces about half of the planet's biomass. It is obvious, therefore, that research in this area is of great scientific and practical interest.

Many authors note the important role of polyteny in cell differentiation in development and its evolutionary significance (Nagl 1976; Edgar et al. 2014; Nozaki and Matsuura 2019; Bomblies 2020). There has been significant progress in the study of genetic and molecular mechanisms of endocycle regulation (Lee et al., 2009, Shakina, Strashnyuk, 2011; Edgar et al. 2014). In recent years, much attention has been paid to the study of signaling pathways that provide epigenetic switching of the cell cycle from mitosis to endoreduplication in various types of tissues (Bandura and Zielke 2017; Øvrebø and Edgar 2018; Ren et al, 2020; Costa et al. 2021).

At the same time, experimental data on the hereditary variability of endoreduplication are very limited. There are a number of studies on this topic in plants (Larkins et al. 2001, Chiniclet et al. 2005; Li et al. 2019; Kobayashi 2019; Frakova et al. 2021; Wos et al. 2022) that report differences between species, cultivars or lines. However, they cannot fully satisfy the interest in these issues. Notable is the lack of research in this area on such a model object as *Drosophila*. The effect of polyteny variability on fitness is also poorly understood. According to (Zielke et al. 2013), the benefit of the endocycle remains to be elucidated.

The purpose of investigation was to study the genetic variability of polyteny degree of giant chromosomes in the salivary glands of *Drosophila melanogaster* Meig. in relation to the differential fitness of different genotypes. The aims were to assess the effect of hereditary factors on the level of endoduplication in the salivary glands of fruit fly larvae, in particular, the influence of different breeding methods, hybridization, adaptively significant selection, some mutations, sex differences, to estimate the degree of variation in the level of chromosome polyteny in different strains, lines and hybrids, as well as the size of the genotype effect on the genome amplification in *Drosophila.*

Materials And Methods

Biological material and experimental conditions

The material for the research were wild-type and mutant strains, selected, inbred and isogenic lines of *Drosophila melanogaster* Meig. from the collection of the Department of Genetics and Cytology, VN Karazin Kharkiv National University. A brief description of the strains, lines and hybrids used in the study is given below.

Low-activity (*LA*) line – highly inbred line, obtained by Kaidanov by inbreeding and selection for the low sexual activity of males from the Essentuki population. The degree of inbreeding at the beginning of the experiment was more than 600 generations. *LA* flies has a complex of inadaptive traits, such as short lifespan, low fertility, low heat resistance, low mobility, etc. (Kaidanov et al. 1997; Iovleva, Mylnikov 2007).

High-activity (*HA*) line – highly inbred line, obtained by Kaidanov from the *LA* line by inbreeding and selection for the high sexual activity of males (Kaidanov et al. 1997; Iovleva and Myl'nikov 2007). The degree of inbreeding at the beginning of the experiment was more than 600 generations. *HA* flies surpass *LA* in fitness components listed above.

Swedish (*Sw*) – wild-type strain, maintained by mass crosses and outbreeding.

Swedish inbred line (Sw_{in}), obtained from the *Sw* strain by crossing siblings. The degree of inbreeding was 40–42 generations.

Oregon-R (*Or*) – wild-type strain, maintained by mass crosses and outbreeding.

Oregon-R inbred line (Or_{in}), obtained from the *Or* strain by crossing siblings for 56 – 76 generations.

Canton-S inbred line ($C\text{-}S_{in}$), obtained from wild-type *Canton-S* strain by crossing siblings for 58 – 78 generations.

Mutant *vestigial* (*vg*) strain. Mutation *vg* (2–67,0) phenotypically manifests in the reduction of the wings in homozygotes. A set of alleles of different phenotypic manifestation is described. Gene vg^+ defines the proliferation of cells in fly's wing. In the absence of expression of the gene vg^+ the cells of the wing and the galters of imaginal discs lose their normal proliferation, leading to the formation of reduced wings in adults (FlyBase). We included this strain in the study due to the known pleiotropic effect of the *vg* mutation on fitness components: *vg* flies are characterized by reduced sexual activity, low fecundity, stress resistance and lifespan (Strashnyuk et al. 1985; Pezzoli et al. 1986; Totskiĭ et al. 1998).

$Bar_{C\text{-}S}$ ($B_{C\text{-}S}$) – the line was obtained by 8 backcrossing of flies of mutant *Bar* strain with the flies of wild-type *Canton-S* strain. Mutation *Bar* (*B*) (1–57,0) is a tandem duplication of 16A1-16A7 region of cromosome X, phenotypically manifested in reduction of eyes to the narrow vertical strip with a number of facets 90 in males and 70 in females, in contrast to the normal amount of about 740 and 780 the facets for males and females, respectively (FlyBase). The pleiotropic effect of *Bar* mutations is manifested in increased embryonic mortality, about half of the individuals do not complete development (Skorobagatko et al. 2015).

iso^{II}; iso^{III} $Bar_{C\text{-}S}$ – the line was derived from the $Bar_{C\text{-}S}$ line by izogenization of chromosomes 2 and 3. The izogenization was carried out according to the classical scheme using the balancer line *Cy/Pm;D/Sb* (Tikhomirova 1990).

In addition, interline F_1 hybrids were investigated: *LA × HA*, *HA × LA*, $Or_{in} \times C\text{-}S_{in}$, $C\text{-}S_{in} \times Or_{in}$, $C\text{-}S_{in} \times vg$, $vg \times C\text{-}S_{in}$.

The flies developed on a standard sugar-yeast medium at a temperature of 24-25°C. For cultivation, 60 ml glass vials containing 10 ml of nutrient medium were used. A pair of parental flies were placed in each vial.

At least ten larvae of each genotype were studied. Females and males were examined separately. In total, 227 females and 228 males of *Drosophila* larvae were examined.

Determination of polyteny degree of giant chromosomes

Polytene chromosomes were studied on squashed preparations of larva salivary glands. Acetoorcein staining was used: 2% of orcein (Merck KGaA, Darmstadt, Germany) in a 45% solution of acetic acid (Reahimtrans, Kyiv, Ukraine). Wandering larvae at the end 3rd instar were taken into the experiment. According to Rodman (1967), the initiation of new endoreduplication cycles in the salivary glands of the

larvae ceases by this time. Giant chromosomes were examined using a light microscope (Granum R 6003, China).

To study the differences in the degree of chromosome polyteny, we used the cytomorphometry method (Strashnyuk et al., 1995). It is known that by the end of larval development, 7 to 10 rounds of endoreduplication occur in the cells of *D. melanogaster* salivary glands. As a result, the cells achieve different values of ploidy or C values, which indicates chromatin amount, as a multiple of the haploid genome (Øvrebø and Edgar 2018). In total, four classes of nuclei have been identified whose C values are 256C, 512C, 1024C and 2048C (Rodman, 1967). On cytological preparations, chromosomes with varying degrees of polyteny differ in thickness, and they are stained by acetoorsein with different intensities (Kiknadze and Gruzdev 1970, Strashnyuk et al. 1995). Control measurements were performed in the region of the 22A band of chromosome 2L at 640 × magnification. Four classes of nuclei with different levels of ploidy contain chromosomes of different thicknesses: about 1.6, 2.3, 3.2, and 4.6 μm, respectively. Nuclei with different ploidy levels were counted on salivary gland preparations at 160 × magnification.

Polyteny was assessed by various indeces. The number of nuclei with different levels of ploidy (256C, 512C, etc.) was counted and their percentage was determined. At least 100 nuclei were examined on each sample.

Mean C value was calculated based on the data on the distribution of nuclei with different C values (Frakova et al. 2021) following the equation:

$$\text{Mean C value} = \frac{\sum \left[\left(n_1 \times 256\text{C} \right) + \left(n_2 \times 512\text{C} \right) + \left(n_3 \times 1024\text{C} \right) + \left(n_4 \times 2048\text{C} \right) \right]}{N}$$

where n_1, n_2, n_3, n_4 are counts of nuclei with chromosomes corresponding polyteny level classes (256C, 512C, 1024C, and 2048C), and N is the total amount of nuclei on the sample: $N = n_1 + n_2 + n_3 + n_4$.

The maximum number of endocycles in salivary gland cells for each genotype that occurred during larval development is reported. Finally, the degree of variation of the mean C value in each strain, line, and hybrid was assessed.

Statistical methods

The data on the ratio of nuclei with different polyteny levels are given in percentages. The statistical significance of differences between sample proportions was assessed using the *F*-test. Mean values of polyteny are presented as mean ± standard error. The verification of data distribution for compliance with the normal law was performed using the Shapiro-Wilk test. The influence of genotype and sex on polyteny degree of chromosomes was determined using Fisher's analysis of variance. We used two-way ANOVA. The effect size (η^2) was determined as the proportion of factorial variation in the total variation of the trait according to the Snedecor method. Student's *t*-test was used to compare individual genotypes.

The degree of variation of the trait was evaluated by the coefficient of variation (C_V). The effects or differences were considered significant at $p \leq 0.05$.

Results

Hereditary variability of polyteny degree of giant chromosomes

Each endoreduplication round leads to a twofold increase in the number of chromatin fibers in the polytene chromosomes. The greater polyteny degree of the chromosomes, the more intense their staining with orcein. Thus, nuclei with different levels of polyteny can be easily distinguished visually. Earlier, we showed a correspondence between the cytomorphometric parameters of chromosomes and their polyteny degrees (Strashnyuk et al., 1995). The location of cells with various ploidy in the salivary gland also differs: nuclei with a lower C value are concentrated in proximal part, and those with a higher ploidy are in the distal part of the gland (Fig. 1).

Analysis of polyteny in different strains, lines and F_1 hybrids of *Drosophila* showed significant genetic variation in this trait. Figure 2 presents data on the percentage ratio of nuclei with different C values in larva salivary glands. As a rule, 1024C nuclei are most frequently represented. However, 512C nuclei prevailed in males of the *LA* inbred line, while in females of this line and in *vestigial* males, the ratio of 512C and 1024C nuclei was at the same level. In the cells of *D. melanogaster* salivary glands, at most 10 endocycles can occur. Such nuclei with a ploidy level of 2048C represented the minor fraction. We did not reveal such nuclei in *LA* and *HA* larvae of both sexes, as well as in males of the *Oregon-R* inbred line and isogenic iso^{II}; iso^{III} Bar_{C-S} line. The 2048C nuclei were also absent in males of the F_1 *C-S* × *vg* hybrid. Thus, in these cases, no more than 9 cycles of endoreduplication occurred.

Data on the distribution of nuclei with different ploidy values were used to calculate the mean C values for different genotypes. This allowed us to evaluate the relative differences between them. The data are presented in Fig. 3. The range of variation of the trait turned out to be quite significant. In males, the minimum levels of polyteny were found in the *vestigial* strain, and the maximum levels were found in the F_1 hybrid *vg* × *C-S*$_{in}$. The differences were 44.6% ($p < 0.001$). Among females, the lowest ploidy values were in the *LA* inbred line, and the highest, in the *Oregon-R* wild type strain, which differed by 33.5% ($p < 0.001$). Given that there is a certain variability within the strains, it is obvious that individual differences can be much greater.

Comparison of wild-type *Swedish* and *Oregon-R* strains, which origin from geographically remote populations, showed the superiority of *Oregon-R* females by an average of 9.5% ($p < 0.05$). No significant differences were found in males.

Different breeding methods affected endoreduplication in different ways. Polyteny levels in inbred lines were lower than in wild-type strains maintained by mass crosses and outbreeding. This is obviously a

manifestation of inbred depression. Thus, the wild-type *Swedish* strain exceeded the inbred line: females by an average of 6.6% ($p < 0.05$), males by 10.9% ($p < 0.05$). Similarly, males of the wild-type *Oregon-R* strain exceeded males of the inbred line by 11.9% ($p < 0.01$). In females, the differences were not significant.

Highly inbred *LA* and *HA* lines, selected on male sexual activity, are also characterized by reduced levels of polyteny. In particular, the low activie *LA* line was characterized by extremely low polyteny values, yielding to the highly active *HA* line: females on average by 15.9% ($p < 0.01$), males – by 12.8% ($p < 0.01$). According to (Kaidanov et al. 1997), the selection for low sexual activity in the *LA* line affected a complex of important adaptive characteristics. This line has reduced fertility, heat resistance, mobility, life expectancy. Thus, polyteny degree of chromosomes in the *LA* and *HA* lines correlates with their fitness properties.

The mutant *vestigial* strain, like the *LA* line, is characterized by a complex of inadaptive traits. These flies have reduced fecundity, stress resistance and life expectancy. The inadaptive properties of the *vestigial* strain correlate with extremely low polyteny values.

The *Bar* mutation (in Bar_{C-S} line), which causes a high level of embryonic mortality, did not reduce the ploidy level. Isogenization of chromosomes, which is similar in nature to inbreeding, had a depressing effect on endoreduplication. In females of the isogenic iso^{II}; iso^{III} Bar_{C-S} line, the polyteny was lower compared to the original Bar_{C-S} line. On average, the differences were 8.2% ($p < 0.001$). In males, the trait did not change.

In hybrids, polyteny values were more aligned compared to strains and inbred lines. This applies to both the distribution of nuclei with different levels of ploidy and the mean C values. Hybrids F_1 *LA* × *HA*, *HA* × *LA* exceeded the parental lines *LA* and *HA*. The excess of mean C values over the best of the parent line (*HA*) was 7.9–11.6% ($p < 0.05$). These data indicate that increased endoreduplication may be one of the possible causes of the manifestation of hybrid vigor. Other F_1 hybrids (Or_{in} × $C\text{-}S_{in}$, $C\text{-}S_{in}$ × Or_{in}, $C\text{-}S_{in}$ × *vg*, *vg* × $C\text{-}S_{in}$) did not show superiority over parent lines. However, it should be noted that this is true only for optimal temperature conditions and in the absence of overpopulation. As was previously shown, under non-optimal conditions, for example, at elevated temperature (Strashnyuk et al. 1997) or culture density (Zhuravleva et al. 2004), hybrids can exceed parent lines by polyteny, although at optimum conditions they did not show differences.

Analysis of variance showed a significant contribution of the genotype to the variability of polyteny in the salivary glands of *Drosophila* larvae (Table 1): the effect size was 45.3% ($p < 0.001$).

Table 1

Effect sizes of genotype and sex on the degree of chromosome polyteny in *Drosophila melanogaster* salivary glands

Acting factors	Indicators of variance analysis		
	η^2 (%)	F_φ	p
Genotype	45.3	32.5	< 0.001
Sex	9.5	53.9	< 0.001
Joint effect of sex and genotype	4.4	2.5	< 0.01

Sex differences in polyteny levels

Sex differences either were not manifested (e.g., in the *Swedish* wild-type strain, *Canton-S* inbred line, iso^{II}; iso^{III} Bar_{C-S} line, in hybrids F_1 Or_{in} × $C\text{-}S_{in}$, $C\text{-}S_{in}$ × Or_{in}, $C\text{-}S_{in}$ × *vg*, and *vg* × $C\text{-}S_{in}$), or females had higher levels of polyteny as for males (e.g., in inbred *LA, HA*, *Oregon-R*, and *Swedish* lines, in the *Oregon-R* wild-type strain, *vestigia*l strain, Bar_{C-S} line, in hybrids F_1 *LA* × *HA*, and *HA* × *LA*). In no case the superiority of males over females on the degree of genome amplification was recorded. The greatest sex differences were found in the *vestigial* strain: the mean C value in females was 20.8% higher than in males (p < 0.001).

Figure 4 shows the sex differences in the average distribution of nuclei with different levels of polyteny by the sum of the data obtained. In females, compared to males, a higher content of nuclei with higher C values, such as 1024C and 2048C, was revealed, nuclei 256C and 512C were found with a lower frequency (p < 0.01–0.001). On average, females were superior to males by 5.8% (p < 0.05) (Fig. 5). The effect size of sex on the degree of chromosome polyteny was 9.5% (p < 0.001). The joint effect of sex and genotype was 4.4% (p < 0.01) (Table 1).

Variations of polyteny in strains, lines and hybrids

Variations in polyteny degree of giant chromosomes varied among different strains, lines, and hybrids (Table 2). In many cases, the method of breeding influenced the variations of the trait. Thus, in the *Swedish* inbred line, the coefficient of variation (C_V) of mean C values in female was 59.5% lower (p < 0.05) than in the *Swedish* wild-type strain; in males the differences were not significant. In the *Oregon-R* inbred line, on the contrary, the variations of polyteny in females were not significant, however, in males the C_V was 2.7 times greater than in the original wild-type strain. In inbred lines, the coefficient of variation of polyteny, as a rule, was higher than in their hybrids. These differences were statistically significant in F_1 hybrids *C-S* × *vg*, *vg* × *C-S* and their parents, the coefficient of variation differed by 1.6–3.7 times (p < 0.001). The Bar_{C-S} line, which has a hybrid origin, also had a low variations of the trait. Isogenization of the line on chromosomes 2 and 3 (the iso^{II}; iso^{III} Bar_{C-S} line) did not affect the C_V values.

Table 2

Coefficients of variation (C_V) of mean C values in *Drosophila melanogaster* salivary glands

Strains, lines, hybrids	Coefficients of variation (C_V)	
	Females	Males
LA	15.5 ± 3.4	11.2 ± 2.5
HA	9.4 ± 2.1	9.7 ± 2.2
Swedish (*Sw)*	11.8 ± 1.8	16.2 ± 2.4
Swedish inbred (Sw_{in})	7.4 ± 1.1	11.0 ± 1.6
Oregon-R (*Or*)	8.2 ± 1.9	5.4 ± 1.3
Oregon-R inbred (Or_{in})	5.0 ± 0.8	14.5 ± 2.8
Canton-S inbred ($C\text{-}S_{in}$)	7.7 ± 1.5	8.3 ± 1.6
vestigial (*vg*)	9.8 ± 1.7	16.0 ± 3.0
$Bar_{C\text{-}S}$ ($B_{C\text{-}S}$)	4.3 ± 1.0	4.9 ± 1.1
iso^{II}; iso^{III} $Bar_{C\text{-}S}$ ($isoBar_{C\text{-}S}$)	6.0 ± 1.3	4.2 ± 0.9
F_1 *LA* × *HA*	5.7 ± 1.3	7.0 ± 1.6
F_1 *HA* × *LA*	5.8 ± 1.3	9.0 ± 1.9
F_1 $C\text{-}S_{in}$ × *vg*	3.7 ± 0.8	4.3 ± 0.9
F_1 *vg* × $C\text{-}S_{in}$	3.8 ± 0.8	5.2 ± 1.1
F_1 Or_{in} × $C\text{-}S_{in}$	5.4 ± 1.0	5.6 ± 1.1
F_1 $C\text{-}S_{in}$ × Or_{in}	6.1 ± 1.2	6.4 ± 1.2

Variations in polyteny in males and females within the strains, lines, or hybrids, as a rule, did not have significant differences. However, in the inbred *Oregon-R* line, the coefficient of variation in males was 2.9 times higher than in females ($p < 0.01$).

Discussion

Endocycle control at the molecular level is carried out by key regulators of the cell cycle, such as cyclins, cyclin-dependent kinases, and modulators of their activity. Studies in *Drosophila* have shown that

switching from the mitotic cycle to endocycling is associated with the loss of mitosis-activating cyclins A and B and the subsequent periodic expression of cyclin E, activating the S-phase (Zielke et al. 2011; Fox and Duronio 2013; Edgar et al. 2014). This transition is part of a developmental program that includes signaling and epigenetic re-programming.

Cell growth in different tissues in *Drosophila* is regulated by several signaling pathways, including Notch, PI3K/TOR, EGFR/MAPK, JAK/STAT, JNK and Hippo (Hpo)/Yki (Deng et al. 2001; Bandura and Zielke 2017; Øvrebø and Edgar 2018; Ren et al. 2020; Costa et al. 2021). In the salivary glands of *Drosophila* larvae, the endocycle rate appears to be controlled downstream of the TOR (target of rapamycin) pathway by the expression of a single *Drosophila* activator E2F: E2F1 (Øvrebø and Edgar 2018). TOR signaling works together with insulin/insulin-like growth factor (IIS) to control cellular responses to nutritional stimuli (Costa et al. 2021). The latest data complement the understanding of genetic networks and transcriptional cascades involved in endocycle regulation (Rotelli et al. 2019; Qian et al. 2020; Wang et al. 2020; Kim et al. 2021; Costa et al. 2021).

Humoral factors play an important role in the regulation of endocycles (Shakina and Strashnyuk 2011; Ren et al. 2020). It is well known that the control of insect development is regulated by two main hormones, juvenile hormone (JH) and ecdysterone (ES). Both hormones have multiple functions, affecting insect growth, metamorphosis and reproduction in different ways. Currently, the key role of JH in the implementation of the genetic program responsible for the amplification of the genome has been proven. JH has been shown to promote cell polyploidization by directly activating genes involved the regulation of G_1/S transition and DNA replication (Guo et al. 2014; Wu et al. 2018). With regard to the role of ES in these processes, the available data are very contradictory. The effect of ES on cellular polyploidy varies in different insect species and depends on the hormone content in the hemolymph (Shakina and Strashnyuk 2011; Moriyama et al. 2016). ES, like JH, is able to bind to appropriate nuclear receptors to initiate expression of cell cycle genes (Ren et al. 2020). The interactions between JH and ES in the regulation of cell polyploidization are also poorly understood. Classically, these two hormones function as incomplete antagonists. According to (Ren et al. 2020), JH and ES can jointly coordinate the timing of DNA reduplication and cell division during the mitotic to endocycle switch process.

Thus, endocycles are regulated by a complex of genetic, molecular, and humoral factors. Certain stages of development are accompanied by specific cytophysiological and epigenetic changes, which creates the necessary conditions for endoreduplication. It is obvious that such a multistep mechanism is influenced by many conditions that are capable of modulating the passage of individual links of this regulation.

According to (Gruntenko et al. 2000, 2007), the levels of JH, ES, and associated biogenic amines in *Drosophila* exhibit hereditary variability. Their content and exchange also differ in females and males. In our study, an example is the *LA* inbred line, which is characterized by a reduced level of JH in the hemolymph (Kaidanov et al. 1997; Iovleva and Myl'nikov 2007). This correlates with extremely low values of polyteny in this line. *In vitro* studies of puffing in polytene chromosomes have also shown that inbred

lines and their hybrids differ in the rate of response to ecdysterone. In inbred lines, a delay in the regression of intermolt puffs and the activation of early ecdysone-induced puffs was observed (Strashnyuk et al. 1991). Thus, the rate of hormonal signal transduction at the level of gene expression varies depending on the genotype.

Endocrine factors can be involved in the modulation of the endocycle functioning in *Drosophila* under stress conditions. The response to stress, as well as development and reproduction in insects, is regulated by hormones (Gruntenko and Rauschenbach 2008). Rauschenbach et al. (1996) showed the presence of polymorphism in the level of JH metabolism and response to stress in natural populations of *D. melanogaster*. According to the authors, the existing polymorphism is a reflection of the existence of a population under conditions of frequent stress effects of low intensity, which can be caused by a wide range of environmental factors, including anthropogenic effects.

Various external stimuli are capable of initiating endocycling or influencing the level of endoreduplication. Switching from mitosis to the endocycle is possible with injuries (Øvrebø and Edgar 2018; Grendler et al. 2019), mutualistic (Bainard et al. 2011), and parasitic (Hesse 1969) interactions. Pavan et al. (1971) observed unicellular tumors and greatly enlarged polytene chromosomes within the nuclei of the cells of the intestinal caeca and the mid-intestine in *Rhynchosciara angelae* when infected with Rhynchosciara polyhedrosis virus (RPV). An increase in endoreduplication was observed in *D. melanogaster* ovarian pseudonurse cells at low temperatures and protein-rich food (Mal`ceva et al. 1995). In contrast, the depleted amino acid composition of the nutrient medium inhibited endoreduplication in the larval tissues of *Drosophila* (Britton and Edgar 1998). Nesterkina et al. (2018, 2020) reported on the action of terpenoids and phenols, which are used in the development of modern insect pest control technologies, on the degree of chromosome polyteny. Lei et al. (2020) found an increase in cell ploidy in tissues of holometabolic insects such as cowpea bruchid (*Callosobruchus maculatus*), corn earworm (*Helicoverpa zea*) and fruit fly (*Drosophila melanogaster*) after electron beam irradiation. Our earlier studies have shown a significant effect on endoreduplication of temperature conditions (Strashnyuk et al. 1997), culture density (Rarog et al. 1999; Zhuravleva et al. 2004), parental age (Rarog et al. 2004). Among man-made factors, microwaves (Dyka et al. 2016) and gamma irradiation (Skorobagatko et al. 2020) had a marked impact. These examples show that induction or modulation of endocycling is a way of adaptation of an organism to changing environmental conditions or a form of a stress response.

It is known that the development, survival and reproduction of plants and animals largely depend on the method of breeding. Genetic distances in inbreeding, outbreeding or hybridization largely determine the selective value of different genotypes. In particular, this is shown in a *Drosophila melanogaster* model system (Houle 1989; Jensen et al. 2018). The data obtained in the current study showed a decrease in the level of endoreduplication under the influence of inbreeding, chromosome isogenization, selection for low sexual activity in males. In contrast, higher levels of endoreduplication occurred with outbreeding, selection for high male sexual activity, and certain combinations of crosses in hybrids. A decrease in the level of polyteny was also observed in the inadaptive mutant *vestigial* line. These results indicate that

variation in the level of polyteny correlates with differential fitness of different genotypes, ie has a selective value.

As for the sex differences in polyteny, they are obviously related to the problem of sexual size dimorphism (SSD) discussed in the literature. The SSD reflects the fundamental differences between the sexes in metabolism that exist in both invertebrates and vertebrates. Both autonomous, associated with the dosage of X chromosomes (Wehr Mathews et al. 2017), and non-autonomous, probably hormonal (Sawala and Gould 2018), mechanisms of SSD control in fruit fly are discussed. The body mass of adult *Drosophila melanogaster* females is 20–60% more than males, depending on the method of measurement (live or dry mass), genotype and environmental conditions (Strashnyuk et al. 1997; Ørsted et al. 2018). Revealed sex differences in polyteny cannot fully explain such a significant difference in size. However, they do contribute to this difference.

In modern literature, in connection with the widespread occurrence of the phenomenon of polyteny, various hypotheses about the biological significance of this phenomenon are discussed. In particular, we are talking about an increase in cell sizes (Sugimoto-Shirasu and Roberts 2003; Chevalier et al. 2011; Marguerat and Bähler 2012; Kobayashi 2019), participation in the mechanisms of cell differentiation and processes of morphogenesis (Anisimov 2005; Lee et al. 2009; Chevalier et al. 2011). It has been suggested that endoreduplication protects against DNA damage and mutations (buffering of genome) (Edgar, Orr-weaver 2001), provides cell tolerance to genotoxic stress (Gandarillas et al. 2018), and modulates the stress response (Cookson et al. 2006). Polyploid cells are resistant to apoptosis when DNA damage (Mehrotra et al. 2008). Polyploidization underlies compensatory cell growth during tissue regeneration in the heart and liver in vertebrates, as well as in the epidermis and gut of *Drosophila* (Bandura and Zielke, 2017; Øvrebø and Edgar 2018; Grendler et al. 2019; Kirillova et al. 2021). According to (Gandarillas et al. 2018), the endoreduplication mechanism can act as a potential developmental timer, and is important for the control of homeostasis. Wos et al. (2022) suggest that endocycles are integrated within the stress response pathways for a fine-tune adjustment of the endoreduplication process to the local environment. Tumor growth that occurs as a result of dysregulation of the cell cycle is also often accompanied by endoreduplication (Chen et al. 2019; Moein et al. 2020; Costa et al. 2021).

Variation in polyteny can be directly related to the function of the corresponding organ or tissue. For example, Nozaki and Matsuura (2019) report on the correlation between fat cell ploidy and fertility in various species of termites. This correlates with the role of the fat body in the vitellogenesis process in these insects. Rangel et al. (2015) discuss age-related changes in ploidy in some tissues in the honey bee, *Apis mellifera* (in particular, in the brain, flight muscles, leg muscles) in connection with age-related polyethism, whereby female workers assume increasingly complex colony tasks as they age.

On the other hand, variations in polyteny can be considered in relation to growth processes. It is known that the tissues of *Drosophila* larvae grow thanks to endocycles. Endoreduplication, which underlies this auxetic growth type, exhibits significant sensitivity to endogenous and exogenous influences. This affects both the growth rate (e.g., at different genotypes, culture density or temperature) and the final

result, that is, the size and fitness of the flies. When discussing the meaning of varying polyteny, Bennett's nucleotype concept (Bennett 1982) can obviously be used. Accordingly, the content of DNA in a cell can have an effect on the phenotype regardless of the hereditary information held in it. It is also obvious that, under stress, cells with a higher degree of polyteny have a greater ability to produce more protective proteins, such as HSP, which provides better resistance.

In conclusion, the level of polyteny in *Drosophila* is under the control of hereditary and non-hereditary factors. This study demonstrates a significant effect of genotype. This is manifested in differences between strains and lines, the effect of inbreeding, chromosome isogenization, hybridization, adaptively significant selection, sex differences and varying degrees of individual variability of the trait in different strains, lines and hybrids. The obtained data allow us to conclude that variations in the level of endoreduplication is a significant factor in the differential fitness of flies. Given the significant variability in the polyteny level, this characteristic can be considered as a manifestation of the structural-functional polymorphism of giant chromosomes. Taking into account the selective significance of polyteny variations, a broader study of this type of chromosomal polymorphism is promising. In particular, it could be research in natural populations, including in connection with genetic drift, population sizes, biological invasions, or in areas which are exposed to intensive anthropogenic influence.

Abbreviations

ES – ecdysterone;

HSP – heat shock proteins;

IIS – insulin/insulin-like growth factor;

JH – juvenile hormone;

SSD – sexual size dimorphism.

Signaling pathways:

EGFR/MAPK – epidermal growth factor receptor/mitogen-activated protein kinase;

Hpo/Yki – Hippo/Yorkie;

JAK/STAT – Janus kinases/signal transducer and activator of transcription proteins;

JNK – c-Jun N-terminal kinases;

PI3K/TOR – phosphoinositide-3-kinase/target of rapamycin.

Declarations

Conflict of interest The authors declare that they have no confict of interest.

Author contributions

All authors contributed to the conception and design. All authors participated in data collection and analysis. Volodymyr Yu. Strashnyuk wrote the first draft of the manuscript. All authors commented on previous versions, read and approved the final manuscript.

Acknowledgements

This work was supported by the Ministry of Education and Science of Ukraine (Project State registration number: 0117U004836).

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Figures

Figure 1

Giant chromosomes of *Drosophila melanogaster* with different levels of polyteny in proximal (*a*) and distal (*b*) parts of the salivary gland (acetonorcein staining)

Figure 2

Genetic variability of polyteny degree of chromosomes in *Drosophila melanog*aster salivary glands: the distribution of cell nuclei with different C values

Figure 3

Genetic variability of polyteny degree of chromosomes in *Drosophila melanogaster* salivary glands: mean C values

Figure 4

Sex differences in polyteny in *Drosophila melanogaster* salivary glands: average distribution of the nuclei with different C values in females (*a*) and males (*b*)

Figure 5

Sex differences in polyteny in *Drosophila melanogaster* salivary glands: mean C values