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Temperature and developmental responses of body and cell size in *Drosophila*; effects of polyploidy and genome configuration

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ABSTRACT

Increased adult body size in *Drosophila* raised at lower temperatures could be attributed both to an increase in the cell volume and cell number. It is not clear, however, whether increased cell size is related to (or even caused by) increased nuclear volume and genome size (or configuration). Experiments with *Drosophila melanogaster* stocks (Oregon-R and w1118) raised at 16, 22, 24, and 28 °C resulted in larger adult body and wing size with lower temperature, while eye size was less affected. The increase in wing size reflected an increase in cell size in both males and females of both stocks. The nucleus size, genome size, and DNA condensation of adult flies, embryos, and Schneider 2 cells (S2 cells, of larval origin) were estimated by flow cytometry. In both adult flies and S2 cells, both nucleus size and DNA condensation varied with temperature, while DNA content appears to be constant. From 12% to 18% of the somatic cells were tetraploid (4C) and 2–5% were octoploid (8C), and for the Oregon strain we observed an increase in the fraction of polyploid cells with decreasing temperature. The observed increase in body size (and wing size) at low temperatures could partly be linked with the cell size and DNA condensation, while corresponding changes in the haploid genome size were not observed.

1. Introduction

A negative correlation between developmental temperature and adult body size has been observed in a large number of ectotherms (Angilletta and Dunham, 2003; Angilletta et al., 2004; Partridge and French, 1996). Similarly, a corresponding relationship is commonly found between temperature and cell size (Arendt, 2007), and this could suggest that the body size responses simply reflect underlying responses in cell size rather than cell numbers, although there are cases where increased cell size is not accompanied with increased body size (Mousseau, 1997, Day and Lawrence, 2000). In general increased genome size also correlates positively with cell size (Cavalier-Smith, 1978, Gregory et al., 2000, Gregory, 2005), meaning that increased genome size could promote increased cell size resulting in increased body size. While an explicit coupling between low temperature and large genome- and body size is reported for many marine invertebrates (Atkinson, 1994; Timoteev, 2001; Rees et al., 2008), the temperature response at the genome level has not been thoroughly addressed, and especially not as a developmental response (Hessen et al., 2013). A recent study on *Daphnia* did however suggest increased genome size in response to developmental temperature, and well as increased incidence of endoreduplication (Jalal et al., 2013; 2014), analogous with response found for plants (Lee et al., 2009).

Thermal plasticity in body size and allometry has been reported in *Drosophila*, although with contrasting cellular responses. While increased adult body size at lower developmental temperatures has been explicitly linked to increased cell size, the increased body size often found in cooler environments along climatic gradients has been attributed to increased cell numbers (French et al., 1998; Partridge et al., 1994). The plastic wing area found in *Drosophila* has also been attributed either to cell size, cell number, or both (Cavicchi et al., 1985; Partridge et al., 1994; Robertson, 1959). In *Drosophila*, changes in the genome size, including increases in somatic ploidy levels (endopolyploidy), may result in corresponding changes in cell size (Dobzhansky, 1929; Held, 1979).

Thus there exist some intriguing relations between genome, cell, and body size that remain unsettled (Blanckenhorn and Llaurens, 2005; Hessen et al., 2013). In principle, the causal relationship could work in both directions (from genome size to cell size and body size, or vice versa). Long-term evolutionary adaptations over ranges in climate could also differ from phenotypic responses over shorter time spans (single generation) (Vieira et al., 2002). Both plants and invertebrates typically have higher prevalence of polyploidy at high latitudes (and low temperatures)

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(Brochmann et al., 2004) and endroreduplication or polyteny that are quite widespread in invertebrates (Lee et al., 2009; Parfrey et al., 2008; Jalal et al., 2014), including *Drosophila* (Smith and Orr-Weaver, 1991). Structural changes like DNA condensation in response to temperature could also affect nuclear volumes or binding properties of fluorochromes used by flow cytometry, as found for *Daphnia* (Jalal et al., 2013). The aim of this study was to explore thermal, developmental responses at the genomic level in *Drosophila*, both in terms of haploid genome size and endoreduplication or endopolyploidy, and further link this to responses in adult body, eye and wing size.

2. Materials and methods

In this study we assessed temperature responses in body size, eye size and wing size of two stocks of *D. melanogaster*. Flow cytometry (FCM) together with confocal laser scanning microscopy was applied to reveal genome- and cellular responses of adult flies as well as the responses of Schneider 2 (S2) cells to temperature could be linked with effects at the genomic level. The S2 cells offered an opportunity to study responses in cell cultures.

2.1. S 2 cell cultures

The S2 cells were grown in Schneider's *Drosophila* medium, which contained 10% heat-inactivated fetal bovine serum and penicillin-streptomycin at a final concentration of 50 units penicillin G and 50 μg streptomycin sulphate per mL of medium (all from Invitrogen). The S2 cell line was derived from a primary culture of late stage (20–24 h old) *Drosophila melanogaster* embryos (Invitrogen). Cells were initially stock cultured in 25 cm^2 flasks until the density reached 10.00×10^6 cells/mL in a 28 °C incubator (without CO_2 supply) two weeks before the experiment. The cell viability was confirmed to be 95–99% by direct cell counting in a hemocytometer after staining with trypan blue (all from Sigma-Aldrich). For the experiment, 3.00×10^6 cells/mL in 75 cm^2 flask were incubated at either 16 °C, 22 °C, or 28 °C for 24 h. S2 cells double in number after 24 h (Kao and Megraw, 2004). Cells were detached from the surface of the culturing flask by using a cell scraper (Sarstedt), stained with trypan blue, and were counted using a hemocytometer. The cell size in micrometers (μm) was measured by CASY (Roche Innovatis AG). The S2 cells were treated with either ethanol or grinding buffer (GB) (Korpelainen et al., 1997) before the FCM analysis. The treatment of S2 cells with GB dissolved both the cytoplasm and the cell membrane, leaving intact nuclei for DNA measurements. However, treatment of S2 cells with ethanol preserved both the cytoplasm and the cell membrane for the cellular DNA measurement.

2.2. S2 cell permeabilization and DNA staining

Next, 5×10^5 cells/mL of each 16 °C, 22 °C, and 28 °C culture were added to a 15 mL tube (Sarstedt), and the same amount of *Gallus gallus domesticus* blood cells (CRBC) in phosphate buffer saline (PBS, Sigma-Aldrich) was added to the sample as an internal standard. Cold PBS was added to the sample to a final volume of 5 mL. The sample was centrifuged for 10 min at 300*g* and 4 °C. The media was taken off, another 5 ml PBS was added, and the sample was again centrifuged for 10 min at 300 g and 4 °C. After the PBS was removed, 1 mL of 70% ethanol in dH_2O was added in droplets while gently vortexing the sample. After 24 h of incubation at 4 °C, the sample was centrifuged for 10 min at 300*g* and 4 °C; then, the ethanol was removed, and the cells were washed with 5 mL cold PBS. After centrifugation, the PBS was removed from the sample, and 0.5 mL cold PBS with 1 mg RNase A (Invitrogen) was added, and the sample was stained with 25 μg propidium iodide (PI, Invitrogen). The sample was further incubated in the dark at 4 °C overnight prior to FCM analysis.

2.3. S2 cell nuclei extraction and DNA staining

An S2 cell sample containing 5×10^5 cells/mL of each 16 °C, 22 °C, and 28 °C culture was added to a 15 mL tube, and cold PBS was added to each sample to a final volume of 5 mL. The sample was centrifuged for 10 min at 300*g* and 4 °C. The medium was removed, another 5 mL PBS was added, and the sample was again centrifuged for 10 min at 300*g* and 4 °C. After the PBS was removed, 0.5 ml cold GB was added in droplets while gently vortexing the sample. Then, 1 mg of RNase A was added to all of the samples, and they were further incubated on ice for 5 min. Next, 5×10^5 cells/mL of CRBC diluted in GB and 25 μg PI were added, and the sample was further incubated in the dark overnight at 4 °C prior to FCM analysis.

2.4. Fly stocks and culturing

Two *Drosophila melanogaster* stocks, Oregon-R (#172100, wild – type, red eye) and w1118, were used. The Oregon-R stock was obtained from Carolina's Easy Fly™ stock center (Carolina Biological Supply Company), and the w1118 strain was obtained from Dr. Rusten stock collection (The Norwegian Radium Hospital, Norway). Between 20 and 40 adult flies (∼ equal numbers of males and females) were cultured at 24 °C in single vials (173120, Carolina Biological Supply Company) and fed on formula 4–24® instant medium, following the Carolina™ *Drosophila* manual (45-2620, Carolina Biological Supply Company). These flies were maintained for 3 (Oregon-R) and 4 (w1118) generations before the experiment. According to c.f. Siddiqui and Barlow (1972) 24 °C is optimal growth temperature for *Drosophila*. To start the experiment, newly emerged flies (12 h after pupal eclosion) of both sexes (∼40 individuals in total) were randomly collected and incubated in new vials with fresh media at 24 °C. Adult flies were removed after ∼24 h and the vials, which now contain first-instar larvae, were incubated at either 16 °C, 22 °C, 24 °C or 28 °C. Temperature shifts affect the final body size when applied before the middle of the third larval instar (Shingleton, 2010). A 12 °C treatment was also performed but was not included in the final analysis because the larval death rates were high and none of the pupae hatched at this temperature. Flies grew and developed faster at high temperature (Table 1; development time in days). Eight flies of each sex were randomly collected under FlyNap® anesthesia and was distributed as following; 4 individuals sex^{-1} to further whole body size measurements (and finally FCM analysis), and 4 individuals sex^{-1} to further wing- and eye-size estimations.

2.5. Fly embryo collection

The fly embryos were collected for DNA analysis comparison with S2 cells which are isolated from embryos. For embryo collection, newly emerged Oregon-R flies of both sexes (∼40 individuals in total) were randomly collected and incubated in new vials with fresh media at 22 °C. Adult flies were removed after 20 h, and 10 embryos were randomly collected under a magnifying lamp by paint brush and washed twice in BD FACSFlow™ solution (BD Biosciences). The nuclei of the embryos were extracted (see Section 2.7) before the FCM analysis.

2.6. Body size measurements

Adult flies collected for FCM were photographed (Veho Discovery Camera VMS-001) before the extraction of nuclei (whole-fly DNA extraction), and the total body size measurements, top