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ORIGINAL ARTICLE

Seasonal variation in endoreduplication and polyteny in the fruit fly *Drosophila melanogaster* (Diptera: Drosophilidae): How does it contribute to adaptation?

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Key words. *Drosophila*, natural population, developmental polyploidy, polytene chromosomes, endocycles, fluctuating selection

Abstract. In temperate regions, the fruit fly *Drosophila melanogaster* (Meigen, 1830) is subject to seasonal changes in natural conditions. Insects exhibit a wide range of adaptive responses to changes in the seasons. In this study, we focused on polyteny, which plays an important role in fruit fly development. Polytene chromosomes are the result of endocycles, a variant of the cell cycle based on endoreduplication. This phenomenon is the basis of postmitotic growth, which is caused by cell expansion. The purpose of the study was to investigate the seasonal dynamics of the levels of endoreduplication and polyteny in fruit flies obtained from the natural population. Flies caught in the spring and autumn of 2019 and 2020 were used as biological material. Chromosomal differences in polyteny were studied by cytomorphometry. We found that patterns of polyteny in *Drosophila* salivary glands undergo seasonal changes. To a certain extent, these variations correlate with changes in the direction of natural selection, which undergoes seasonal fluctuations. Indices of endoreduplication and cell ploidy acquire the greatest values after overwintering, during which there is an extreme decline in population size and flies undergo selection for viability and stress resistance. During the growing season, endoreduplication and ploidy indicators are reduced. We believe that this facilitates population growth by resulting in smaller flies with accelerated development and faster change of generations. The seasonal component in the total variation of chromosome polyteny in the salivary glands of *Drosophila* larvae was 21.9%. No significant sex differences were found for this trait.

INTRODUCTION

In temperate regions, the fruit fly *Drosophila melanogaster* (Meigen, 1830) is subject to seasonal changes in natural conditions. This causes seasonal population boom and busts (Bertram & Masel, 2019; Nunez et al., 2024), as well as changes in the direction of selection, which has been called "fluctuating selection" (Bell, 2010; Johnson et al., 2023). The latter is related to seasonal adaptations acting through a compromise of resource allocation between reproduction in favourable conditions and survival in unfavourable ones (Schmidt & Conde, 2006).

Plastic responses of insects to environmental signals associated with seasonal changes attract the attention of many researchers (Danks, 2007; Denlinger et al., 2017; Erickson et al., 2020). Interest in this problem is growing due to ongoing climate change. In this regard, variations in cell ploidy in insect somatic tissues should become an interesting object of study.

Studies of chromosomal polymorphisms are common in population biology (Dobzhansky & Ayala, 1973; Garcia & Valente, 2018; Zivanovic et al., 2023). Polytene chromosomes in Diptera are often the subject of study, since they allow us to detect subtle changes in the structure of interphase chromosomes, of which they are a modified form (Zhimulev et al., 2004; Stormo & Fox, 2017). Such studies have a rich history and tradition. However, an important aspect in polytene chromosome research – variations in polyteny patterns in insect tissues within natural populations – has not yet been properly developed.

Polytene chromosomes are the result of endocycles, a variant of the cell cycle based on endoreduplication (Shakina & Strashnyuk, 2011; Ren et al., 2020). Endocycles are characterised by the loss of mitosis**;** everything comes down to alternating G- and S-phases. In fact, endoreduplication is successive rounds of DNA replication inside a non-dividing nucleus. This leads to somatic (endo-) polyploidy or developmental polyploidy, a variant of which is polyteny. Endopolyploidy is extremely common in plants and animals (Bandura & Zielke, 2017; Peterson & Fox, 2021). This phenomenon is the basis of postmitotic, so-

called auxetic growth, which is caused by cell expansion (Edgar et al., 2014; Pinto et al., 2024). In *Drosophila*, this type of growth is characteristic of many tissues and organs and occurs across all four stages of the life cycle: embryo, larva, pupa and adult (Peterson & Fox, 2021; Costa et al., 2022). The biological role of developmental polyploidy can be diverse, depending on tissue types and local conditions. This issue is currently being actively discussed in the literature (Fox & Duronio, 2013; Bandura & Zielke, 2017; Gandarillas et al., 2018; Øvrebø & Edgar, 2018; Peterson & Fox, 2021).

Variation in the level of endoreduplication in *Drosophila* includes a significant heritable component (Strashnyuk et al., 2023), and is also subject to substantial modifying influences (Dyka et al., 2016; Strashnyuk et al., 1997; Britton & Edgar, 1998; Nesterkina et al., 2020, 2023). It should be noted that the adaptive value of variations in the levels of polyteny has not been properly investigated. The study of somatic cell ploidy patterns in natural populations of organisms is extremely rare (Nozaki & Matsuura, 2019; Tavares et al., 2024). Such studies, furthermore, are completely absent with regard to *Drosophila*.

The purpose of this study was to investigate the seasonal dynamics of the levels of endoreduplication and polyteny in *Drosophila melanogaster* fruit flies obtained from a natural population. The aims were to examine seasonal variations in endoreduplication indices and ploidy patterns in the salivary glands of fruit fly larvae and to explore how these variations may contribute to seasonal adaptations of the flies.

MATERIAL AND METHODS

Flies

The study material consisted of *Drosophila melanogaster* lines, obtained from a natural population at the biological station of V.N. Karazin Kharkiv National University in the village of Haidary, Chuhuiv City Council, Kharkiv Region (49°38′13″N, 36°18′46″E), during different seasons:

– the Spring 2019 line, obtained from the Haidary population in the spring of 2019, 11 females and 10 males were caught;

– the Autumn 2019 line, obtained from the Haidary population in the autumn of 2019, 50 females and 50 males were taken;

– the Spring 2020 line, obtained from the Haidary population in the spring of 2020, 19 females and 11 males were caught;

– the Autumn 2020 line, obtained from the Haidary population in the autumn of 2020, 24 females and 7 males were caught.

Spring flies were caught at the end of May – beginning of June, and autumn flies – in the second half of September in 2019 and 2020.

To avoid the presence of the sibling species *Drosophila simulans* in the experiment, a genetic test was employed. Isofemale lines were obtained from the captured flies. In the next generation, males were selected and crossed with females of the *Oregon-R* laboratory strain of *D. melanogaster* (the *Oregon-R* strain was taken from the collection of the Department of Genetics and Cytology of the V.N. Karazin Kharkiv National University). It is known that interspecific crosses between *D. melanogaster* and *D. simulans* leads to unisexual inviability of hybrids (Yamamoto, 1993). The progeny of the isofemale *D. melanogaster* lines were then mixed, and the fly lines were subsequently maintained by mass crosses and outbreeding. Thus, the preparation of the material took three generations. In the two subsequent generations, ploidy patterns in the salivary glands of the larvae were examined.

Experimental conditions

The flies developed on a standard sugar-yeast nutrient medium at a temperature of 24–25°C. In the experiments, flies were placed in pairs in 60 ml glass vials containing 10 ml of the nutrient medium for mating. Larvae for the experiment were taken in the first 2 days after the start of emerging from the nutrient medium on the vial wall for pupation.

Determination of endoreduplication indices and polyteny levels of giant chromosomes

Polytene chromosomes were examined in the salivary glands of fruit fly larvae at the end of the 3rd instar, the so-called wandering larvae. According to Rodman (1967), the initiation of new endoreduplication cycles in the larval salivary glands stops at this stage, due to changes in the levels of juvenile hormone and ecdysterone in the hemolymph (Shakina & Strashnyuk, 2011; Ren et al., 2020). The pressed acetoorcein preparation method was used for chromosome analysis. For staining, 2% orcein (Merck KGaA, Darmstadt, Germany) in a 45% acetic acid solution (Reachimtrans, Kyiv, Ukraine) was used. Polytene chromosomes were studied using a Granum R 6003 light microscope (China).

Differences in the degree of polyteny were analysed using cytomorphometry (Strashnyuk et al., 1995). It is known that 7 to 10 rounds of endoreduplication (endocycles) take place in the salivary gland cells of *D. melanogaster* at the end of larval development. As a result, cells reach different levels of ploidy. The level of ploidy in polytene chromosomes is characterised by the C value, which indicates the amount of chromatin as multiples of the haploid genome (Øvrebø & Edgar, 2018). According to (Rodman, 1967), two to four classes of nuclei with C values of 256C, 512C, 1024C and 2048C are found in wandering larvae of *D. melanogaster*. On cytological preparations, chromosomes with different levels of polyteny differ in the thickness and intensity of acetoorcein staining. Control measurements of chromosome thickness were performed at locus 22A of chromosome 2L at a magnification of 640×. In this area, giant chromosomes with different ploidy levels have different widths: about 1.6, 2.3, 3.2, and 4.6 μm, respectively. The number of nuclei with different levels of ploidy was counted on salivary gland preparations at 160× magnification.

Polytene chromosomes were assessed by various indices. The number of nuclei with different levels of ploidy (256C, 512C, etc.) was counted and their percentage was determined.

Endoreduplication indices (EI) were calculated as the average number of endocycles in salivary gland cells, following the equation (Frakova et al., 2021):

$$\mathrm{EI} = \frac{\sum [(n_1 \times 7) + (n_2 \times 8) + (n_3 \times 9) + (n_4 \times 10)]}{N}$$

where n_1, n_2, n_3, n_4 are the number of nuclei in which 7, 8, 9, or 10 endocycles occurred, respectively, and N is the total number of nuclei in the sample: $N = n_1 + n_2 + n_3 + n_4$.

Mean C value (MCV) was calculated based on the data on the distribution of nuclei with different C values, according to the formula (Frakova et al., 2021):

$$\mathrm{MCV} = \frac{\sum [(n_1 \times 256\mathrm{C}) + (n_2 \times 512\mathrm{C}) + (n_3 \times 1024\mathrm{C}) + (n_4 \times 2048\mathrm{C})]}{N}$$

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

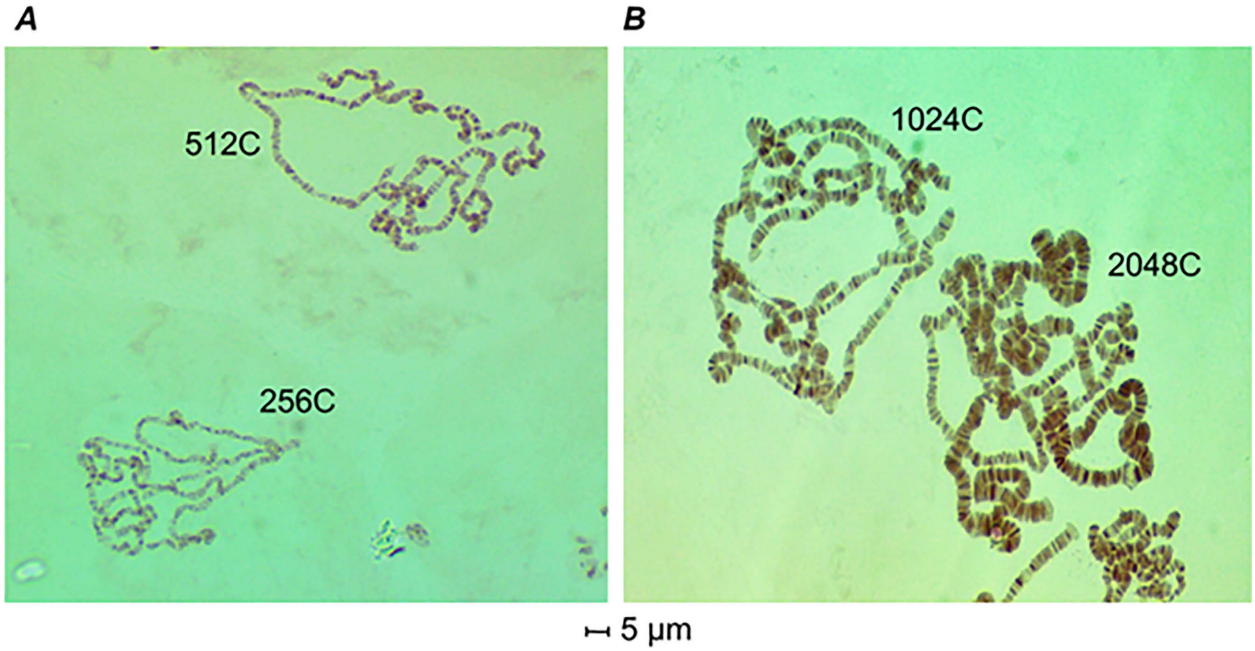

Fig. 1. Giant chromosomes in the salivary glands of *Drosophila melanogaster* larvae with different levels of polyteny in proximal (A) and distal (B) parts of the gland (staining with acetoorcein).

Finally, the degrees of variation of EI and MCV in each variant of the experiment were evaluated.

In each experimental variant, 9–13 samples were studied. At least 75 nuclei were examined on each sample. A total of 10557 nuclei in 82 samples were studied.

Statistical analysis

The ratio of nuclei with different levels of polyteny is given as a percentage. The significance of seasonal differences in this indicator was assessed using Pearson's χ^2 test. Distributions of endoreduplication indices (EI) and polyteny levels (MCV) were checked for normality using the Shapiro-Wilk test. EI and MCV values are presented as mean ± confidence interval. We also used BoxPlotR: a web tool for box plot statistics and generation of box plots (Spitzer et al., 2014). To assess the influence of the season on EI, the non-parametric Kruskal-Wallis test was used. The effect of seasons and sex on the degree of chromosome polyteny was determined using Fisher's analysis of variance. Two-way ANOVA was used. We calculated the effect size (η^2), which characterises the proportion of factorial variation in the total variation of the trait. For this, Plokhinsky's method was used. The degree of variation of EI and MCV was estimated by the coefficient of variation (C_V). Student's *t*-test was used to compare individual groups by EI, MCV, and C_V. Bonferroni correction was used for multiple comparisons. In some cases, when the data distribution did not correspond to the normal law, we used median values for comparison. Effects were considered significant at $p \leq 0.05$. All data are available in the supplementary material (Table S1).

ABBREVIATIONS. C_V – coefficient of variation; EI – endoreduplication index; HSPs – heat shock proteins; MCV – mean C value; SNP – single nucleotide polymorphism.

RESULTS

Seasonal variations of ploidy patterns in *Drosophila melanogaster* salivary glands

The ploidy level in salivary gland cells doubles with each round of endoreduplication. This makes it easy to visually distinguish chromosomes with different levels of polyteny by their thickness. In addition, chromosomes with greater polyteny exhibit more intense staining with acetoorcein. Previously, we demonstrated the correlation between the cytomorphometric parameters of chromosomes and their degree of polyteny (Strashnyuk et al., 1995). Nuclei with lower ploidy are located in the proximal part of the gland, while those with a higher C value are in its distal part (Fig. 1).

In *Drosophila* lines obtained from the natural population of Haidary in different seasons, the percentage distribution of nuclei of different ploidy was studied. The results are presented in Fig. 2. As is usually the case, 1024C nuclei were most abundant in the salivary glands of larvae, with their proportion varying depending on sex and season within the range of 66.4–82.6%. Nuclei 512C were found with a frequency of 9.1–28.0%. 256C nuclei were less represented, their numbers between 3.8–7.7%. Nuclei with a ploidy level of 2048C were a minor fraction with a frequency of 0–3.8%.

We found no significant changes in ploidy patterns between fly larvae of the spring and autumn generations of 2019. This applies to both females and males. It is possible to note the absence of the fraction of nuclei 2048C in the autumn males, but even in spring their proportion did not exceed 1%. Instead, substantial changes in the ratio of nuclei of different ploidy occurred after overwintering. In the spring of 2020, the number of nuclei of higher ploidy – 1024C and 2048C – increased in both females and males, while the proportion of nuclei 256C and 512C decreased compared to autumn 2019. According to Pearson's χ^2 test, the distributions of nuclei of different ploidy in autumn 2019 and spring 2020 differ significantly in individuals of both sexes ($p < 0.001$). Later, in the autumn of 2020, the ratio of nuclei of different ploidy returned to last year's

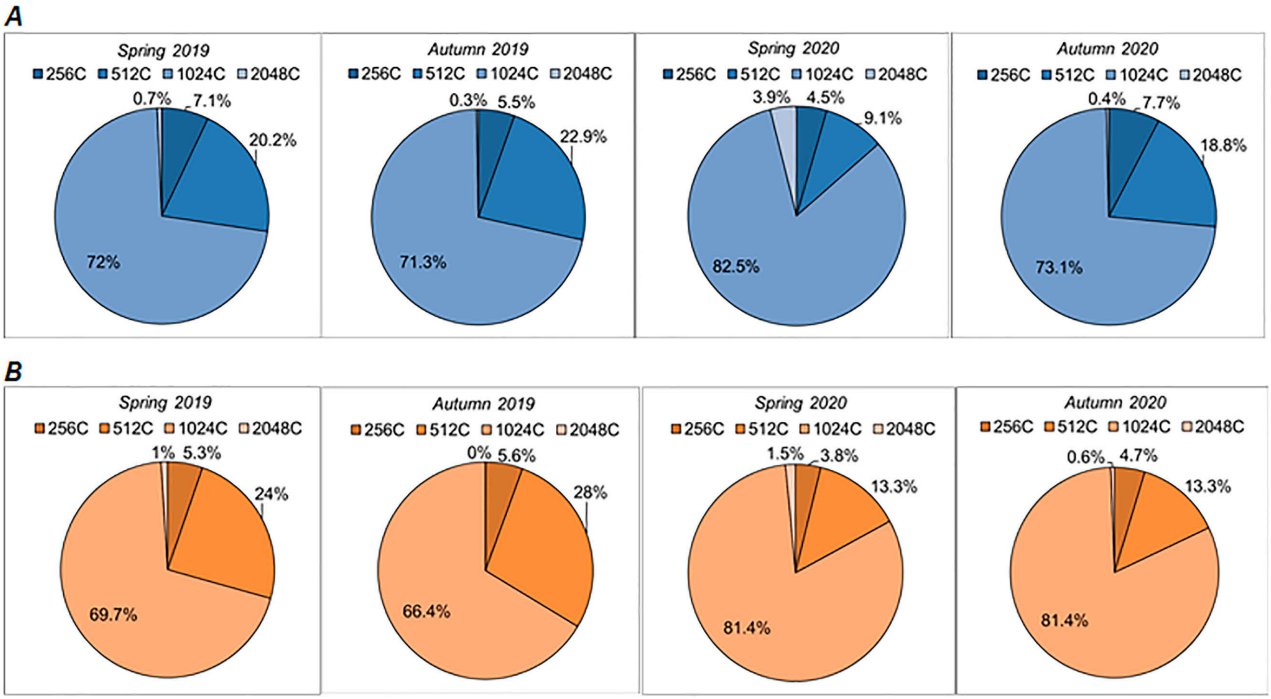

Fig. 2. Seasonal variation in polyteny in *Drosophila melanogaster* salivary glands: the distribution of cell nuclei with different C values in females (A) and males (B).

level in females. In males, this ratio practically did not differ in the spring and autumn generations of 2020, i.e., having changed after overwintering, it remained the same in autumn.

Thus, we observed seasonal changes in the distribution of nuclei of different ploidy in the salivary glands of fruit fly larvae. After overwintering, the effect was observed in both sexes, and in females also after the subsequent growing season.

Seasonal variability of endoreduplication levels in *Drosophila melanogaster* salivary glands

Based on the data on the distribution of nuclei with different ploidy, we calculated endoreduplication indices (EI), that is, the average number of endocycles for larvae salivary glands in different seasons. The data are presented in Fig. 3.

No difference in EI was found between spring and autumn 2019 flies. We also did not observe sex differences in this indicator. Instead, we found an increase in endoreduplication indices after overwintering. In both females and males, the EI index increased in the spring of 2020 compared to the autumn of 2019 by 2.3% ($p < 0.03$). That doesn't seem like much of an increase. But it is worth considering that the ratio between the number of endocycles and ploidy level is defined as C value = 2^{m+1}, where m is the number of endocycles. Therefore, even small changes in EI can significantly affect the ploidy value. Later, during 2020, the EI indicator in females decreased in autumn compared to spring by the same 2.3%, i.e. it returned to the level of 2019. It remained unchanged in males.

In one of the variants of the experiment, namely in the Spring 2020 females, the Shapiro-Wilk test showed that the data were not normally distributed ($W = 0.717$). Therefore, for the statistical analysis of the data in this case, we used the Kruskal-Wallis test, which is a non-parametric analogue of variance analysis. The results are shown in Table 1. The test showed the significance of seasonal changes in the level of endoreduplication in the salivary glands of *Drosophila* larvae. The proportion of seasonal variability in the total variability of EI was 25.6% in females and 23.8% in males.

The coefficients of variation (C_V) of the average values of EI were not significantly different across the seasons of 2019–2020 (Table 2). Females and males also did not differ in this indicator.

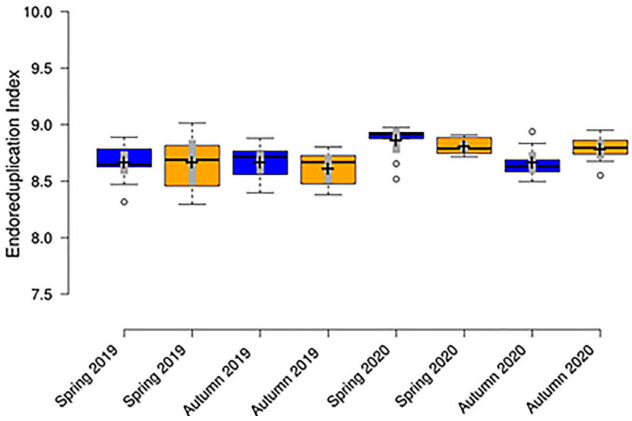

Fig. 3. Seasonal variation in endoreduplication levels in *Drosophila melanogaster* salivary glands: endoreduplication indices in females (blue boxes) and males (orange boxes). Box plot description for figure legend: centre lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, outliers are represented by dots; crosses represent sample means; bars indicate 95% confidence intervals of the means.

Table 1. Effect size of seasons on endoreduplication indices in *Drosophila melanogaster* salivary glands.

Sex	Indicators of statistical analysis		
	η^2 (%)	F_φ	p
Females	25.6	4.6	< 0.01
Males	23.8	3.6	< 0.05

Thus, we found an increase in the level of endoreduplication in *Drosophila* larvae of both sexes after overwintering and a further decrease in females during the growing season. The degree of variation of the trait did not change.

Seasonal variability of chromosome polyteny in *Drosophila melanogaster* salivary glands

The seasonal dynamics of polyteny levels in the giant chromosomes of *Drosophila* salivary glands (Fig. 4) correlate with variations in EI. There were no significant changes in the mean C values (MCV) during 2019. However, after overwintering, this indicator increased by 13.3% in females and by 12.4% in males ($p < 0.03$). Subsequently, during 2020, the MCV indicator in females decreased in autumn, compared to spring, by 9.2% ($p < 0.03$) and did not differ from 2019 levels. For males, this indicator remained unchanged and was at the same level as in the spring of 2020.

The coefficients of variation of polyteny levels (C_V, Table 3) in females did not differ throughout the seasons of 2019–2020. At the same time, in males, the MCV coefficients of variation in spring and autumn 2020 were significantly lower than in spring 2019. This may have been caused by a decrease in genetic variability after overwintering due to population decline, genetic drift and selection. No significant sex differences in the degree of variation of the MCV indicator were found.

The use of variation coefficients allows us to compare the degree of variability between quantitative traits of different type, since C_V is defined as the ratio of the standard deviation to the mean value of the trait ($C_V = s/X_m \times 100\%$). It is worth noting that the variation of MCV significantly exceeds the variation of EI with a difference of 4.2–5.4 times ($p < 0.05$). In light of this, the MCV indicator is more sensitive to the action of certain factors, in particular, seasonal influences, compared to EI.

The Shapiro-Wilk test showed that the distribution of the MCV indicator conformed to the normal law (in different variants of the experiment, the W value varied in the range of 0.821–0.963, $p < 0.05$–0.01). Analysis of variance confirmed a seasonal effect on the degree of chromosome polyteny in the salivary glands of fruit fly larvae (Table 4). The proportion of seasonal variations in the total MCV variation was 21.9% ($p < 0.05$). Sex did not have a significant effect on the ploidy level, as well as the joint effect of sex and seasons.

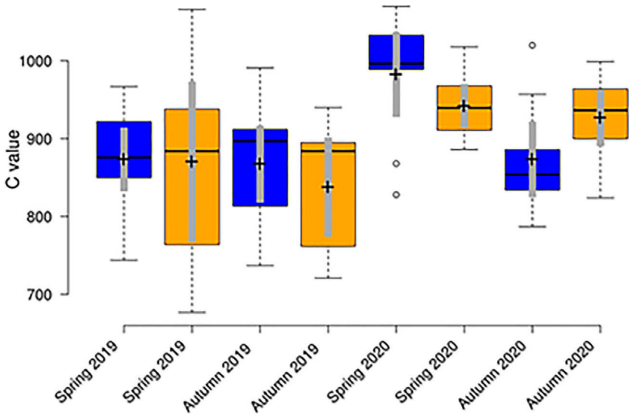

Fig. 4. Seasonal variation in polyteny in *Drosophila melanogaster* salivary glands: mean C values in females (blue boxes) and males (orange boxes). Box plot description for figure legend: centre lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, outliers are represented by dots; crosses represent sample means; bars indicate 95% confidence intervals of the means.

Thus, the average level of polyteny in the salivary glands of *Drosophila* larvae increased after overwintering in both females and males. It subsequently decreased in females during the growing season. The degree of trait variability in females did not change, while in males of 2020 it was lower compared to the spring of 2019. Similarly to the endoreduplication index, MCV exhibits significant seasonal variability.

DISCUSSION

Insects living in seasonally variable environments demonstrate a wide range of adaptive responses to those seasonal changes, relating to morphology and physiology (Nijhout, 2003; Danks, 2006; Denlinger, 2022), development (Koštál, 2006; Erickson et al., 2020), and behaviour (Danks, 2007; Takata et al., 2023). This allows them to use favourable seasons for reproduction and survive unfavourable ones. *Drosophila* has a short generation period (~10–15 days, depending on temperature), and produces about 15 generations per year (Pool, 2015). Due to changing conditions in different seasons, fruit flies experience fluctuations in selection: variations in stress tolerance and life history are the objects of selection in the winter months, while increased reproductive potential and more

Table 2. Coefficients of variation (C_V) of endoreduplication indices in *Drosophila melanogaster* salivary glands.

Seasons	Coefficients of variation, C_V(%)	
	Females	Males
Spring 2019	1.9 ± 0.5	3.0 ± 1.2
Autumn 2019	1.7 ± 0.6	1.9 ± 0.8
Spring 2020	1.7 ± 0.5	0.8 ± 0.2
Autumn 2020	1.5 ± 0.5	1.3 ± 0.4

Table 3. Coefficients of variation (C_V) of mean C values in *Drosophila melanogaster* salivary glands.

Seasons	Coefficients of variation, C_V(%)	
	Females	Males
Spring 2019	7.7 ± 1.5	15.3 ± 3.6
Autumn 2019	8.4 ± 1.8	9.8 ± 2.3
Spring 2020	7.7 ± 1.7	4.2 ± 0.9
Autumn 2020	7.7 ± 1.7	5.5 ± 1.2

Table 4. Effect sizes of seasons and sex on the degree of chromosome polyteny in *Drosophila melanogaster* salivary glands.

Acting factors	Indicators of variance analysis		
	η^2 (%)	F_φ	p
Seasons	21.9	7.44	< 0.01
Sex	0.1	0.09	> 0.05
Joint effect of seasons and sex	5.3	1.78	> 0.05

efficient use of resources are more favourable in the growing season (Behrman et al., 2015; Erickson et al., 2020).

According to (Nunez et al., 2024), the temporal structure of the population is determined by seasonal changes in population booms and busts, in which overwintering is the main determinant. For example, in the Charlottesville population studied by the authors, the size of the winter collapse was about 98% of the maximum summer size.

Seasonal fluctuations in population size lead to changes in the genetic structure of fruit fly populations, for which there is a lot of evidence. It has been shown that the amount of genetic differentiation accumulated during the growing season is less than that accumulated in winter (Nunez et al., 2024). Changes in the frequencies of genotypes from one year to the next indicate that genetic drift occurs in fly populations and can be particularly affected by seasonal fluctuations in population size and selection (Bell, 2010). One of the first examples of adaptive tracking was chromosomal inversions (Behrman et al., 2015), which are usually seen as facilitators of adaptation to fluctuating ecosystems (Garcia & Valente, 2018; Nunez et al., 2024). Convincing evidence of multilocus fluctuating selection was obtained in a study of fruit fly populations from the temperate latitudes of North America, by employing SNP frequency analysis (Bergland et al., 2014).

In this study, we investigated the variation in endoreduplication as one of the possible mechanisms by which flies adapt to changing seasonal conditions. The adaptive role of endopolyploidy is currently being actively debated. In particular, this refers to an increase in cell size, which affects cell metabolism (Edgar & Orr-Weaver, 2001; Leitch & Leitch, 2022), participation in the mechanisms of cell differentiation, growth, and morphogenesis processes (Lee et al., 2009; Edgar et al., 2014). Due to the increased content of genomic DNA, endocycling cells can achieve massive output of secreted protein products (Edgar et al., 2014). It is assumed that endoreduplication protects DNA from damage and mutations (Edgar & Orr-Weaver, 2001) and provides cell tolerance to genotoxic stress (Gandarillas et al., 2018). Polyploid cells are resistant to apoptosis upon DNA damage (Mehrotra et al., 2008). Somatic polyploidy underlies compensatory cell growth during tissue regeneration (Øvrebø & Edgar, 2018; Grendler et al., 2019). Endoreduplication modulates the stress response and its mechanism is important for the control of homeostasis (Gandarillas et al., 2018; Øvrebø & Edgar, 2018) and tissue plasticity (Nagai et al., 2022). Wos et al. (2022) suggest that endocycles are important for fine-tuning the endoreduplication process to the local environment. Many authors note the important evolutionary significance of endopolyploidy (Nagl, 1976; Edgar et al., 2014; Nozaki & Matsuura, 2019).

With regard to research on *Drosophila*, a significant effect of genotype on polyteny level in the salivary gland cells of fruit fly larvae has previously been shown (Strashnyuk et al., 2023). In particular, the hereditary component in the total variability of the trait was 45.3%. At the same time, the level of endoreduplication can vary significantly depending on the conditions of fruit fly development. The modification variability of this indicator was investigated under the influence of a wide range of environmental factors. In particular, the influence of temperature (Strashnyuk et al., 1997), culture density (Rarog et al., 1999; Zhuravleva et al., 2004), nutrient composition (Britton & Edgar 1998; Nagai et al., 2022), exposure to toxic substances (Nesterkina et al., 2020, 2023), were considered.

For insects, the temperature of the environment is extremely important. Evidently, this factor is one of the determinants in seasonal fluctuations of fitness and selection in natural populations of fruit flies. The effect size of temperature on the degree of chromosome polyteny in *Drosophila*, estimated in controlled laboratory conditions, was close to the genetic component and amounted to 40.6% (Strashnyuk, 2012).

An important piece of evidence on the adaptive role of polyteny variations is the correlation of this indicator with a number of adaptively important traits. Under different temperature conditions and depending on the genotype, the degree of polyteny had a close positive correlation with adult body weight, stress resistance, and the duration of development in flies (Strashnyuk et al., 1997).

Thus, variations in the level of chromosome polyteny in *Drosophila* have both genetic and modification components. This gives reason to consider them as an adaptive response to the conditions in which fruit flies develop. On the other hand, a significant hereditary component and correlations of polyteny with a complex of adaptively important traits make it a potential target for natural selection.

Evidently, the selection on the level of ploidy in natural populations should coincide with adaptive modifications, in terms of direction. If temperature is taken into account as the main factor accompanying seasonal changes, then low temperatures lead to an increase in the level of polyteny, which correlates with an increase in the body size of adult flies (Strashnyuk et al., 1997). This is achieved due to a significant increase in the duration of the development of flies as a result of slowed metabolism. In this case, gene expression occurs at a lower level compared to optimal temperatures, as evidenced, in particular, by changes in the sizes of polytene chromosome puffs (Strashnyuk, 2012). It is likely that the increase in ploidy under these conditions compensates to a certain extent for the decrease in gene expression. An increase in temperature has the opposite effect. It is obvious that through this gene dosage/gene expression balance the size of flies is achieved that is most suitable (adaptive) for certain temperature conditions.

The correspondence between the direction of selection and adaptive modifications is confirmed by the data of the

present study, which showed changes in the endoreduplication index and ploidy level in the fruit fly larvae of spring 2020, i.e., those that were selected for survival under unfavourable low temperatures during overwintering. As a result, we observed an increase in EI and MCV indicators compared to samples obtained from the larvae of the previous year's autumn generation.

As already noted, polyploidisation allows cells to regulate their size. This in turn affects fly body size. Metamorphosis in flies occurs due to resources released from the lysis of larval tissues. Larval tissues are characterised by postmitotic growth caused by cell expansion. Their cells do not proliferate. Only cells of the imaginal discs and small collections of diploid epidermal cells retain the ability to proliferate (Truman, 2019). Thus, larval growth is based on endoreduplication. The higher the degree of polyteny, the larger the larvae. The larger the larvae, the larger the adult flies. This has physiological consequences, as surface area grows more slowly than volume. This surface-to-volume ratio affects interactions with the environment at both the cellular and organismal levels. In particular, this occurs when flies develop under different temperature conditions. According to (Szabla et al., 2024), flies that evolved and developed under low temperature conditions (16°C) had larger cell sizes in the Malpighian tubules and wing epithelium. At higher temperatures (25°C), smaller flies with smaller cells developed. Genotypes from the cold selective regime were less sensitive to hypoxia. The authors conclude that there is a relationship between cell size, tissue oxygenation, and the energy cost for tissue maintenance. According to (Roberts et al., 2023), seasonal energetics optimisation is an important trait associated with insect fitness.

Another aspect of the influence of cell ploidy level on physiological processes concerns gene expression, which is enhanced by an increase in the number of genomic DNA copies. This increases the yield of protein products, in particular heat shock proteins (HSPs), which are upregulated during overwintering conditions (Denlinger, 2022). This enhances defence responses in general, since HSPs protect cells from a wide range of stressors, including cold stress and oxygen limitations that accompany overwintering. Thus, an increase in cell ploidy can contribute to winter survival in various ways.

Comparing the 2019 and 2020 data, there is some stability in endoreduplication and polyteny levels throughout 2019, while the 2020 spring flies outperform the 2019 and 2020 autumn flies on these measures. In this regard, it is worth noting that the winter of 2019/2020 in the studied area turned out to be abnormally warm. According to meteorological observations (Meteopost archive, 2024), the average winter temperature was 2–3°C, and in general the winter temperature did not fall below –10°C. Such a "warm" winter had not been observed in the previous 2018/2019 season. That is, the overwintering conditions differed, which could affect the selection parameters.

It is known that ongoing climate change has a multifaceted effect on natural groups of organisms. A "warm" winter does not mean that it is more favourable for the survival of insects, whose physiology depends on the temperature of the environment. Warming in the middle of winter can simulate the onset of spring and provoke an untimely exit from diapause. This can have fatal consequences for insects. In other words, global warming creates new challenges for natural populations (Denlinger et al., 2017; Roberts et al., 2023; Zivanovic et al., 2023) and can create additional selection pressure, in particular for overwintering insects (Rozsypal, 2024). The increase in EI and MCV we found in spring 2020 may reflect this increased selection pressure.

As is known, various species of insects survive adverse conditions by stopping development and entering diapause (Denlinger, 2022). In *Drosophila melanogaster*, diapause occurs at the adult stage. The ability to diapause in *D. melanogaster* has moderate heritability and is more common in populations at high latitudes where winters are more severe (Erickson et al., 2020). Diapause in *D. melanogaster* is shallow compared to that of other insects, including other drosophilids, and is easily disrupted if the temperature or day length increases. It has been suggested that seasonal diapause in *D. melanogaster* may be a recently evolved trait, and instability of fruit fly diapause may reflect its initial evolution (Saunders et al., 1989; Tauber et al., 2007). Therefore, *D. melanogaster* may be quite sensitive to climate change.

It should also be understood that the entry of flies into the winter season is in any case accompanied by an increase in their size and cell ploidy due to development at lower temperatures. The modification component is present in any case. This study demonstrates the presence of a genetic component in seasonal variability due to selection. However, this variability may be irregular and depends on the level of selective pressure.

The return of EI and MCV parameters to the previous year's level in female fruit flies in the fall of 2020 may indicate that selection during the population growth period does not favour an increase in cell ploidy. This does not necessarily mean that cell ploidy is negatively correlated with fly fecundity. We have not previously found such a correlation in laboratory studies (Strashnyuk et al., 1997). There may be another kind of dependence here. The rate of fly development under different temperature conditions is negatively correlated with the level of endoreduplication (Strashnyuk et al., 1997). Endocycles take time. At higher temperatures, smaller flies develop, and their development takes less time (Strashnyuk et al., 1997; Szabla et al., 2024). As a result, generations replace each other faster. And in this way, it should contribute to population growth.

In this study, we did not find significant sex differences in the level of endoreduplication and polyteny. Overall, as we found earlier, females have slightly higher MCV values compared to males (Strashnyuk et al., 2023). However, this difference is not evident in every genetic strain or line. Obviously, this requires additional research. This question concerns the mechanisms underlying sexual dimorphism, and in particular, sexual size dimorphism. But it can also

relate to sex differences in fitness and survival, and therefore directly affect seasonal fluctuations of selection in natural populations.

In conclusion, we found that polyteny patterns in *Drosophila* salivary glands undergo seasonal changes. To a certain extent, these variations correlate with changes in the direction of natural selection in natural populations, which undergoes seasonal fluctuations. Indices of endoreduplication and cell ploidy acquire the greatest values after overwintering, during which there is an extreme decline in population size and flies undergo selection for viability and stress resistance. This is observed in both female and male fruit flies. During the growing season, indicators of endoreduplication and ploidy in females decrease. We believe that this facilitates population growth by resulting in smaller flies with accelerated development and faster change of generations. Sex differences in the level of polyteny in salivary gland cells of *Drosophila* larvae were not detected. The combined effect of seasons and sex was also not significant. Thus, fruit flies have seasonal variations in the level of endoreduplication and cell ploidy, which we believe may facilitate adaptation to changing seasons.

Regarding research perspectives, as already noted, data on cell ploidy patterns for the phenomena of endopolyploidy in natural populations are extremely limited. *Drosophila*, as a model species, provides great opportunities for such research. If we talk about seasonal variations, then such studies also involve the use of material from different places, obtained from different latitudes or altitudes, which differ in environmental conditions. In general, it would be promising to study the variation in somatic polyploidy in natural populations and clarify the role of such variation in terms of fitness, reproduction, survival and adaptation, i.e. in connection with various forms and directions of natural selection.

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CONFLICTS OF INTEREST. The authors declare that they have no conflict of interest.

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Table S1. Seasonal variations in endoreduplication level and polyteny in *Drosophila melanogaster* salivary glands.

Seasons	Sex	*n*	Number of nuclei with different C values (%)				Endoreduplication Index (Median)	Endoreduplication Index (Mean)	Mean C value
			256C	512C	1024C	2048C			
Spring 2019	♀	13	7.1 ± 2.0	20.2 ± 2.2	72.0 ± 3.0	0.7 ± 0.4	8.65	8.66 ± 0.04	873.4 ± 18.6
	♂	9	5.3 ± 1.2	24.0 ± 6.8	69.7 ± 7.0	1.0 ± 0.8	8.69	8.66 ± 0.09	870.3 ± 44.5
Autumn 2019	♀	11	5.5 ± 1.3	22.9 ± 4.2	71.3 ± 3.9	0.3 ± 0.2	8.72	8.66 ± 0.04	867.5 ± 22.0
	♂	9	5.6 ± 1.2	28.0 ± 5.8	66.4 ± 5.4	0 ± 0	8.67	8.61 ± 0.05	837.7 ± 27.5
Spring 2020	♀	10	4.5 ± 2.1	9.1 ± 1.6	82.6 ± 2.9	3.8 ± 1.5	8.92	8.86 ± 0.05	982.4 ± 23.9
	♂	10	3.8 ± 1.0	13.3 ± 2.5	81.4 ± 2.0	1.5 ± 0.6	8.79	8.81 ± 0.02	941.8 ± 12.4
Autumn 2020	♀	10	7.7 ± 0.7	18.8 ± 2.9	73.1 ± 3.1	0.4 ± 0.4	8.63	8.66 ± 0.04	873.3 ± 21.3
	♂	10	4.7 ± 1.2	13.3 ± 2.4	81.4 ± 3.0	0.6 ± 0.6	8.80	8.78 ± 0.04	926.8 ± 16.0

Note: Data are presented as mean ± standard error; *n* – number of specimens examined.