Genome and cell size responses to temperature in ectotherms

Marwa Jalal

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Centre for Ecological and Evolutionary Synthesis (CEES)
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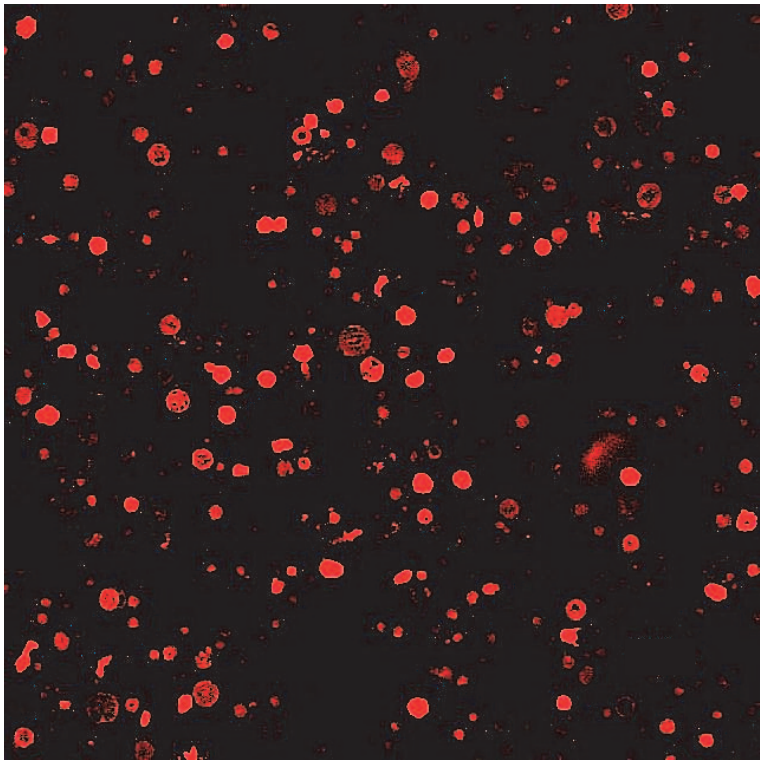

"*Daphnia's tiny somatic nucleus with its genome wrapped sometimes very tightly inside*"

by Marwa Jalal

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Marwa Jalal

Oslo, 12. September, 2013

TABLE OF CONTENTS

LIST OF PAPERS

This thesis is based on the following list of papers. They will be referred to in the remainder of the introduction by using the roman numerals assigned to them:

I. Jalal, M., Wojewodzic, M.W., Laane, C.M.M., and Hessen, D.O. Larger *Daphnia* at lower temperature; A role for cell size and genome configuration?
Genome, 2013 Sep; 56(9): 511-9. Doi: 10.1139/gen-2013-0004. Epub 2013 Jun 5.

II. Jalal, M., Shala, N., Wojewodzic, M.W., Andersen, T., and Hessen, D.O. Multigenerational genomic responses to dietary P and temperature in *Daphnia*
Submitted to ***Genome***, 2013.

III. Leinaas, H.P., Jalal, M., Gabrielsen, T., and Hessen, D.O. Inter- and intra-specific variation in body- and genome size in calanoid copepods; temperature as a key driver?
Manuscript.

IV. Jalal, M. and Hessen, D.O. Links between genome, cell, and body size in *Drosophila melanogaster* raised at different temperatures.
Submitted to ***Journal of Thermal Biology***, 2013.

V. Hessen, D.O., Jalal, M., and Svenning, M.A. Erythrocyte size and genome size in Arctic charr (*Salvelinus alpinus*): A linkage to temperature and body size.
Manuscript.

ABBREVIATIONS

ATP	Adenosine triphosphate
C	Carbon
C:N:P	Carbon to nitrogen to phosphorous ratio (molar)
C:P	Carbon to phosphorous ratio (molar)
CRBC	*Gallus gallus domesticus*
CV	Coefficient of variation (flow cytometry)
DAPI	4',6-diamidino-2-phenylindole
DNA	Deoxyribonucleic acid
EB	Ethidium bromide
FCM	Flow cytometry
FSC	Forward light scatter
GB	Grinding buffer
GLM	Generalized linear model
HP	Phosphorous sufficient diet
HtHf	High temperature and high food
HtLf	High temperature and low food
LP	Phosphorous deficient diet
LtLf	Low temperature and low food
Mb	Mega base pairs
mm	Millimeter
µm	Micrometer
N	Nitrogen
P	Phosphorous
pg	Picogram
PI	Propidium iodide
RNA	Ribonucleic acid
S2 cells	Schnider 2 cells
SD	Standard deviation
SSC	Side light scatter
TSR	Temperature size rule

ABSTRACT

The relationship between temperature and body size has attracted wide interest since the "Bergmann's rule" was introduced. While this rule originally dealt with endotherms, later studies have focussed ectotherms, including cell- and genome sizes. Because the final body size of an organism is largely the sum of its cells, any increase in cell size would lead to an overall increase in body size. For many ectotherms, the negative correlation between body size and temperature is also reflected in a corresponding relationship between temperature and cell- or genome size. For example changes in body size of ectothermic metazoans may partly reflect changes in cell size rather than cell number. While changes in genome size is generally expected to occur over longer time period (evolutionary), except for the case of polyploidization, changes in cell size (cytoplasmic volume) could occur at shorter time scales. For example the responses reflecting geographical (temperatures) clines may differ from those that occur during ontogeny. The main aim of this study was to test whether temperature could affect genome- and cell size in selected ectotherms. The experiments were performed on the following taxa and species; *Daphnia* (papers I and II), calanoid copepods (paper III), *Drosophila melanogaster* (paper IV), and Arctic charr (*Salvelinus alpinus*) (paper V). Genome and cell (nucleus) size showed that the strongest temperature responses were in *Daphnia* (papers I and II) compared with the other species. Increased body size of *Daphnia* at low temperatures could, at least partly, be caused by an increase in both DNA condensation and increased cell volume at low temperature (paper I). Our genome size estimates of *Daphnia* clones (papers I and II), some calanoids (paper III), and *Drosophila* (embryo and Schnider 2 cells; paper IV) are novel findings. In addition to the temperature effect, we also tested dietary stoichiometric effect on the genome and cell size of *Daphnia*, by growing it in phosphorus (P) limited versus P complete diet for several generations (paper II). Our genome and cell size results show that *Daphnia magna* and *Daphnia pulex* respond

differently to dietary P concentration change at different growth temperatures (paper II). We further show that diet with low P, negatively effect both genome and cell size in *Daphnia* (*Daphnia magna*), which supports our hypothesis; that small genome size may be an evolutionary consequence of P allocation from DNA to RNA under P deficiency (paper II). Experiments with *Daphnia* (papers I and II) and *Drosophila* (paper IV) were conducted in the laboratory, while calanoid copepods (paper III) and Arctic charr (paper V) were analysed from the field samples.

1. INTRODUCTION

1.1. Ectothermic organisms

An ectotherm, from the Greek *εκτός* (*ektós*) "outside" and *θερμός* (*thermós*) "hot", is an organism whose regulation of body temperature depends on external sources, such as sunlight or a heated rock surface (Davenport, 1992). The ectotherms include the fishes, amphibians, reptiles, and invertebrates. The body temperatures of aquatic ectotherms are usually very close to those of the ambient water. Ectotherms constitute the vast majority of organism biomass and about 99% of all species worldwide (Atkinson and Sibly, 1997; Wilson, 1999). Ectotherms acclimate by adjusting their biochemical composition and physiological rates, thereby favouring the maintenance of function at the acclimation temperature, but not necessarily at other temperatures (Guderley, 2004). For instance, seasonal changes in environmental temperature may lead to acclamatory responses that enhance performance under seasonal conditions (Packard *et al.*, 2001; Guderley, 2004). The potential for acclimation thus influences an organism's response to climate warming.

1.2. The temperature-size rule in ectotherms

In 1847, Carl Bergmann first proposed a general rule for organism size with temperature based on intraspecific comparisons of size among endotherms; he noted that organism size tends to increase with latitude (Bergmann, 1848). Bergmann surmised that a smaller surface area to volume ratio, associated with a larger mass, might have evolved to reduce heat loss in colder environments. However, this biological rule was also found to apply to ectotherms. Approximately 80% of the ectotherms reviewed by Ray (1960) obeyed Bergmann's rule. Moreover, the response of ectotherms to latitude was found, at least partly, to be a phenotypic response to rearing temperature. In general, animals found in colder climates tend to be larger as adults than their conspecifics in warmer climates. This pattern even holds

when altitude or latitude is used as a proxy for environmental temperature (Ashton, 2001; 2002a; b; Ashton and Feldman, 2003). Laboratory studies support the notion that animals reared at lower temperatures grow to a larger body size. Studies show that more than 80% of ectothermic species studied in laboratories exhibited faster growth but smaller adult body size at higher rearing temperatures (Atkinson, 1994; Atkinson, 1995). This trend, dubbed the temperature - size rule (TSR) (Atkinson *et al.*, 1996; Atkinson and Sibly, 1996), is a special case of Bergmann's rule, where the relationship between environmental temperature and body size is the product of phenotypic plasticity (von Bertalanffy, 1960). Recognition of the TSR has caused a resurgence of efforts to understand how temperature affects growth and body size of organisms, as reviewed by Atkinson and Sibly (1997).

1.3. Temperature, growth rate, and body size in ectotherms

Ectotherms generally grow slower but often mature at a larger body size in colder environments. The relationships between environmental temperature, organismal growth, and adult body size have intrigued biologists for over a century, but a resurgence of interest in the last decade with the discovery of widespread patterns in diverse taxa as well as the potential size effect of global warming. Ectothermic species distributed over broad geographic ranges often exhibit thermal clines in body size, with the majority of species exhibiting larger adult size in colder environments (Partridge and French, 1996; Ashton, 2004). During the last decade, intensive theoretical and empirical research has generated various explanations, both adaptive and non-adaptive, for phenotypic plasticity. Non-adaptive plasticity of body size is hypothesized to result from thermal constraints on cellular growth that cause smaller cells at higher temperatures (Ghalambor *et al.*, 2007), but the generality of this theory is poorly supported. Adaptive plasticity is hypothesized to result from greater benefits or lesser costs of delayed maturation in colder environments

(Ghalambor *et al.*, 2007). These theories seem to apply well to some species, but not others (Mousseau, 1997; Arendt, 2007), which may reflect that TSR actually is a "concept cluster" with different drivers (Watt *et al.*, 2010). Thus, no single theory has been able to explain the temperature-size relationships in ectotherms, and its relationships with life-history (Berrigan and Charnov, 1994). Other environmental variables that affect growth rate (e.g., food availability) may have a parallel effect on adult body size, such that better conditions result in faster growth to a larger final size. The fact that there may be various confounding factors operating argues for controlled experiments to reveal the net effect of temperature alone, or in combinations with parameters such as food quantity or quality, to explore these effects.

1.4. Temperature, genome, and cell size in ectotherms

For a wide range of ectothermic metazoans it has been demonstrated that individuals reared under reduced temperatures reach larger cell sizes than conspecifics reared at higher temperatures (Robertson, 1959; Van Voorhies, 1996; Arendt, 2007; Kammenga *et al.*, 2007; Daufresne *et al.*, 2009). Individual body growth may occur either by increasing cell size or cell number (Timofeev, 2001; Arendt, 2007), or through both strategies (Partridge *et al.*, 1994) (Fig. 1). For both ectotherms with variable and fixed cell numbers, variation in body size may partly be attributed to changes in cell size (Partridge *et al.*, 1994). Similarly, differences in adult body size among individuals and populations, or between closely related species are caused either by differences in cell number or cell size (Calboli *et al.*, 2003). In principle, growth during certain life stages, e.g. until maturity, may be determined primarily by cell number (Fig. 1). For organisms with fixed cell number (e.g. nematodes and rotifers), body growth can only occur through changes in cell size (Stelzer, 2002; Kammenga *et al.*, 2007), while the situation is more complex in organisms with variable cell numbers. In *Drosophila melanogaster*, the observed phenotypic response of increased adult body size at

lower developmental temperatures has been explicitly linked to increased cell size, while conspecific populations sampled across climatic gradients, show increased body size in cooler environments mainly attributed to increased cell numbers (Partridge *et al.*, 1994; French *et al.*, 1998).

The positive correlation between genome size and body size commonly observed among invertebrate taxa (McLaren *et al.*, 1988; Ferrari and Rai, 1989; Finston *et al.*, 1995; Gregory, 2005; Rasch and Wyngaard, 2006) indicates that the contribution from cell size to the difference in body size between related species may be significantly related. A coupling between low temperature, large genome, and large body size is typically found in many marine invertebrates (Atkinson, 1994; Timofeev, 2001; Rees *et al.*, 2007; Hessen and Persson, 2009). Further, a positive correlation between genome size and cell size appears to be rather universal in both plants and animals (Cavalier-Smith, 1978; Bennett, 1987; Gregory *et al.*, 2000; Gregory, 2005), also supported by the observation that expansion of genome size causes increased cell size (Gregory, 2001).

Genome size may increase by an increase in number of base pairs (mainly in the intron regions), causing larger diploid genomes, or by partial or whole-genome duplication, the latter also known as polyploidization. However, genome structure that affects nuclear volume, e.g. chromatin packaging, mitotic processes or aneuploidy may also affect cell volume. Both on the intra- and interspecific levels, it is well documented that increases in genome size through polyploidization generally results in increased cell size (Gregory, 2005). While both increased diploid genome size and polyploidization are potential means of increasing cell size, and both seem somehow related to low temperatures, the evolutionary drivers may be widely different and occur at different time scales.

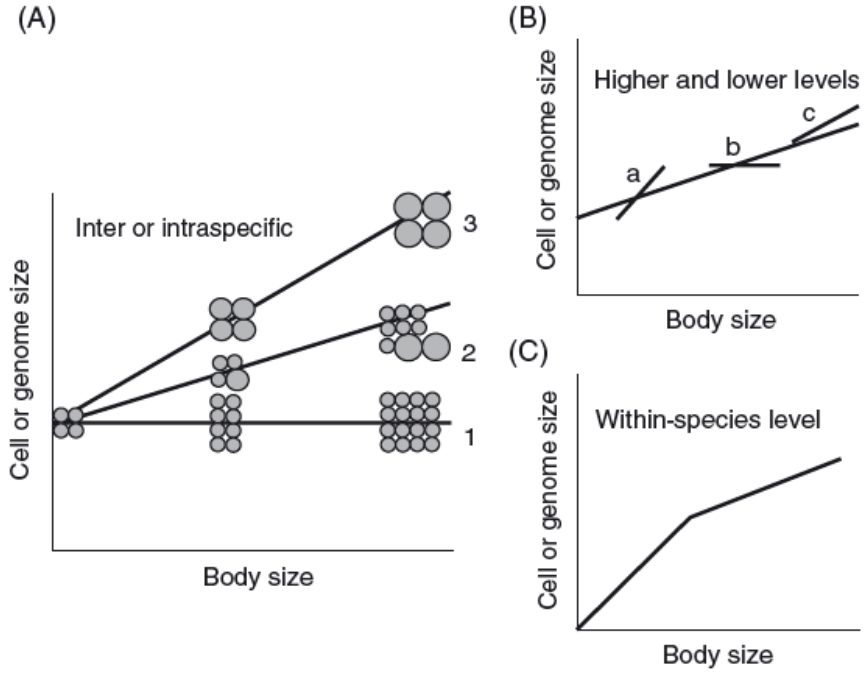

Figure 1. The relationships between cell or genome size and body size. Body size may increase (A) by increase in cell number (1), or by a combination of increased cell numbers and cell size by genome size expansion or polyploidy (2), or by increase in cell size (3). Modified from Kozłowski et al. (2003). Potentially different slopes for cell or genome size versus body size (B) at different taxonomic levels (e.g. classes a, b and c within a phylum or order). Within a given class (a, b and c) may yield different slopes compared with the higher level, indicating different evolutionary strategies. Allometric effects in cell or genome size versus body size may occur during ontogeny, e.g. by somatic endopolyploidy (C). Figure obtained from Hessen et al. (2013).

1.5. Genomic responses to different dietary phosphorous and temperature

It is hypothesized that temperature and phosphorus (P) limitation can drive changes in genome size, which in many cases scale with cell size and body size. Increased risk of P limitation has been shown to reduced growth efficiency at high temperatures (Persson *et al.*, 2011). The specific content of P in food is important for consumer growth and metabolism because it is a key component of DNA, RNA, ATP, and membrane phospholipids (Sterner and Elser, 2002). Nucleic acids are especially P-rich compared with other molecules, and it has also recently been suggested that that an evolutionary reallocation of P from non-coding

DNA to RNA could results in decreased genome size and elevated RNA: DNA ratio (Fig. 2), boosting growth rate under P-limited conditions (Hessen *et al.*, 2008; Hessen *et al.*, 2010). In fact, even closely related organisms may show a striking variability in their genome size, and at least for invertebrates high growth rates generally go along with small genomes (Gregory, 2005) and high levels of RNA (Elser *et al.*, 2000; Hessen *et al.*, 2008) has been taken as support of this hypothesis (Hessen *et al.*, 2010). Also, previous studies with plants show that ploidy level could be reduced in response to P-limitation (Leitch and Bennett, 2004; Leitch and Leitch, 2008), suggesting a selective pressure to reduce material costs associated with DNA under P-scarcity. Similar responses have been detected in snails (Neiman *et al.*, 2012).

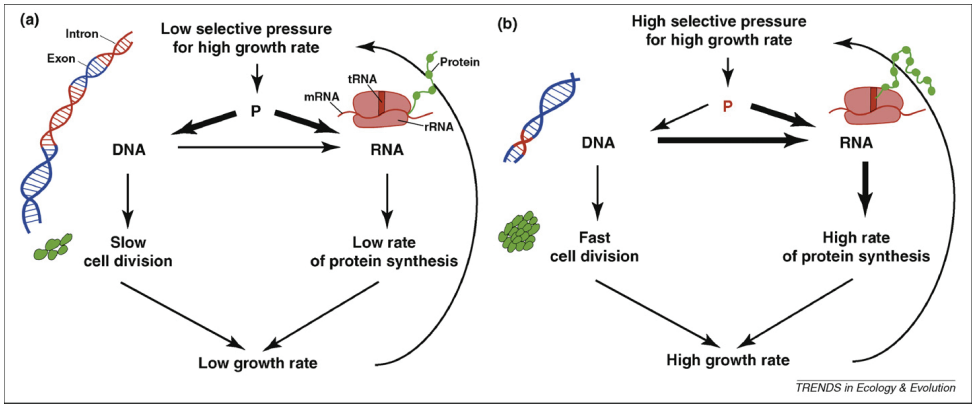

Figure 2. The effects of different evolutionary allocations of P to DNA or RNA. Under low selective pressure for high growth rate, a significant portion of P is allocated to DNA (and implicit large genome size) causing slow growth rate and low rate of protein synthesis (a). Under strong selection for high growth rate, there will be selective pressure for reallocating P from non-coding DNA to RNA, promoting high growth rate (b). Thickness of arrows indicates relative importance of P allocation or causality. Scenario (a) indicates large genome, high intron:exon ratio, slow rate of protein synthesis and slow cell division as opposed to scenario (b). The figure was obtained from Hessen et al. (2010).

2. MATERIALS AND METHODS

2.1. Model organisms

We used selected ectotherms partly as a model organism and partly as representative "cases" to study and understand particular biological phenomena of the link between genome and cell size at different temperatures, with the expectation that discoveries made in the organism model will provide insight into the working of other organisms. The following ectotherms (by order or genus) were used as model organisms in our study; *Daphnia*, calanoida, *Drosophila* and *Salvelinus*. The choice of *Drosophila*, *Daphnia*, calanoids, and *Salvelinus* as model organisms in our study was mainly based on their frequent use in temperature-size studies (reviewed in Angilletta Jr and Dunham, 2003; Angilletta *et al.*, 2004; Jonsson and Jonsson, 2009).

2.1.1. *Daphnia* (papers I and II)

Daphnia (commonly called water fleas) is a genus of small, planktonic crustaceans, between 0.2 and 5 mm in body length. They live in various freshwater environments ranging from acidic swamps to freshwater lakes, ponds, streams and rivers. The two most commonly used experimental "model" species of *Daphnia* are *Daphnia pulex* and *Daphnia magna*. These species are commonly used in ecophysiological studies, toxicological assays, genetic studies etc. The lifespan of a *Daphnia* is largely temperature-dependent, where some individuals can live up to 108 days at 3 °C, while others live for only 29 days at 28 °C (Lampert and Kinne, 2011). They generally grow at a slower rate at low temperatures, but are larger than those under normal conditions (Lampert and Kinne, 2011). The genome of *D. pulex* is very small compared to many organism and was first sequenced in 2011, and is estimated to be 200-227 Mb (Colbourne *et al.*, 2011). Sequencing of *D. magna* genome is under progress. *Daphnia* are usually filter feeders, ingesting mainly unicellular algae and various sorts of organic

detritus including protists and bacteria. Reproduction is normally clonal (allowing the maintenance of genetic lineages), but sex can be induced environmentally (allowing the production of inbred or outbred lineages). The clonal nature of the organism provides an exceptional opportunity to study genomic and phenotypic responses to environmental stimuli, in our case change of growth temperature and dietary P concentrations.

2.1.2. Calanoida (paper III)

Calanoid copepods are planktonic crustaceans, comprising around 40 families with about 1800 species of both marine and freshwater copepods (Blaxter *et al.*, 1998). There are about 2300 described species of calanoid copepods worldwide, of which some 25% occur in freshwater (Bowman and Abele, 1982). The calanoids are primarily suspension feeders eating mainly phytoplankton and reproduction is only sexual (e.g. Mauchline, 1988). The largest specimens reach 18 mm long, but most are 0.5–2.0 mm long (Blaxter *et al.*, 1998). The duration of development from egg to adult is primarily temperature dependent (Breteler *et al.*, 1982; Uye, 1988; Ban, 1994). According to Animal Genome Size Database, calanoid copepods show enormous genome diversity with genome size ranging from 616 to 12185 Mb (Gregory, 2013).

2.1.3. *Drosophila* (paper IV)

Drosophila is a genus of small flies, belonging to the family *Drosophilidae*, whose members are often called "the fruit flies". In nature, *Drosophila* feed on microorganisms, particularly yeast, on the surface of fruits. The life cycle of the fruit fly is temperature dependent and takes about 9 days to complete at 25 °C (Ashburner and Thompson Jr, 1978). After the egg is fertilized, the embryo emerges in ~ 24 hours. The embryo undergoes successive molts to become the first, second, and third instar larva. In particular, one species of *Drosophila*, *Drosophila melanogaster*, has been heavily used in genetics research and is a common model organism in developmental biology and temperature response studies (Partridge *et al.*,

1994; Pétavy, 1994; Nunney and Cheung, 1997; Reeve *et al.*, 2000). The fly genome, which was sequenced in the year 2001, is 165 Mb (spread over four chromosomes) (Adams *et al.*, 2000). The most commonly used *Drosophila* cell line is Schneider 2 cells (S2 cells). The S2 cells are derived from a primary culture of late stage (20–24 hours old) *Drosophila melanogaster* embryos, likely from a macrophage-like lineage (Schneider, 1972).

2.1.4. *Salvelinus* (paper V)

Salvelinus alpinus (Arctic charr) is a cold-water fish in the *Salmonidae* family, native to Arctic (66° 33'N) and sub-Arctic (50°N and 70°N). It breeds in fresh water and populations can be either landlocked or anadromous, migrating to the sea (Webster and Lim, 2002). Individual fish can weigh 0.91 kg or more, and the body size up to 107 cm in length. According to Animal Genome Size Database the genome size of Arctic charr is ~ 3246 Mb (Gregory, 2013). Temperature is one of the most important environmental cues for Arctic charr and effects feeding, growth, and maturation (Jobling *et al.*, 1993). Arctic charr is considered the most cold-adapted species within the salmonid family (Balon, 1980). From field studies, Arctic charr seems able to feed, grow, and have relatively low mortality rates during winter periods with low water temperatures (Klemetsen *et al.*, 2003; Byström *et al.*, 2006; Svenning *et al.*, 2007; Amundsen and Knudsen, 2009; Siikavuopio *et al.*, 2009).

2.2. Culture and sampling setup

2.2.1. *Daphnia* cultures

In paper I and II, *D. magna* and *D. pulex* clones were used in our experiments. *Daphnia* clone Clone A, German, and LL4-15 was used in paper I, while only the two latter clones were used in paper II. All clones originated from temperate and functionally diploid populations (Baird *et al.*, 1991; Weider *et al.*, 2004; Pulkkinen, 2007). Prior to the experiments, all clones were raised at 20 °C for at least three generations and fed *ad libitum*

with the chemostat-grown chlorophyte *Selenastrum capricornutum* (Kilham *et al.*, 1998). In paper I, *Daphnia* were analysed after one generation, while paper II presents a multigenerational study of *Daphnia*. Female juvenile *D. magna* and *D. pulex* were allowed to grow at either high or low temperature (20 or 10 ºC) (papers I and II). In paper II, the *Daphnia* were also fed high and low P diets for up to 35 generations to assess the eventual multigenerational impacts of genome- and nucleus size at different temperatures and diets. The high and low P diets were prepared in chemostats, according to previous experiments by Hessen et al. (2002), before fed to *Daphnia*.

2.2.2. Calanoida sampling

The calanoid copepods (five species) samples, in paper III, were collected from either the Oslo fjord on the southern coast (59°19.0'N; 10°35.0'E), the Lurefjorden on the western coast (60°41.0'N 5°8.0'E), the Arctic fjords Billefjorden (78°66.0'N; 16°7.0'E) and Rijpfjorden (80°66'N; 22°18.15'E) or the Fram Strait (78°0.8'N 8°0.2W). All samples were taken from the deeper part of the location (> 100 m) by use of WP2 nets with 0.5 m diameter and 200 µm mesh size (Gabrielsen *et al.*, 2012). The exact temperatures depend on depth and season as well as annually measurements; the typical summer temperature is 0 - 1.5 °C for the Arctic sites, while 5 – 10 °C for the southern fjords.

2.2.3. *Drosophila* cultures

In paper IV, we used both *Drosophila melanogaster* fly stocks and S2 cells. Both fly stocks, Oregon-R and w1118, were cultured at 24 °C and fed formula 4-24® instant medium following the Carolina™ *Drosophila* manual before start of experiment (Flagg, 1988). For the experiment, a cohort of first-instar larvae was produced by incubating flies (both sexes) in new vials at 24 °C followed by removing the flies from the vials after ~ 24 hours and further randomly incubated at either 16 °C, 22 °C, 24 °C or 28 °C. Flies were randomly collected under anaesthesia and distributed to further flow cytometry (FCM) and

microscopic analysis. We also analysed S2 cells and 24 hour old Oregon-R embryos for cellular and genomic comparison between parameters. The S2 cells were grown in Schneider's *Drosophila* medium initially stock cultured at 28 °C two weeks before the experiment. For the experiment, S2 cells were incubated at 16 °C, 22 °C, and 28 °C for 24 hours.

2.2.4. Arctic charr sampling

In paper V, we wanted to test for eventually changes in Arctic charr erythrocyte volume and genome size during early development. Newly hatched Arctic charr juveniles from the same population were raised at different temperatures and two food regimes; HtHf, HtLf, LtHf, and LtLf. The low temperature group was held at 6 - 3 °C from October to late November, and at 2.5 - 1.4 °C from late November to late December. The other two groups were held at temperatures at 13.3 - 15.0 °C during the whole period. The experiment was run at Tromsø Aquaculture Research Station (70°39'N; 23°41'E). Blood samples were taken initially before the split of the three test groups, and after 1 and 2 months. To assess the variability in erythrocyte volume and genome size within and between populations that varies strongly in habitat, size and life history, individuals were analysed from 4 different wild charr populations in northern Norway; Koifjordvatn (70^{o}56'N; 28^{o}09'E), Oksfjordvatn (69^{o}53'N; 21^{o}23'E), Tromvikvatn (69^{o}44'N; 18^{o}24'E), Laksvatn (69^{o}38'N; 19^{o}40'E), plus the souther, large lake Tyrifjord (60^{o}02'N; 10^{o}08'E) which is known to have unusually large species of charr.

2.3. Body size measurements

We measured body size of *Daphnia* (paper I), calanoid copepods (paper III), *Drosophila* (paper IV), and Arctic charr (paper V) at different temperatures (and treatments). Body size of *Daphnia*, calanoids, and *Drosophila* was directly measured from photographs taken by

light microscope. Body size measurements of *Daphnia* in paper II was not included since the animals were not in the same physiological age at the time before FCM analysis. *Drosophila* (paper IV) was also collected for wing and eye size measurements by microscopy. Measurements of wing and eye cell area were also included in paper IV. In paper V, weight and length was measured of four wild anadromous (migratory) or resident charr populations individuals.

2.4. Nuclei extraction and DNA staining

The nuclei extraction steps were performed on *Daphnia* (papers I and II), calanoid copepods (paper III), and *Drosophila* (IV) following the protocol of Korpelainen *et al.* (1997), but with modifications. Whole animals were ground in grinding buffer (Korpelainen *et al.*, 1997) followed by RNase A treatment and DNA staining with propidium iodide (PI) (papers I – IV) or ethidium bromide (EB) (paper I) or DAPI (paper I). FCM analysis was performed on FACS Calibur (papers I – IV) and BD LSR II (paper I) machines. Fresh blood cells of *Gallus gallus domesticus* (CRBC) and 2.5 μm alignment beads were used as standard (Galbraith *et al.*, 1983; Galbraith *et al.*, 2001). Similar nuclei extraction steps were also preformed on *Drosophila* S2 cells (paper IV). Treatment of cells with GB dissolved both the cytoplasm and the cell membrane, leaving intact nuclei for DNA measurements (papers I – IV). To access the potential of DNA condensation status in *Daphnia* (paper I and II) at low and high growth temperature, DNA was also stained with nuclear-ID green which specifically binds to condensed DNA (Park, 2011). Condensation status of *Drosophila* (fly and S2 cell) and copepod nuclei were also tested, but not included in this study.

2.5. The temperature reversal experiment

In paper I, we tested whether temperature during *Daphnia* sample preparation could induce effects on genome and nucleus size estimates by FCM analysis. Nuclei suspension from clones raised at low temperature were prepared following the standard nuclei extraction protocol (see section 2.4.), stained with PI, and split in two parts before the incubation procedure. One part was cold incubated following our standard protocol, and the other part was incubated at high temperature (i.e. "reversal incubation"; paper I).

2.6. Cellular permeabilization and DNA staining

Cellular permeabilization was performed of *Drosophila* S2 cells (paper IV) and Arctic charr blood cells (paper V). Phosphate buffered saline solution and ethanol was used to permeabilize Arctic charr and S2 cells, respectively. Treatment of S2 cells with ethanol preserved both the cytoplasm and cell membrane for cellular DNA measurement.

2.7. Genome, nucleus, and cell size estimation by flow cytometry

The genome and cell (or nucleus) size estimations in papers I – V were measured by FCM. FCM, which is commonly used in the medical field and in plant biology, provides an accurate determination of differences in genome size (Ulrich, 1990; Michaelson *et al.*, 1991; Lauzon *et al.*, 2000) and is considered to be highly reliable for detecting tiny differences in genome size, such as a difference of 1.5% (Kent *et al.*, 1988). According to Animal Genome Size Database, FCM is the second most widely used method for estimating genome size (Gregory, 2013). The recorded fluorescent signal of a fluorochrome bound to DNA is assumed to be directly proportional to the amount of DNA in the nucleus (Shapiro, 2003). The measurements of relative fluorescence intensity of stained nuclei were performed on a linear scale and 10 000 nuclei (or cells) were analysed for each sample (Galbraith *et al.*,

1983). The absolute DNA amount of a sample was calculated based on the values of the 2C peak means. C-value = pg DNA nucleus^{-1} was calculated following method of Galbraith et al. (2001): Sample 2C DNA content (pg nucleus^{-1}) = [(Sample 2C peak mean) / (Standard 2C peak mean)] * Standard DNA content (pg DNA nucleus^{-1}). The standard DNA content used was CRBC = 2.5 pg (Vergilino *et al.*, 2009) for all experiments.

Cell or nucleus size was measured in forward light scatter (FSC) detector (cf. Swat *et al.*, 1991; Neufeld *et al.*, 1998; Johnston *et al.*, 1999; Weinkove *et al.*, 1999). Cell granularity or complexity was measures by side light scatter (SSC) detector.

The quality of a DNA histogram is usually estimated from the width of the peak of DNA of 2C cells (Ormerod and Imrie, 1990). This is measured by the coefficient of variation (CV) across the peak and is calculated from the standard deviation (SD): CV = 100 x SD / (peak channel) %. The peak channel is the mean channel number of the DNA peak. Thus, the peak channel represent the "midpoint" of fluorochrome intensity distribution of each ploidy peak (Ormerod and Imrie, 1990). In theory, acceptable CVs for DNA estimates has been set to 6% (Baretton *et al.*, 1994; Vilhar *et al.*, 2001; Darzynkiewicz *et al.*, 2010), with CVs < 3% as ideal (Marie and Brown, 1993).

2.8. Confocal laser scanning microscopy

Fluorochrome staining of *Daphnia* (paper I), calanoid copepods (paper III), and *Drosophila* (paper IV) DNA was also confirmed by observation of nuclei using confocal laser scanning microscopy. Confocal images were obtained using an Inverted Olympus FluoView 1000 confocal laser scanning microscope - IX81 (Olympus Imaging America Inc., USA) equipped with 488 nm and ultra violet laser lines. Confocal microscopy settings were calibrated with 2.5 μm alignment beads and CRBC nuclei.

2.9. Statistics

The comparison between groups of body size, genome size, and nucleus (cell) size of *Daphnia* (paper I) and *Drosophila* (paper IV) at different temperatures were computed by one-way ANOVA after testing for homogeneity of variance and normal distribution in Sigmaplot software. The pairwise multiple comparison procedures for genome size estimations were also measured by Holm-Sidak method in paper I and IV.

In paper II, comparison between groups of genome size, and nucleus size of *Daphnia* species at different temperatures vs. diets vs. generations were computed using the generalized linear model* (GLM) (Nelder and Wedderburn, 1972) in R (Team, 2005).

In paper III, the relations between the five different species of calanoid copepods body length and genome size was tested by linear regression. Also the correlation between genome size and nucleus size was tested by linear regression. Differences between species were tested by non-parametric all-pair Tukey HSD-test. One-way analysis of calanoid copepods genome estimates was included by category.

In paper V, the relations between cell size (volume) or genome size and weight in both the experimental and wild populations was tested by linear regression. Population differences were tested by non-parametric all-pair Tukey HSD-test due to rather small and non-homogenous sample size between populations.

* The dependent variable in the GLM model is linearly related to the factors and covariates by a specified link function. The model allows for the dependent variable to have a non-normal distribution. Each outcome in GLM of the dependent variables, Y, is assumed to be generated from a particular distribution in the exponential family, a large range of probability distributions that includes the normal, binomial, Poisson and gamma distributions, among others. The mean, μ, of the distribution depends on the independent variables, X, through: $E(Y) = \mu = g^{-1}(X\beta)$. $E(Y)$ is the expected value of Y; $X\beta$ is the linear predictor, a linear combination of unknown parameters, β; g is the link function. In this framework, the variance is typically a function, V, of the mean: $Var(Y) = V(\mu) = V(g^{-1}(X\beta))$. It is convenient if V follows from the exponential family distribution, but it may simply be that the variance is a function of the predicted value. The unknown parameters, β, are typically estimated with maximum likelihood, maximum quasi-likelihood, or Bayesian techniques.

3. MAIN FINDINGS

3.1. Temperature, cell size and genome configuration of *Daphnia* (paper I)

In this study we assessed responses of adult body size at different temperatures in two species of *Daphnia,* and applied FCM together with confocal laser scanning microscopy to reveal whether the body size response to temperature could be related to structural effect at the genomic level.

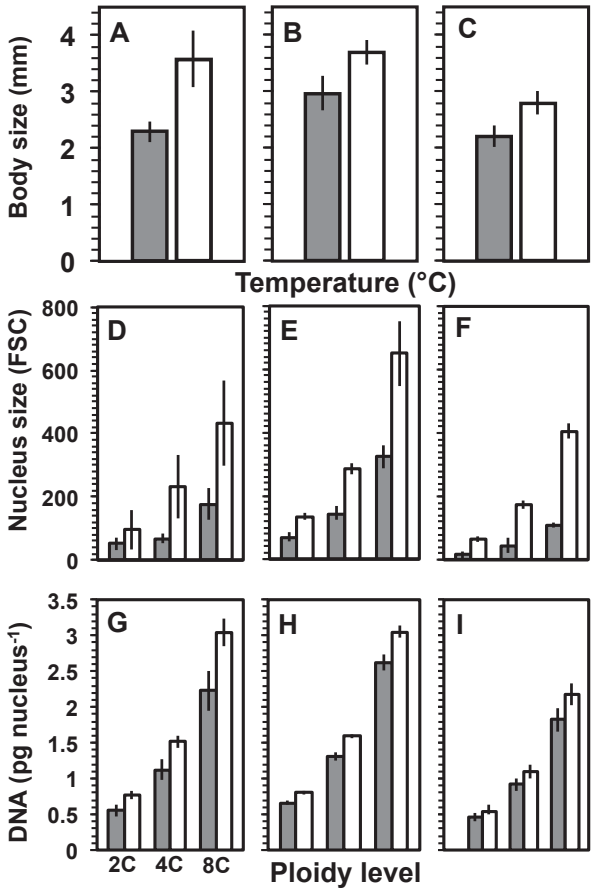

Figure 3. Adult body size (A-C), nucleus size (D-F) and DNA content (G-I) of three *Daphnia* clones from 20 °C (shaded bars) and 10 °C (open bars) growth. The *Daphnia* clones include; *D. magna* Clone A (A, D, and G), *D. magna* German clone (B, E, and H), and *D. pulex* LL4-15 (C, F, and I). Error bars represent SD of the mean of three independent experiments. (Paper I)

The experiments with *D. magna* and *D. pulex* raised at 10 and 20 °C yielded larger adult body size at the lower temperature (Fig. 3). FCM results of both nucleus and genome size estimates were elevated at low temperature, (Fig. 3), suggesting that larger body size at low temperature could partly be accredited to an enlarged nucleus and thus cell size. Confocal microscopy observations confirmed the staining properties of fluorochromes. As differences in nucleotide numbers in response of growth temperature within a life span is unlikely, these results seem accredited to changes in DNA–fluorochrome binding properties, presumably reflecting increased DNA condensation at low temperature.

3.2. Long term effect of P and temperature on *Daphnia* genomics (paper II)

In this study we addressed the multigenerational impact of both dietary P and temperature on genome size (Fig. 4), nucleus size (Fig. 5) and the prevalence of endopolyploidy in two species of *Daphnia* that have different sensitivity to P limitation. *D. magna* and *D. pulex* were kept for up to 35 generations at high and low temperature and fed high and low P diets. FCM revealed significant increases in nucleus size for both species in response to low temperature (Fig. 5). Under dietary P deficiency, *D. magna*, but not *D. pulex*, showed a reduced genome size (Fig. 4), most likely reflecting structural changes in DNA (as previously confirmed with same clones in paper I). The larger nuclei found at 10 °C also had increased CVs of FCM DNA histograms, especially in *D. magna*. In this species, the nucleus size was also elevated in individuals raised on P sufficient compared to P deficient food, while the opposite effect was found in *D. pulex*. Additionally, the degree of endopolyploidy, measured as cycle value, was species specific and responded to temperature and dietary composition. Dietary effects on endopolyploidy were observed in *D. magna* at both temperatures, with increasing prevalence in the P deficient.

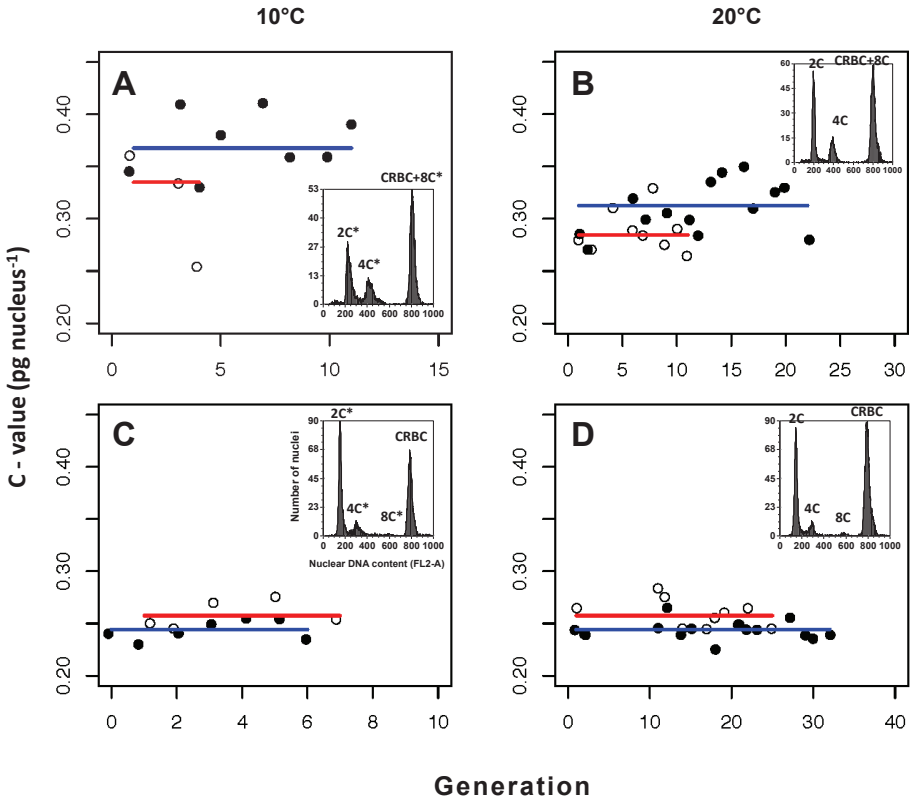

Figure 4. Responses to diet and temperature in C-values (y-axis) versus generation (x-axis) in *D. magna* (A and B) and *D. pulex* (C and D) at 10 and 20 ˚C. Each data point represents one replicate, containing 6-10 pooled individuals. Measurements (circles) and model predictions (curves) from daphniids consuming the low-phosphorus diet (LP; open circles and red line) and daphniids consuming the high-phosphorus diet (HP; solid circles and blue line). Inset of a representative DNA histogram from *D. magna* at generations 10 (10°C) and 20 (20°C) and *D. pulex* at generations 5 (10°C) and 32 (20°C) consuming the HP diet, with CRBC as an internal standard. (Paper II)

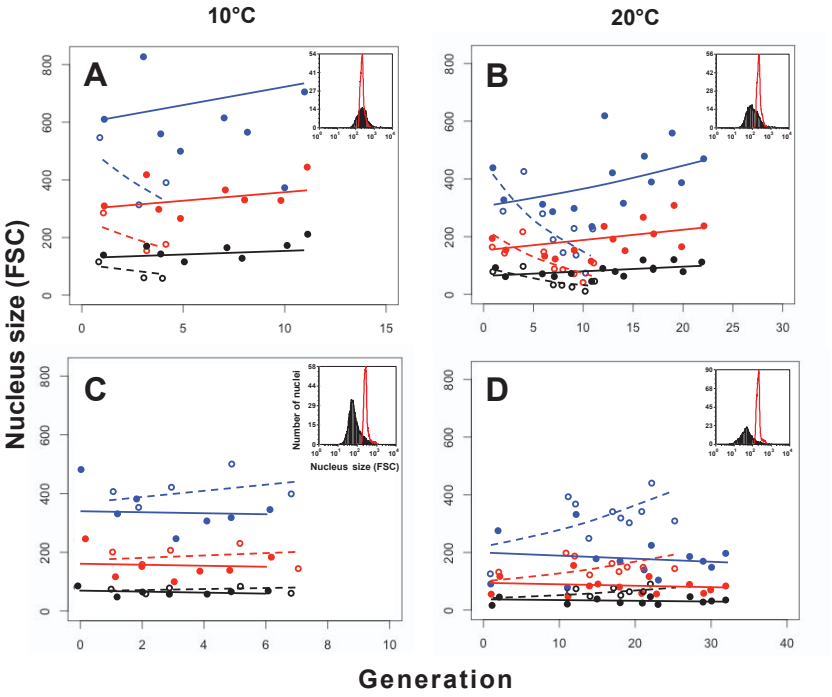

Figure 5. Nucleus size (FSC; y-axis) versus generation (x-axis) in *D. magna* (A and B) and *D. pulex* (C and D) at 10 and 20 °C. Measurements (circles) and model predictions (curves) of 2C (black), 4C (red), and 8C (blue) nuclei from daphniids consuming the low-phosphorus (LP; open circles and dashed curves) and high-phosphorus (HP; solid circles and solid curves) diets are shown at each temperature. Inset of FSC histogram of *D. magna* at generations 10 (10 °C) and 20 (20 °C) and *D. pulex* at generations 5 (10 °C) and 32 (20 °C) fed the HP diet, with CRBC as an internal standard (red line). (Paper II)

3.3. Body and genome size variation in calanoid copepods (paper III)

In accordance with temperature-size expectations (i.e. Bergmann′s rule), larger species generally had the northernmost distribution, and the same was seen at the population levels. Our data showed that size of the surveyed species covered 2.5 - 6.6 mm body length, including substantial intra-specific (population) differences. Genome and nucleus size was measured by FCM, and confocal microscopy observations confirmed the flourochrome staining of nuclei (Fig. 6). Species genome sizes (C-value) ranged from 5.5 – 33.8 pg haploid DNA cell^{-1}, the latter by far the largest ever recorded for copepods, and we also found strong intraspecific variability in nucleus and genome size.

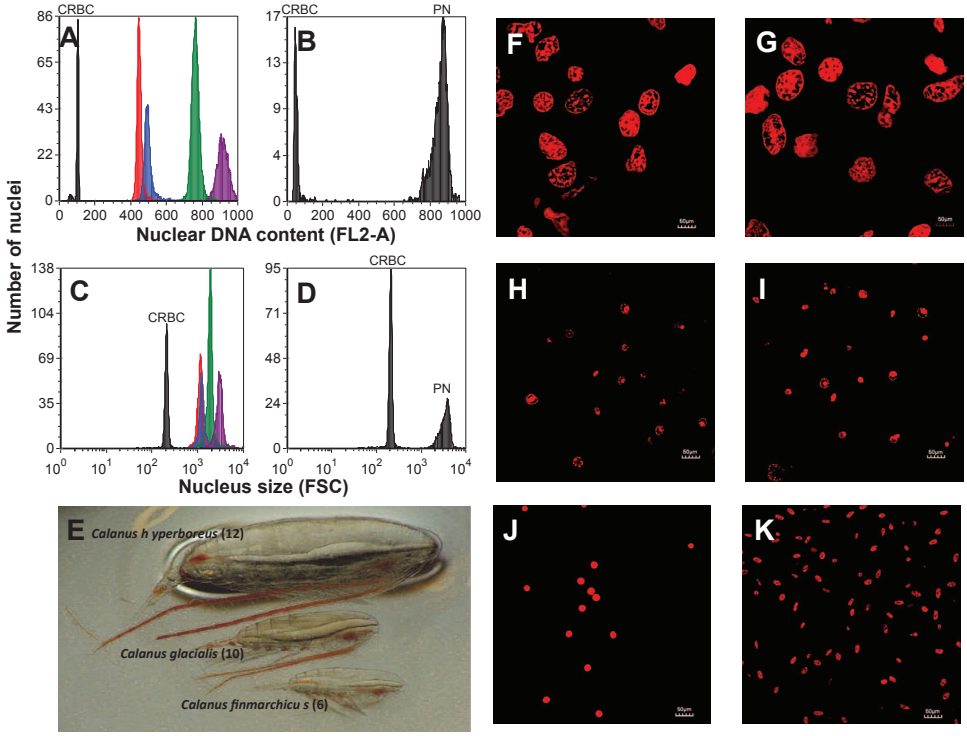

Figure 6. Representative DNA (A and B) and nucleus size (C and D) histograms of copepods and CRBC; *C. finmarchicus* (red), *Metridia longa* (blue), *C. glacialis* (green), *C. hyperboreus* (purple), and *Paraeuchaeta norvegica* (PN). (Paper III). Example of three closely related marine copepod species that show both a body-size and genome-size variability along a temperature gradient, with the larger species living in the coldest waters and also having the lowest growth rate and most prolonged life cycle (E). Average genome size in pg DNA $cell^{-1}$ (in parentheses) is from the Animal Genome Size Database: http://www.genomesize.com/ (Gregory, 2013). Picture obtained from Hessen *et al.* (2013). Confocal microscopy images of *C. glacialis* (F and G), *D. magna* (H and I), 2.5µm beads (J), and CRBC nuclei (K). All stained with PI. Scale bar 50 µm. (Paper III)

3.4. Temperature, body, cell and genome size of *Drosophila* (paper IV)

In this study we assessed responses in adult body, eye and wing size on temperature in two *Drosophila melanogaster* stocks (Oregon-R and w1118). The fly stocks raised at 16, 22, 24, and 28 °C yielded larger adult body size at the lower temperature. Female flies showed larger body and cell size at all temperatures. Development at lower temperature also resulted in larger wing size, but size of the eye was not as much affected as the size of the wing at lower

temperatures (Fig. 7). The effect of temperature on wing size was caused by an increase in cell area in both males and females (Fig. 7).

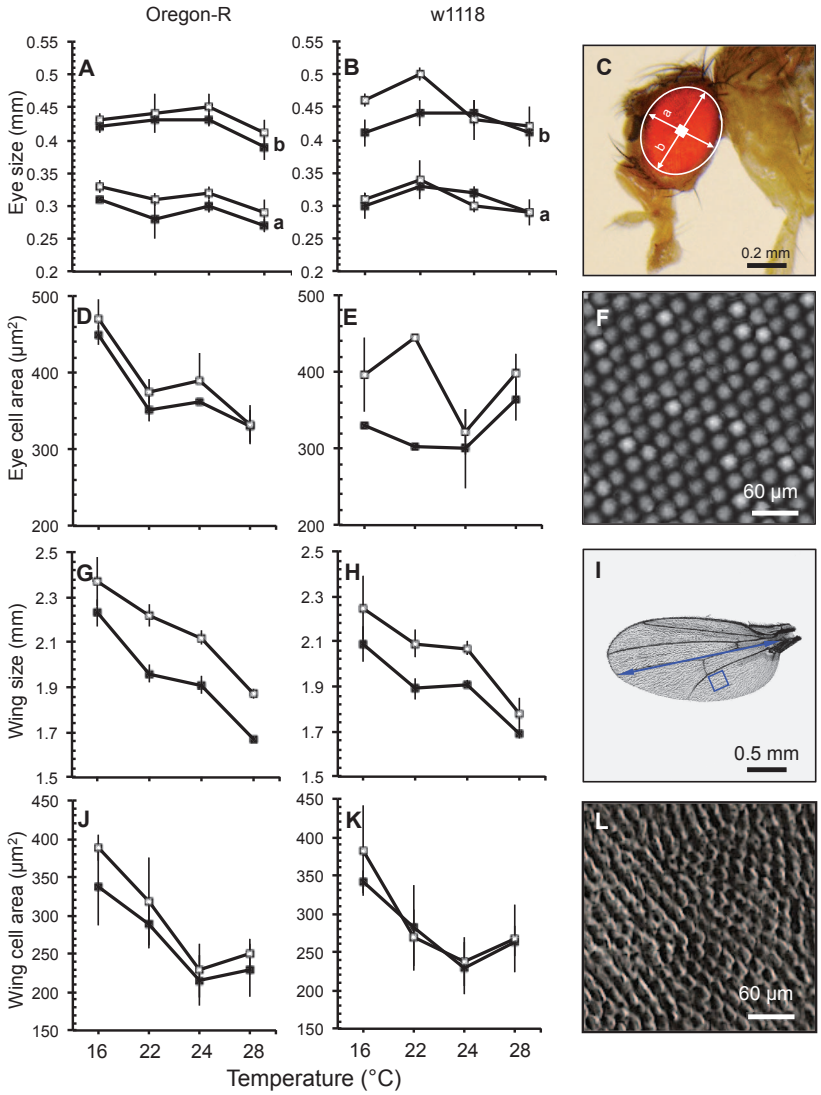

Figure 7. *Drosophila* wing and eye size from different growth temperatures. Eye (A-F) and wing size (G-L) estimates of male (solid) and female (open) flies from growth temperatures (x-axis). The average size and cell area values of both stocks are listed in table 1. Error bars represent standard deviations of the means of three independent experiments. Presented is sample pictures of a female Oregon-R eye (C and F) and wing (I and L), all from 16 °C; the vertical (C; a) and horizontal (C; b) lines of the eye indicate the size, and the square indicates the average cell area (C) of the cells counted (F; magnified). The length of the fourth longitudinal vein of the wing (I; line) represents the size, and the square represents the average cell area (I) of the cells counted (L; magnified). Scale bars are shown on pictures. (Paper IV)

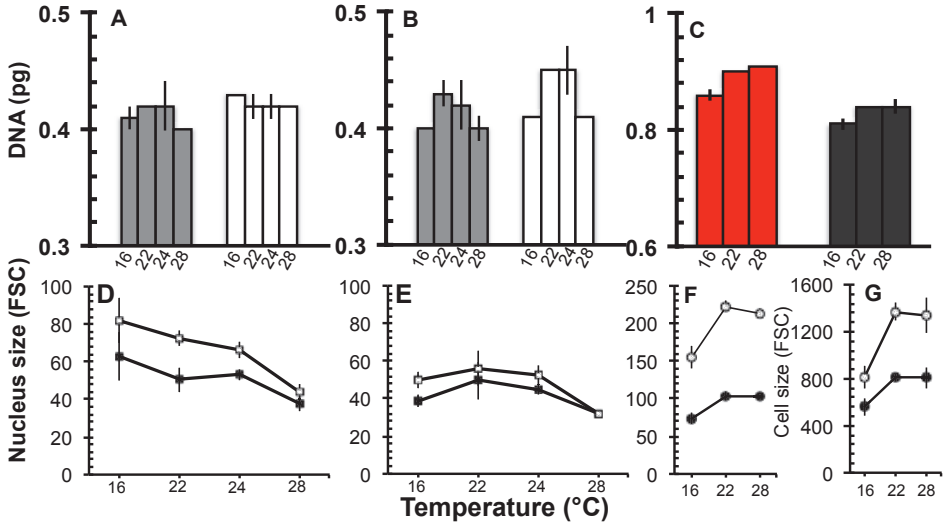

Figure 8. DNA content and nucleus (cell) size of *Drosophila* flies and S2 cells from different growth temperatures. 2C DNA content shown for male (shaded bars) and female (open bars) Oregon-R (A) and w1118 (B) flies from growth temperatures. S2 cells 2C nuclear DNA (pg) from ethanol-treated (red bars) and GB-treated (dark bars) treatment (C). The 2C nucleus size of the male (solid quadrat) and the female (open quadrat) Oregon-R (D) and w1118 (E). S2 cells 2C (solid circles) and 4C (open circles) nucleus sizes of GB (F) and ethanol (G) treated S2 cells. (Paper IV)

The two fold larger genome size of S2 cells (C ~ 0.42 pg) compared with fly DNA (C ~ 0.21 pg) was confirmed to be due to development stage, by analyzing Oregon-R embryos (C ~ 0.40 pg) from 22 °C growth. Any change in nucleus size is directly linked to change in size of rest of cells membrane and cytoplasm, as documented by cellular versus nuclear size change of S2 cells with temperature (Fig. 8). While nucleus and cell size varied, genome size was kept relatively constant at all temperatures for fly stocks and S2 cells (Fig. 8). The observed increase of body size (and wing size) at low temperature may thus at least be linked with cell size change, while corresponding changes in genome size was not observed.

3.5. Temperature, cell- and genome size in Arctic charr (paper V)

In this study we assessed the linkage between temperature, food ration, body size, erythrocyte cell volume, and genome size in Arctic charr raised under different temperatures and populations with strong intrapopulation variability in body size. We found differences in cell volume related to age, but not to temperature or final body size. These differences were recorded in cell volume between wild populations, but not associated with body size. Genome size, assessed by FCM, did not vary at all under experimental conditions, but again some interpopulation differences were recorded. No strong correlation was found between genome size and cell size (Table 1). Assuming that erythrocytes serve as a proxy of somatic cell volume in general, these results suggest minor effect of body size and temperature on cell- or genome size at least in early stages, while intriguing inter-population cellular differences do occur.

Table 1. C-value (in pg cell-1), cell volume (FSC) and cellular complexity (SSC) of erythrocytes from the temperature versus food treatments. FSC and SSC mean values in arbitrary units. Standard deviation in brackets. (Paper V)

	29.11.2011			**22.12.2011**		
Treatment	C-value (pg/cell)	Cell size (FSC)	Cell complexity (SSC)	C-value (pg/cell)	Cell volume (FSC)	Cell complexity (SSC)
HtHf	2.78 [0.154]	85.8 [3.747]	6.53 [0.469]	2.75 [0.096]	94.35 [3.304]	6.34 [0.254]
LtHf	2.77 [0.225]	83.42 [4.812]	5.92 [0.464]	2.80 [0.058]	92.88 [3.255]	5.72 [0.204]
HtLf	2.67 [0.237]	84.375 [2.744]	5.475 [0.618]	2.84 [0.096]	92.51 [2.293]	6.43 [0.353]

4. DISCUSSION

4.1. Ectotherms obtain larger body size at low temperature

The enlargement of body and nucleus size in *Daphnia* (paper I) and *Drosophila* (paper IV) when raised at low temperature (body size; Fig. 9) is consistent with previous findings (Atkinson, 1994; Van Voorhies, 1996; Angilletta *et al.*, 2004; Blanckenhorn and Llaurens, 2005; Atkinson *et al.*, 2006; Arendt, 2007). Like most ectotherms, both *Daphnia* (papers I and II) and *Drosophila* (IV) grew and matured faster at high temperature (Mc Kee and Ebert, 1996; Seebacher, 2009). Also, a number of studies have demonstrated that aquatic ectotherms obtain larger body size in colder environments in support of a Bergmann-type TSR (Bergmann, 1848; Van Voorhies, 1996; Angilletta *et al.*, 2004). Body size at maturation can also vary across different populations and clones of *Daphnia*, independently of food concentration (Ebert, 1992; Pangle and Peacor, 2010), but body size may reflect a fitness-promoting adaptation to lower temperature (Mitchell and Lampert, 2000). Also, evolutionary oriented experiments with *Drosophila* raised under different thermal regimes has highlighted the importance of temperature as a selective force increased body size seem to be a general adaptation to lower temperatures (Partridge *et al.*, 1994; Partridge and French, 1996; Nunney and Cheung, 1997; Kari and Huey, 2000).

In addition to body size response, the enlargement of *Drosophila* wing size at low rearing temperature (paper IV) has been shown to be mainly a consequence of an increase in cell size and delayed growth rate (Partridge *et al.*, 1994; French *et al.*, 1998; Azevedo *et al.*, 2002; Arendt, 2007). Robertson (1959) and James et al. (1995) found that genetically controlled differences in wing size under optimal conditions were mainly due to differences in cell number. In consistence with our findings (paper IV), several studies have also shown that the change in wing area in response to rearing temperature is mediated mainly by a change in cell area (Alpatov, 1930; Partridge *et al.*, 1994; James *et al.*, 1995; Potter *et al.*,

2001). On the other hand, cell number seem to be predominantly effected by food abundance and quality (Robertson, 1959).

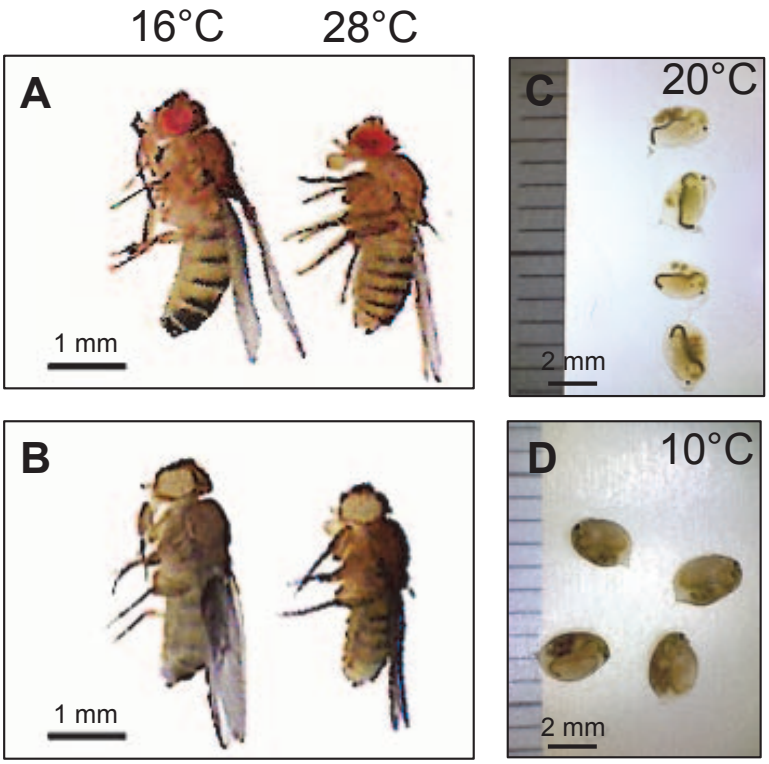

Figure 9. The presented sample pictures of *Drosophila* and *Daphnia* at low and high temperature. *Drosophila* Oregon-R (A) and w1118 (B) show each stock from both 16 °C and 28 °C growth temperatures (paper IV). *Daphnia* sample pictures of *D. magna* (German clone) from 20 °C (C) and 10 °C (D) growth (paper I). Scale bar shown on pictures.

Female *Drosophila* body and wing size was larger than males at all temperatures, which indicate that the temperature - size response happens in parallel order during development (paper IV); meaning that female flies are still larger than males at all temperatures, because they grow in parallel order. Interestingly, size of the eye was not as much affected as the size of the wing at lower temperature, which may be due to the fact that the structures originally are derived from different imaginal disc (Hartenstein, 1993). The two structures may therefore respond independently to rearing temperature.

Typically, TSR predict lower growth rate but larger adult size at low. Our study revealed a striking variability calanoid copepods body size both between and within the surveyed species (paper III). Three of the species from both southern and Arctic population also followed typical "Bergmann clines" with larger species and genomes in the northern populations (paper III). At the intraspecific level, notably the *Calanus*-species displayed a strong inter-population variability more attributed to body size and geographical site. Temperature is likely to play a role, also because it affects growth rate and life cycle but there may be several confounding factors. However, larger *Calanus* species at high latitude may also reflect differential predation regimes in these waters, and that the dominance of the large *C. glacialis* in the temperate western coast (Norway) is preferred by the lack of visual predator in the turbid water (Kaartvedt, 2000; Eiane *et al.*, 2002).

Our study, in paper V, showed that neither body size nor erythrocyte or genome size did adhere to TSR as judged from our data. Hence to fully reveal TSR in fish, body size development should be assessed at various temperatures for the entire life span, and clearly species with a faster growth and maturation than Arctic charr could be for this purpose.

4.2. Ectotherms obtained larger cell and nucleus size at low temperature

The positive correlation between nucleus, cell, and body sizes of *Daphnia* (papers I and II) and *Drosophila* (IV) has been shown to be a general trend widespread among ectotherms (Gregory, 2001; Rees *et al.*, 2007; Hessen and Persson, 2009). For a wide range of ectotherms metazoans it has been demonstrated that individuals reared under reduced temperatures reach larger cell sizes than conspecifics reared at higher temperatures (Robertson, 1959; Van Voorhies, 1996; Arendt, 2007; Kammenga *et al.*, 2007; Daufresne *et al.*, 2009). This fits the argument from van der Have and De Jong (1996) , that cell growth is more sensitive to thermal constraints than is cell division, meaning that organisms with a

rather constant cell number would be smaller due to reduced cell size at elevated temperatures. Our study revealed that the apparent enlargement of *Daphnia* somatic nuclei at low temperature (paper I) came along with increased DNA condensation (see section 4.4). The DNA condensation was more obvious in *D. magna* compare with *D. pulex* at low temperature (paper I). Thus, we propose a theory that temperature response of nucleus size may be somewhat linked to modified cell shape owing to "local" chromatin condensation (Versaevel *et al.*, 2012). The link between condensation status, rearrangements within the nucleus, and concomitant nucleus size effects could then be responsible for the observed effects in larger animals, where the cell changes could again impact their body size.

4.3. Genome and cell size variation in calanoids

Our data revealed variability in genome and nucleus size both between and within the measured copepod species (paper III). Three of the species from southern and Arctic population also followed typical "Bergmann clines" with larger species and genomes in the northern populations. Large genome size is common found among organisms in arctic habitats (Rees *et al.*, 2007; Rees *et al.*, 2008), or extreme habitats, such as fluctuating habitats or habitats causing some physiological factors like osmotic stress that could be more pronounced in semi-terrestrial or freshwater habitats (cf. Libertini *et al.*, 2008). Also, the commonly occurring gigantism in deep-water crustaceans is tightly linked with low metabolic rates, extended life span and large cell size. In fact this has been advocated as a general explanation for the Bergmann's rule (Timofeev, 2001). Generally, calanoid copepods have large genome size compared with other typical crustacean zooplankton species (cf. comparison with *Daphnia*). Especially the genome size found for *Paraeuchaeta* is truly remarkable, although very large genome has also been found in some in amphipods and decapods (Hessen and Persson, 2009; Gregory, 2013). While many crustaceans, especially

cladocerans, have high levels of somatic endopolyploidy (Beaton and Hebert, 1989), this seems not to be the case for copepods.

4.4. Genome size of ectotherms at low temperature

Increased genome size and polyploidization are potential means of increasing cell size, and both seem somehow related to low temperatures, the evolutionary drivers may be widely different and occur at different time scales (cf. Hessen *et al.*, 2013). Elevated genome size estimates at low growth temperature was confirmed in *Daphnia* (papers I and II), but not in calanoid copepods (paper III), *Drosophila* (paper IV) and Arctic charr (paper V). The genome size estimates of calanoid copepods (paper III), *Drosophila* (paper IV) and Arctic charr (paper V) were relatively constant at all temperatures with coefficient of variation of DNA histogram 2C peak within the acceptable range (Marie and Brown, 1993; Baretton *et al.*, 1994; Vilhar *et al.*, 2001; Darzynkiewicz *et al.*, 2010). The elevated *Daphnia* genome size estimates came along with elevated coefficient of variation (CV > 6%) of DNA histogram 2C peak at low compared with high temperature (papers I and II). These genome size estimates were further shown to be due to change between DNA - flourochrome binding prosperities caused by elevated DNA condensation at low temperature (Fig. 10 and paper I). The DNA condensation status was also tested in *Drosophila* and copepods nuclei, and showed (similar to that genome size) no effect at all temperatures (data is not included in this study).

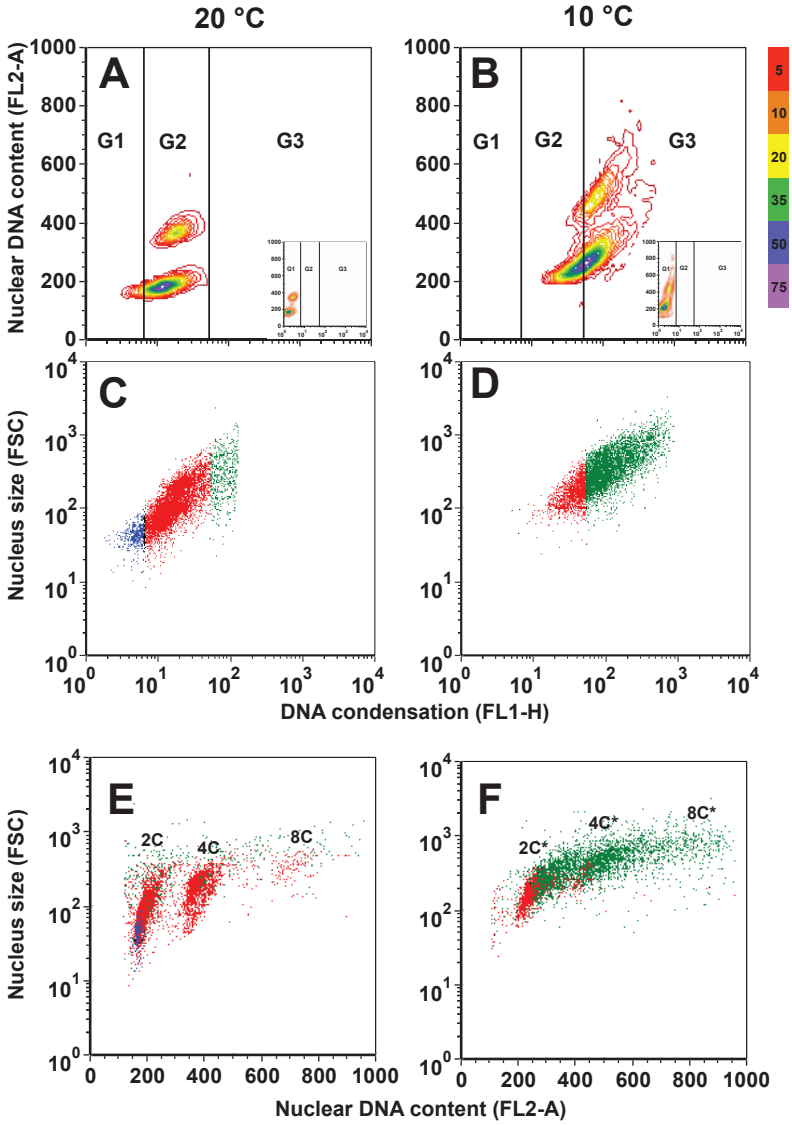

Figure 10. Nuclear DNA content versus DNA condensation for *D. magna*. FCM contour plot shows nuclear-ID green negative (no DNA condensation) nuclei as gate 1 (G1), as set by gating PI-stained nuclei of nuclear-ID green negative control sample (inset) of 20 °C (A) and 10 °C (B). Gate 2 (G2) is positive-stained nuclear-ID green nuclei and was set by gating nuclear-ID green positive 20 °C nuclei, and gate 3 (G3) thus represent high DNA condensation. The G1–G3 populations were further displayed in nucleus size versus DNA condensation (C & D) and nucleus size versus nuclear DNA content (E & F) colour dot plot as blue = G1 nuclei; red = G2 nuclei; green = G3 nuclei. Nuclei of 20 °C (C) and 10 °C (D) with high DNA condensation (green) were larger in size compared with nuclei of low DNA condensation (red). G1 nuclei were identified as part of 2C population at (E) 20 °C and were smaller in size compared with nuclei with condensed DNA (red and green; E). 2C–8C at 10 °C are labelled with asterisks, indicating artefact values. (Paper I)

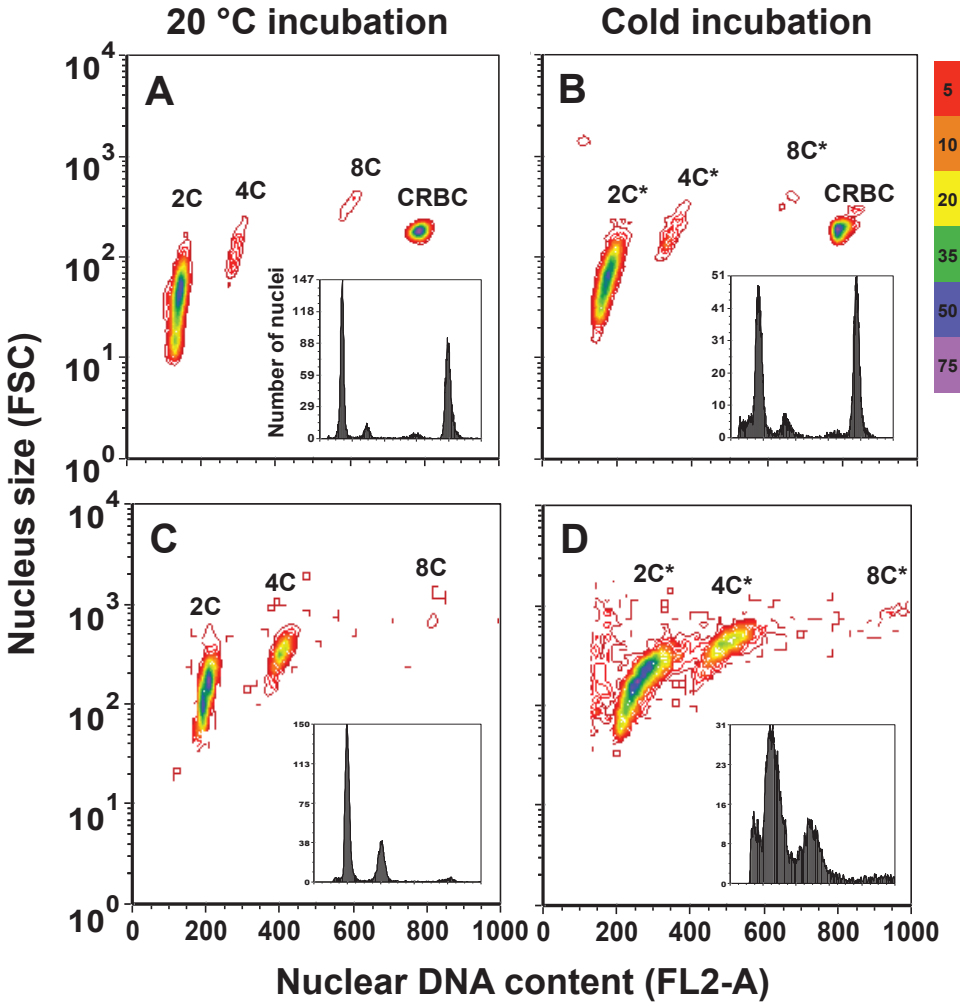

Figure 11. The "reversal incubation" experiment; nucleus size versus DNA presented as FCM contour plot of PI stained *D. pulex* (A & B) and *D. magna* (C & D) nuclei from 10 °C growth were incubated at 20 °C or cold prior FCM analysis. DNA content as measured from histogram (insets) show narrower peaks of 20 °C compared with cold incubation. 2C DNA content / CV values decreased from 0.56 ± 0.00 pg / 8.90 ± 0.06 % to 0.44 ± 0.01 pg / 5.90 ± 0.06% for *D. pulex*, and from 0.81 ± 0.03 pg / 9.06 ± 0.40 % to 0.66 ± 0.00 pg / 4.73 ± 0.06 % for *D. magna* at 20 °C incubation, respectively. The size of nuclei decreased by 11.40 ± 0.62 % for *D. pulex* and 8.80 ± 1.79 % for *D. magna* when incubated at 20 °C. The density (%) of nuclei per ploidy level was digitized in colour codes (from very to less dense: purple, blue, green, yellow, orange, and red). 2C – 8C at 10 °C are labelled with asterisk indicating artefact values. (Paper I)

Furthermore, “the temperature reversal experiment” (Fig. 11) revealed that the elevated *Daphnia* genome size estimations at low temperature was not linked to increased number of nucleotides, but is rather due to artefact genome size estimations (paper I). However, the genome size estimations of *Daphnia* (all clones) were nearly consistent with previous published values (Korpelainen *et al.*, 1997; Vergilino *et al.*, 2009; Gregory, 2013), thus minor differences could be ascribed as clonal and methodical (papers I and II).

Drosophila genome size estimation correspond well to those previously estimated by FCM analysis (Bennett *et al.*, 2003), but somehow higher than estimated from sequencing (Adams *et al.*, 2000) (paper IV). Furthermore, FCM results of fly DNA also showed somatic polyploidy up to 8C at all temperatures, which has been previously observed in *Drosophila* cell line cultures (Mosna and Dolfini, 1972). These 8C cells were not observed during DNA analysis of S2 cells from all treatments and growth temperatures. Similar to fly DNA, the S2 cells did not show low temperature response on genome level at all treatment (paper IV). However, the genome size of S2 cells was estimated to be twice as large as fly DNA at all temperatures and cellular treatments. This genome doubling was further shown to be approximately similar to (Oregon-R) embryo genome size estimates, which confirmed that the genome size difference between flies and S2 cells is due to different stage of development (paper IV). With this we would like to remark that it currently does not exist any published data on genome size of S2 cells, so our genome size estimates are genuine (paper IV).

The genome size estimates for all Arctic charr populations under all treatments was somewhat lower than previously reported for Arctic charr (Hartley, 1990; Hardie and Hebert, 2003; 2004), which may reflect methodological disparities since the former studies were all based on Feulgen staining and densiometry (paper V).

4.5. Dietary P also effects genome and cell size of *Daphnia*

The multigenerational study of *Daphnia* suggests responses in genomic size, nucleus size and distribution of 2C, 4C and 8C cell populations on temperature and P diet (paper II). The differing responses between the two species may reflect their different demands for P, where *D. magna* is far more susceptible to P limitation than *D. pulex* (Mcfeeters and Frost, 2011). Diet also seems to effect 2C CV estimates, as lower CV´s is observed for P deficient diet of *Daphnia*. The elevated CV and its bearings on DNA fluorescence and structure may hinge on different mechanisms for temperature and dietary P (paper II). In both cases DNA condensation could play a role. A higher degree of DNA condensation may require higher quantities of P since P-concentration has been found to affect the distribution of chromatin compaction within nucleosomes (Bazett-Jones and Ottensmeyer, 1981; Bazett-Jones *et al.*, 1999; Fussner *et al.*, 2011). Previous experiments found corresponding temperature effects within one single generation for both species (paper I), while this response was maintained in *D. magna* over the generations, it was not observed for *D. pulex.* Also the two species different in their dietary response, where only *D. magna* (with the larger genome) responded clearly to P limitation both in genome and nucleus (paper II). While this study point to an intriguing structural response at the subcellular level, with potential effects on cell size and even body size (paper I), the mechanistic behind these effects will have to await following-up studies and sequencing.

5. CONCLUSIONS AND FUTURE PERSPECTIVES

This thesis provides novel insights in to the link between genome, cell, and body size of popular ectotherms at different temperatures. Paper I highlights the importance of FCM as a fast and reliable method in measuring instant change in genomic, nuclear, and cellular level at low vs. high temperatures. This, I believe, is essential when studying fluctuating parameters such as temperature change in ectotherms, especially in light of increasing global warming. The fact that lower temperature may increase condensation of the DNA molecule in ectotherms is novel theory and should call for caution when estimating genome size from FCM, as well as from other methods. Of course, as for all methods, FCM has its advantages and disadvantages, but at least FCM is the fastest method in genome size estimation with minor modification of DNA helix, which make it easy to reveal "natural" modifications of the DNA helix. According to Davy and Kell (1996) there are three main advantages of FCM, which include multiparameter data acquisition and multivariate data analysis, high-speed analysis, and the ability to effect cell sorting. Furthermore, they mention that the only disadvantageous of FCM is mainly the highly cost of many of the various FCM machines and the need of skilled operator to run samples by the sophisticated FCM instruments (Davey and Kell, 1996).

Because *D. magna* showed highest DNA condensation response at low temperature compared with *D. pulex* (paper I), indicate that our method is important to evaluate the critical aspect in genome size analysis of different ectotherms. However, the term genome can refer to either all the nuclear DNA or to only some of it, and a completely sequenced genome is a relative concept (Bork and Copley, 2001). It can mean that every type of sequence in an organism has been sequenced, but it need not mean that all copies of all types have been sequenced, or that their copy numbers are all known. Without this information total genome size (C‑value) cannot be determined based on genome sequencing. There

fore, from my point of view, it is important to at least include FCM as method when estimating genome size in thermal studies, because that will strength the estimation by excluding artefact measurements (such as condensation) as shown it this study.

Another important aspect is the effect of DNA condensation on cell size as response to low temperature, should at least considered to be the major cause of enlargement of body size in ectotherms (paper I). In fact, increased polyploidy have been shown to cause severe damage to organisms, and is also linked to cancer (by production of aneuploidy cells). A review by Comai (2005) indicate that becoming polyploid has several disadvantages compared to only three advantages. The main disadvantages of polyploidy include the disrupting effects of nuclear and cell enlargement, the propensity of polyploid mitosis and meiosis to produce aneuploid cells, and the epigenetic instability that results in non-additive gene regulation (Comai, 2005). Our study shows that ectotherms (e.g. *Daphnia*) prefer to enlarge the largest cell pool (diploid cells) by DNA condensation, rather than polyploidization at low temperature. This is because some ectotherms (e.g. *Daphnia*) may find it easily to shift to more relaxed DNA structure when temperature rises in a sudden within a generation, as documented by "the temperature reversal experiment" (paper I). This, I think, is especially effective among *Daphnia* (*magna*), because of its actively swimming behaviour across different temperature gradients during life time (Gerritsen, 1982).

Further knowledge about the DNA sequences responsible for the temperature dependent genome configuration can be identified by more nucleotide selective experiments such as sequencing. Also culturing of cells with microscopically analysis might give more information about the kind of cell size enlargement at different temperatures, for instance; is it cell expansion or cell deformation? Is the condensation local, scattered as foci, or is it aggregated in the cell? Which chromosome is condensed at low temperature? Is the condensation epigenetically related? How to place this condensation in evolutionary

perspective? Why highly condensation (or FCM CV estimation) of DNA in *Daphnia* compared to other ectotherms at low temperature?

Final conclusions on the temperature effects on genome size, and not the least the corresponding effects on cell and body sizes, are premature. If one can demonstrate that natural selection favours a reaction norm in which organisms at low temperatures delay maturity until reaching a relatively large body size, then a single explanation might account for much of the variation in body size observed in laboratories and natural populations. However, the TSR is still one of the most taxonomically widespread "rules" in biology. As with all biological "rules," clear exceptions to Bergmann's rule and the TSR exist. Still, biologists have had more difficulty finding plausible explanations for these rules than they have had finding causes for exceptions.

6. REFERENCES

Adams, M. D., Celniker, S. E., Holt, R. A., Evans, C. A., Gocayne, J. D., Amanatides, P. G., Scherer, S. E., Li, P. W., Hoskins, R. A. & Galle, R. F. (2000). The genome sequence of *Drosophila melanogaster*. *Science* **287**, 2185-2195.

Alpatov, W. (1930). Phenotypical variation in body and cell size of *Drosophila* melanogaster. *The Biological Bulletin* **58**, 85-103.

Amundsen, P.-A. & Knudsen, R. (2009). Winter ecology of Arctic charr (*Salvelinus alpinus*) and brown trout (*Salmo trutta*) in a subarctic lake, Norway. *Aquatic Ecology* **43**, 765-775.

Angilletta Jr, M. J. & Dunham, A. E. (2003). The temperature-size rule in ectotherms: simple evolutionary explanations may not be general. *The American Naturalist* **162**, 332-342.

Angilletta, M. J., Steury, T. D. & Sears, M. W. (2004). Temperature, growth rate, and body size in ectotherms: fitting pieces of a life-history puzzle. *Integrative and Comparative Biology* **44**, 498-509.

Arendt, J. (2007). Ecological correlates of body size in relation to cell size and cell number: patterns in flies, fish, fruits and foliage. *Biological Reviews* **82**, 241-256.

Ashburner, M. & Thompson Jr, J. (1978). The laboratory culture of *Drosophila*. *The genetics and biology of Drosophila* **2**, 1-109.

Ashton, K. G. (2001). Are ecological and evolutionary rules being dismissed prematurely? *Diversity and Distributions* **7**, 289-295.

Ashton, K. G. (2002a). Do amphibians follow Bergmann's rule? *canadian Journal of Zoology* **80**, 708-716.

Ashton, K. G. (2002b). Patterns of within-species body size variation of birds: strong evidence for Bergmann's rule. *Global Ecology and Biogeography* **11**, 505-523.

Ashton, K. G. (2004). Sensitivity of intraspecific latitudinal clines of body size for tetrapods to sampling, latitude and body size. *Integrative and Comparative Biology* **44**, 403-412.

Ashton, K. G. & Feldman, C. R. (2003). Bergmann's rule in nonavian reptiles: turtles follow it, lizards and snakes reverse it. *Evolution* **57**, 1151-1163.

Atkinson, D. (1994). Temperature and organism size,a biological law for ectotherms? *Advances in ecological research* **25**, 1-58.

Atkinson, D. (1995). Effects of temperature on the size of aquatic ectotherms: exceptions to the general rule. *Journal of Thermal Biology* **20**, 61-74.

Atkinson, D., Johnston, I. & Bennett, A. (1996). Ectotherm life-history responses to developmental temperature. *Animals and temperature: phenotypic and evolutionary adaptation*, 183-204.

Atkinson, D., Morley, S. A. & Hughes, R. N. (2006). From cells to colonies: at what levels of body organization does the 'temperature-size rule' apply? *Evolution & development* **8**, 202-214.

Atkinson, D. & Sibly, R. (1996). On the solutions to a major life-history puzzle. *Oikos* **77**, 359-365.

Atkinson, D. & Sibly, R. M. (1997). Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. *Trends in ecology & evolution* **12**, 235-239.

Azevedo, R., French, V. & Partridge, L. (2002). Temperature modulates epidermal cell size in *Drosophila melanogaster*. *Journal of insect physiology* **48**, 231-237.

Baird, D. J., Barber, I., Bradley, M., Soares, A. M. V. M. & Calow, P. (1991). A comparative study of genotype sensitivity to acute toxic stress using clones of *Daphnia magna* straus. *Ecotoxicology and Environmental Safety* **21**, 257-265.

Balon, E. K. (1980). *Charrs. Salmonid fishes of the genus Salvelinus*: Dr. W. Junk bv Publishers.

Ban, S. (1994). Effect of temperature and food concentration on post-embryonic development, egg production and adult body size of calanoid copepod *Eurytemora affinis*. *Journal of plankton research* **16**, 721-735.

Baretton, G., Blasenbreu, S., Li, X., DePascale, T. & Löhrs, U. (1994). DNA cytometry using paraffin embedded tumor tissues. Comparison of flow cytometry (FCM) and image cytometry (ICM). *Zentralblatt für Pathologie* **139**, 419.

Bazett-Jones, D., Hendzel, M. & Kruhlak, M. (1999). Stoichiometric analysis of protein-and nucleic acid-based structures in the cell nucleus. *Micron* **30**, 151-157.

Bazett-Jones, D. & Ottensmeyer, F. (1981). Phosphorus distribution in the nucleosome. *Science* **211**, 169-170.

Beaton, M. J. & Hebert, P. D. N. (1989). Miniature genomes and endopolyploidy in cladoceran crustaceans. *Genome* **32**, 1048-1053.

Bennett, M. D. (1987). Variation in genomic form in plants and its ecological implications. *New phytologist* **106**, 177-200.

Bennett, M. D., Leitch, I. J., Price, H. J. & Johnston, J. S. (2003). Comparisons with *Caenorhabditis* (~100 Mb) and *Drosophila* (~ 175 Mb) using flow cytometry show genome size in *Arabidopsis* to be ~ 157 Mb and thus ~ 25% larger than the *Arabidopsis* genome initiative estimate of ~ 125 Mb. *Annals of Botany* **91**, 547-557.

Bergmann, C. (1848). *Über die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grösse*: Göttinger Studien.

Berrigan, D. & Charnov, E. L. (1994). Reaction norms for age and size at maturity in response to temperature: a puzzle for life historians. *Oikos*, 474-478.

Blanckenhorn, W. & Llaurens, V. (2005). Effects of temperature on cell size and number in the yellow dung fly *Scathophaga stercoraria*. *Journal of Thermal Biology* **30**, 213-219.

Blaxter, J. H., Douglas, B., Tyler, P. A. & Mauchline, J. (1998). *The Biology of Calanoid Copepods: The Biology of Calanoid Copepods*: Access Online via Elsevier.

Bork, P. & Copley, R. (2001). The draft sequences: filling in the gaps. *Nature* **409**, 818-820.

Bowman, T. E. & Abele, L. G. (1982). Classification of the recent Crustacea. *The biology of Crustacea* **1**, 1-27.

Breteler, W., Fransz, H. & Gonzalez, S. (1982). Growth and development of four calanoid copepod species under experimental and natural conditions. *Netherlands Journal of Sea Research* **16**, 195-207.

Byström, P., Andersson, J., Kiessling, A. & Eriksson, L.-O. (2006). Size and temperature dependent foraging capacities and metabolism: consequences for winter starvation mortality in fish. *Oikos* **115**, 43-52.

Calboli, F. C., Gilchrist, G. W. & Partridge, L. (2003). Different cell size and cell number contribution in two newly established and one ancient body size cline of *Drosophila subobscura*. *Evolution* **57**, 566-573.

Cavalier-Smith, T. (1978). Nuclear volume control by nucleoskeletal DNA, selection for cell volume and cell growth rate, and the solution of the DNA C-value paradox. *Journal of Cell Science* **34**, 247-278.

Colbourne, J. K., Pfrender, M. E., Gilbert, D., Thomas, W. K., Tucker, A., Oakley, T. H., Tokishita, S., Aerts, A., Arnold, G. J. & Basu, M. K. (2011). The ecoresponsive genome of *Daphnia pulex*. *Science* **331**, 555-561.

Comai, L. (2005). The advantages and disadvantages of being polyploid. *Nature Reviews Genetics* **6**, 836-846.

Darzynkiewicz, Z., Halicka, H. D. & Zhao, H. (2010). Analysis of cellular DNA content by flow and laser scanning cytometry. *Polyploidization and Cancer*, 137-147.

Daufresne, M., Lengfellner, K. & Sommer, U. (2009). Global warming benefits the small in aquatic ecosystems. *Proceedings of the National Academy of Sciences* **106**, 12788-12793.

Davenport, J. (1992). *Animal life at low temperature*: Springer.

Davey, H. M. & Kell, D. B. (1996). Flow cytometry and cell sorting of heterogeneous microbial populations: the importance of single-cell analyses. *Microbiological reviews* **60**, 641-696.

Ebert, D. (1992). A food-independent maturation threshold and size at maturity in *Daphnia magna*. *Limnology and oceanography*, 878-881.

Eiane, K., Aksnes, D. L., Ohman, M. D., Wood, S. & Martinussen, M. B. (2002). Stage-specific mortality of Calanus spp. under different predation regimes. *Limnology and oceanography* **47**, 636-645.

Elser, J., Sterner, R., Gorokhova, E., Fagan, W., Markow, T., Cotner, J., Harrison, J., Hobbie, S., Odell, G. & Weider, L. (2000). Biological stoichiometry from genes to ecosystems. *Ecology Letters* **3**, 540-550.

Ferrari, J. A. & Rai, K. S. (1989). Phenotypic correlates of genome size variation in *Aedes albopictus*. *Evolution* **43**, 895-899.

Finston, T. L., Hebert, P. D. & Foottit, R. B. (1995). Genome size variation in aphids. *Insect Biochemistry and Molecular Biology* **25**, 189-196.

Flagg, R. O. (1988). *Carolina Drosophila Manual*: Carolina Biological Supply Company Burlington.

French, V., Feast, M. & Partridge, L. (1998). Body size and cell size in *Drosophila*: the developmental response to temperature. *Journal of insect physiology* **44**, 1081-1089.

Fussner, E., Djuric, U., Strauss, M., Hotta, A., Perez-Iratxeta, C., Lanner, F., Dilworth, F. J., Ellis, J. & Bazett-Jones, D. P. (2011). Constitutive heterochromatin reorganization during somatic cell reprogramming. *The EMBO journal* **30**, 1778-1789.

Gabrielsen, T. M., Merkel, B., Søreide, J., Johansson-Karlsson, E., Bailey, A., Vogedes, D., Nygård, H., Varpe, Ø. & Berge, J. (2012). Potential misidentifications of two climate indicator species of the marine arctic ecosystem: *Calanus glacialis* and *C. finmarchicus*. *Polar biology* **35**, 1621-1628.

Galbraith, D. W., Harkins, K. R., Maddox, J. M., Ayres, N. M., Sharma, D. P. & Firoozabady, E. (1983). Rapid flow cytometric analysis of the cell cycle in intact plant tissues. *Science (New York, NY)* **220**, 1049.

Galbraith, D. W., Lambert, G. M., Macas, J. & Dolezel, J. (2001). Analysis of nuclear DNA content and ploidy in higher plants. *Current protocols in cytometry*, 7.6. 1-7.6. 22.

Gerritsen, J. (1982). Behavioral response of *Daphnia* to rate of temperature change: Possible enhancement of vertical migration. *Limnology and oceanography* **27**, 254-261.

Ghalambor, C. K., McKay, J. K., Carroll, S. P. & Reznick, D. N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology* **21**, 394-407.

Gregory, T. (2001). Coincidence, coevolution, or causation? DNA content, cell size, and the C-value enigma. *Biological Reviews* **76**, 65-101.

Gregory, T. R. (2005). Genome size evolution in animals. *The evolution of the genome* **1**, 4-87.

Gregory, T. R. (2013). Animal genome size database. http://www.genomesize.com.

Gregory, T. R., Hebert, P. D. N. & Kolasa, J. (2000). Evolutionary implications of the relationship between genome size and body size in flatworms and copepods. *Heredity* **84**, 201-208.

Guderley, H. (2004). Locomotor performance and muscle metabolic capacities: impact of temperature and energetic status. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **139**, 371-382.

Hardie, D. C. & Hebert, P. D. N. (2003). The nucleotypic effects of cellular DNA content in cartilaginous and ray-finned fishes. *Genome* **46**, 683-706.

Hardie, D. C. & Hebert, P. D. N. (2004). Genome-size evolution in fishes. *Canadian Journal of Fisheries and Aquatic Sciences* **61**, 1636-1646.

Hartenstein, V. (1993). *Atlas of Drosophila development*. Plainview, N.Y. Cold Spring Harbor Laboratory Press.

Hartley, S. (1990). Variation in cellular DNA content in Arctic charr, *Salvelinus alpinus* (L.). *Journal of Fish Biology* **37**, 189-190.

Hessen, D. O., Daufresne, M. & Leinaas, H. P. (2013). Temperature size relations from the cellular genomic perspective. *Biological Reviews*.

Hessen, D. O., Færøvig, P. J. & Andersen, T. (2002). Light, nutrients, and P: C ratios in algae: grazer performance related to food quality and quantity. *Ecology* **83**, 1886-1898.

Hessen, D. O., Jeyasingh, P. D., Neiman, M. & Weider, L. J. (2010). Genome streamlining and the elemental costs of growth. *Trends in ecology & evolution* **25**, 75-80.

Hessen, D. O. & Persson, J. (2009). Genome size as a determinant of growth and life-history traits in crustaceans. *Biological Journal of the Linnean Society* **98**, 393-399.

Hessen, D. O., Ventura, M. & Elser, J. J. (2008). Do phosphorus requirements for RNA limit genome size in crustacean zooplankton? *Genome* **51**, 685-691.

James, A. C., Azevedo, R. & Partridge, L. (1995). Cellular basis and developmental timing in a size cline of *Drosophila melanogaster*. *Genetics* **140**, 659-666.

Jobling, M., Jørgensen, E. H., Arnesen, A. M. & Ringø, E. (1993). Feeding, growth and environmental requirements of Arctic charr: a review of aquaculture potential. *Aquaculture international* **1**, 20-46.

Johnston, L. A., Prober, D. A., Edgar, B. A., Eisenman, R. N. & Gallant, P. (1999). *Drosophila* myc regulates cellular growth during development. *Cell* **98**, 779-790.

Jonsson, B. & Jonsson, N. (2009). A review of the likely effects of climate change on anadromous Atlantic salmon *Salmo salar* and brown trout *Salmo trutta*, with particular reference to water temperature and flow. *Journal of Fish Biology* **75**, 2381-2447.

Kaartvedt, S. (2000). Life history of *Calanus finmarchicus* in the Norwegian Sea in relation to planktivorous fish. *ICES Journal of Marine Science: Journal du Conseil* **57**, 1819-1824.

Kammenga, J. E., Doroszuk, A., Riksen, J. A. G., Hazendonk, E., Spiridon, L., Petrescu, A. J., Tijsterman, M., Plasterk, R. H. A. & Bakker, J. (2007). A *Caenorhabditis elegans* wild type defies the temperature-size rule owing to a single nucleotide polymorphism in tra-3. *PLoS genetics* **3**, e34.

Kari, J. & Huey, R. B. (2000). Size and seasonal temperature in free-ranging *Drosophila subobscura*. *Journal of Thermal Biology* **25**, 267-272.

Kent, M., Chandler, R. & Wachtel, S. (1988). DNA analysis by flow cytometry. *Cytogenetic and Genome Research* **47**, 88-89.

Kilham, S. S., Kreeger, D. A., Lynn, S. G., Goulden, C. E. & Herrera, L. (1998). COMBO: a defined freshwater culture medium for algae and zooplankton. *Hydrobiologia* **377**, 147-159.

Klemetsen, A., Amundsen, P. A., Dempson, J., Jonsson, B., Jonsson, N., O'connell, M. & Mortensen, E. (2003). Atlantic salmon *Salmo salar* L., brown trout *Salmo trutta* L. and Arctic charr *Salvelinus alpinus* (L.): a review of aspects of their life histories. *Ecology of freshwater fish* **12**, 1-59.

Korpelainen, H., Ketola, M. & Hietala, J. (1997). Somatic polyploidy examined by flow cytometry in *Daphnia*. *Journal of plankton research* **19**, 2031-2040.

Kozłowski, J., Konarzewski, M. & Gawelczyk, A. (2003). Cell size as a link between noncoding DNA and metabolic rate scaling. *Proceedings of the National Academy of Sciences of the United States of America* **100**, 14080.

Lampert, W. & Kinne, O. (2011). *Daphnia: development of a model organism in ecology and evolution*: International Ecology Institute Oedendorf.

Lauzon, W., Sanchez Dardon, J., Cameron, D. W. & Badley, A. D. (2000). Flow cytometric measurement of telomere length. *Cytometry* **42**, 159-164.

Leitch, A. & Leitch, I. (2008). Genomic plasticity and the diversity of polyploid plants. *Science* **320**, 481-483.

Leitch, I. & Bennett, M. (2004). Genome downsizing in polyploid plants. *Biological Journal of the Linnean Society* **82**, 651-663.

Libertini, A., Sola, L., Rampin, M., Rossi, A. R., Iijima, K. & Ueda, T. (2008). Classical and molecular cytogenetic characterization of allochthonous European bitterling *Rhodeus amarus* (*Cyprinidae, Acheilognathinae*) from Northern Italy. *Genes & genetic systems* **83**, 417-422.

Marie, D. & Brown, S. C. (1993). A cytometric exercise in plant DNA histograms, with 2C values for 70 species. *Biology of the Cell* **78**, 41-51.

Mauchline, J. (1988). Taxonomic value of pore pattern in the integument of calanoid copepods (Crustacea). *Journal of Zoology* **214**, 697-749.

Mc Kee, D. & Ebert, D. (1996). The effect of temperature on maturation threshold body-length in *Daphnia magna*. *Oecologia* **108**, 627-630.

Mcfeeters, B. J. & Frost, P. C. (2011). Temperature and the effects of elemental food quality on *Daphnia*. *Freshwater Biology* **56**, 1447-1455.

McLaren, I., Sevigny, J. & Corkett, C. (1988). Body sizes, development rates, and genome sizes among Calanus species. *Hydrobiologia* **167**, 275-284.

Michaelson, M. J., Price, H. J., Ellison, J. R. & Johnston, J. S. (1991). Comparison of plant DNA contents determined by Feulgen microspectrophotometry and laser flow cytometry. *American journal of botany*, 183-188.

Mitchell, S. & Lampert, W. (2000). Temperature adaptation in a geographically widespread zooplankter, *Daphnia magna*. *Journal of Evolutionary Biology* **13**, 371-382.

Mosna, G. & Dolfini, S. (1972). Morphological and chromosomal characterization of three new continuous cell lines of *Drosophila melanogaster*. *Chromosoma* **38**, 1-9.

Mousseau, T. A. (1997). Ectotherms follow the converse to Bergmann's rule. *Evolution* **51**, 630-632.

Neiman, M., Kay, A. D. & Krist, A. C. (2012). Sensitivity to phosphorus limitation increases with ploidy level in a New Zealand snail. *Evolution*.

Nelder, J. A. & Wedderburn, R. W. (1972). Generalized linear models. *Journal of the Royal Statistical Society. Series A (General)*, 370-384.

Neufeld, T. P., de la Cruz, A. F. A., Johnston, L. A. & Edgar, B. A. (1998). Coordination of Growth and Cell Division in the *Drosophila* Wing. *Cell* **93**, 1183-1193.

Nunney, L. & Cheung, W. (1997). The effect of temperature on body size and fecundity in female *Drosophila melanogaster*: evidence for adaptive plasticity. *Evolution*, 1529-1535.

Ormerod, M. G. & Imrie, P. R. (1990). *Flow cytometry*: Springer.

Packard, G. C., Packard, M. J. & McDaniel, L. L. (2001). Seasonal change in the capacity for supercooling by neonatal painted turtles. *Journal of experimental biology* **204**, 1667-1672.

Pangle, K. L. & Peacor, S. D. (2010). Temperature gradients, not food resource gradients, affect growth rate of migrating *Daphnia mendotae* in Lake Michigan. *Journal of Great Lakes Research* **36**, 345-350.

Park, J. B. (2011). Protective effects of veskamide, enferamide, becatamide, and oretamide on H_2O_2 induced apoptosis of PC-12 cells. *Phytomedicine* **18**, 843-847.

Partridge, L., Barrie, B., Fowler, K. & French, V. (1994). Evolution and development of body size and cell size in *Drosophila melanogaster* in response to temperature. *Evolution*, 1269-1276.

Partridge, L. & French, V. (1996). Thermal evolution of ectotherm body size: why get big in the cold. *Animals and temperature: phenotypic and evolutionary adaptation*, 265-292.

Persson, J., Wojewodzic, M. W., Hessen, D. O. & Andersen, T. (2011). Increased risk of phosphorus limitation at higher temperatures for *Daphnia magna*. *Oecologia* **165**, 123-129.

Pétavy, G. (1994). in relation to growth temperature in *Drosophila melanogaster*: an isofemale lines analysis.

Potter, C. J., Huang, H. & Xu, T. (2001). *Drosophila Tsc1* Functions with *Tsc2* to Antagonize Insulin Signaling in Regulating Cell Growth, Cell Proliferation, and Organ Size. *Cell* **105**, 357-368.

Pulkkinen, K. (2007). Microparasite transmission to *Daphnia magna* decreases in the presence of conspecifics. *Oecologia* **154**, 45-53.

Rasch, E. M. & Wyngaard, G. A. (2006). Genome sizes of cyclopoid copepods (Crustacea): evidence of evolutionary constraint. *Biological Journal of the Linnean Society* **87**, 625-635.

Ray, C. (1960). The application of Bergmann's and Allen's rules to the poikilotherms. *Journal of Morphology* **106**, 85-108.

Rees, D. J., Belzile, C., Glémet, H. & Dufresne, F. (2008). Large genomes among caridean shrimp. *Genome* **51**, 159-163.

Rees, D. J. R. D. J., Dufresne, F. D. F., Glémet, H. G. H. & Belzile, C. B. C. (2007). Amphipod genome sizes: first estimates for Arctic species reveal genomic giants. *Genome* **50**, 151-158.

Reeve, M., Fowler, K. & Partridge, L. (2000). Increased body size confers greater fitness at lower experimental temperature in male *Drosophila melanogaster*. *Journal of Evolutionary Biology* **13**, 836-844.

Robertson, F. W. (1959). Studies in quantitative inheritance. XII. Cell size and number in relation to genetic and environmental variation of body size in *Drosophila*. *Genetics* **44**, 869.

Schneider, I. (1972). Cell lines derived from late embryonic stages of *Drosophila melanogaster*. *Journal of embryology and experimental morphology* **27**, 353-365.

Seebacher, F. (2009). Responses to temperature variation: integration of thermoregulation and metabolism in vertebrates. *Science Signalling* **212**, 2885.

Shapiro, H. M. (2003). *Practical flow cytometry*: Wiley-Liss.

Siikavuopio, S. I., Skybakmoen, S. & Sæther, B.-S. (2009). Comparative growth study of wild-and hatchery-produced Arctic charr (*Salvelinus alpinus* L.) in a coldwater recirculation system. *Aquacultural Engineering* **41**, 122-126.

Stelzer, C. (2002). Phenotypic plasticity of body size at different temperatures in a planktonic rotifer: mechanisms and adaptive significance. *Functional Ecology* **16**, 835-841.

Sterner, R. W. & Elser, J. J. (2002). *Ecological stoichiometry: the biology of elements from molecules to the biosphere*: Princeton University Press.

Svenning, M.-A., Klemetsen, A. & Olsen, T. (2007). Habitat and food choice of Arctic charr in Linnévatn on Spitsbergen, Svalbard: the first year-round investigation in a high Arctic lake. *Ecology of freshwater fish* **16**, 70-77.

Swat, W., Ignatowicz, L. & Kisielow, P. (1991). Detection of apoptosis of immature $CD4^+ 8^+$ thymocytes by flow cytometry. *Journal of immunological methods* **137**, 79-87.

Team, R. D. C. (2005). R: A language and environment for statistical computing. ISBN 3-900051-07-0. R Foundation for Statistical Computing. Vienna, Austria, 2013. url: http://www. R-project. org.

Timofeev, S. (2001). Bergmann's principle and deep-water gigantism in marine crustaceans. *Biology Bulletin* **28**, 646-650.

Ulrich, W. (1990). Aneuploid and polyploid cellular DNA heterogeneity in insect cell material of Diptera species analyzed by flow cytometry. *Zeitschrift für Naturforschung. Section C, Biosciences* **45**, 1026-1030.

Uye, S.-i. (1988). Temperature-dependent development and growth of *Calanus sinicus* (Copepoda: Calanoida) in the laboratory. In *Biology of Copepods*, pp. 285-293: Springer.

Van der Have, T. & De Jong, G. (1996). Adult size in ectotherms: temperature effects on growth and differentiation. *Journal of Theoretical Biology* **183**, 329-340.

Van Voorhies, W. A. (1996). Bergmann size clines: a simple explanation for their occurrence in ectotherms. *Evolution*, 1259-1264.

Vergilino, R., Belzile, C. & Dufresne, F. (2009). Genome size evolution and polyploidy in the *Daphnia pulex* complex (Cladocera: Daphniidae). *Biological Journal of the Linnean Society* **97**, 68-79.

Versaevel, M., Grevesse, T. & Gabriele, S. (2012). Spatial coordination between cell and nuclear shape within micropatterned endothelial cells. *Nature communications* **3**, 671.

Vilhar, B., Greilhuber, J., Koce, J. D., Temsch, E. M. & Dermastia, M. (2001). Plant genome size measurement with DNA image cytometry. *Annals of Botany* **87**, 719-728.

von Bertalanffy, L. (1960). Principles and theory of growth. *Fundamental aspects of normal and malignant growth* **493**.

Watt, C., Mitchell, S. & Salewski, V. (2010). Bergmann's rule; a concept cluster? *Oikos* **119**, 89-100.

Webster, C. D. & Lim, C. (2002). *Nutrient requirements and feeding of finfish for aquaculture [electronic resource]*: CAB International.

Weider, L. J., Glenn, K. L., Kyle, M. & Elser, J. J. (2004). Associations among ribosomal(r) DNA intergenic spacer length, growth rate, and C: N: P stoichiometry in the genus *Daphnia*. *Limnology and oceanography* **49**, 1417-1423.

Weinkove, D., Neufeld, T. P., Twardzik, T., Waterfield, M. D. & Leevers, S. J. (1999). Regulation of imaginal disc cell size, cell number and organ size by *Drosophila* class I(A) phosphoinositide 3-kinase and its adaptor. *Current biology* **9**, 1019-1029.

Wilson, E. O. (1999). *The diversity of life*: WW Norton & Company.

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