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Research article

Polyploidy confers better cold tolerance in *Daphnia*

Ivan Pecl[1], Pierre U. Blier[1] and, France Dufresne[1]

[1]Département de Biologie, Université du Québec à Rimouski, Rimouski, QC, Canada

Correspondence: France Dufresne (france_dufresne@uqar.ca)

Oikos

2025: e10991

doi: 10.1111/oik.10991

Subject Editor: Dan Mayor

Editor-in-Chief: Pedro Peres-Neto

Accepted 30 October 2024

Despite decades of studies on the differential distribution of polyploid organisms, the causes of this pattern have yet to be elucidated. This study aimed to explore some of the possible physiological mechanisms explaining the differential northern distribution of polyploid clones of *Daphnia pulex* compared to the one of the diploid parental species. The critical thermal minimum (CTmin) was measured in 17 *D. pulex* clones of contrasted ploidy (diploid and triploid) and geographic origins (temperate and subarctic climates) reared under low and high temperatures (16 and 24°C). Triploid clones had better cold tolerance (lower CTmin) than both sympatric and temperate diploid clones. No significant association was found between CTmin and body size nor with cell size. We suggest that triploids might express a cold shock resistant phenotype related to higher gene expression and/or fatty acid profiles. Cold tolerance can be viewed as one of the possible reasons for polyploid preponderance in subarctic climates.

Keywords: body size, cell size, CTmin, *Daphnia*, ploidy, temperature

Introduction

Polyploidy, the possession of more than two sets of chromosomes is considered a common mode of speciation with significant ecological and evolutionary consequences in plants and animals (Van de Peer et al. 2017). This process has been pivotal in shaping the evolutionary trajectories of plant and animal taxa as it is associated with increased genetic diversity, ecological adaptation and biological complexity (Van de Peer et al. 2017). Despite the apparent success of well-established polyploids, nascent ones face many challenges including minority cytotype exclusion, mitotic and meiotic abnormalities, and genomic instability (Comai 2005, Madlung et al. 2005, Morgan et al. 2020). To overcome these problems, polyploid species often colonize areas unavailable to parental species. Polyploids are frequently found in habitats different from those of their diploid progenitors, indicating that their evolutionary success could be tied to their occupation of new ecological niches (Hegarty and Hiscock 2008, Edgeloe et al. 2022).

In species where diploid and polyploid individuals co-exist, differential distribution patterns can often be observed with polyploids being found in environments that are considered as more extreme (higher altitudes or latitudes, colder and drier environments)

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than that of their diploid counterparts (Ehrendorfer 1979, Lorch et al. 2016, Van de Peer et al, 2017, Rice et al. 2019, Mata et al. 2023). In plants, polyploids are often invasive species that dominate fluctuating and/or marginal environments (Te Beest et al. 2012). A clear gradient of polyploidy with latitude has been found in insects, amphibians and ray-finned fish, with the frequency of polyploids increasing with distance from the equator (David 2022). Within the Arctic, the frequency of higher order plant polyploids increases with latitude (Brochmann et al. 2004). It is not clear if polyploids have higher fitness at higher latitudes or if conditions conducive to their origins such as higher rates of unreduced gametes and/or hybridization potentials are prevalent in these (Dufresne and Hebert 1997, David 2022). Furthermore, as polyploidy is often accompanied by asexual reproduction, it is often difficult to disentangle the role of each factor in shaping distribution patterns (Tilquin and Kokko 2016).

Genome duplications have major consequences on cell size, cell physiology, whole-organism phenotype, life history traits and ecology (Comai 2005, Doyle and Coate 2020). The sheer amount of DNA in a genome can affect organismal phenotype through its nucleotypic effects (i.e. effects on cells that are due to the sole amount of DNA). Increases in bulk DNA amount bring increased nuclei and cell size with concomitant changes in cell surface area to volume ratios (Gregory 2001, Glazier 2022). Larger cells exhibit reduced resource supply and demand due to reduced surface area per volume. Positive relationships between body size and genome size have been reported in numerous invertebrates (Hessen and Persson 2009, Beaudreau et al. 2021). Ploidy-induced increase in cell surface area in carps leads to changes in metabolic scaling (Zhu et al. 2021). Some studies have shown that under cold conditions, animal polyploids have faster development rates (Dufresne and Hebert 1998, Hermaniuk et al. 2016) or higher metabolic rates (Hermaniuk et al. 2021) and suggest that this cold advantage could be mediated by cell size. Cold stress induces cell membrane depolarization, impairment of energy metabolism, disruption in ion homeostasis and water balance (Hayward et al. 2014, Overgaard et al. 2021, Reid et al. 2022). The larger cells of polyploid might reduce energetic costs of membrane polarization at low temperatures and confer a better cold tolerance to polyploids. Considering the observed distributional patterns of polyploids and physiological changes linked to genome duplication, it is of interest to study whether the distribution of ploidy is linked to ploidy-induced physiological changes. The observation that polyploid organisms and/or organisms with bigger genomes and cell sizes are more common at higher latitudes and altitudes then sparked the question if polyploids are more tolerant to cold temperatures (Riseth et al. 2020).

North American *Daphnia pulex* complex displays a clear pattern of latitudinal distribution of ploidy, with diploid hybrids between *D. pulex* and *Daphnia pulicaria* found in temperate and subarctic regions while triploid hybrids between the same species are found in subarctic and arctic regions only (Beaton and Hebert 1988, Dufresne and Hebert 1994). Bernier (2020) explored the thermal tolerance hypothesis, which proposes that polyploid distribution is partly related to differences in heat tolerance. The study showed that diploids have a higher heat tolerance than triploids, likely allowing them to live in warmer environments. Bernier (2020) found a negative relationship between heat tolerance and cell size suggesting that the higher heat tolerance of diploids might be linked to their smaller cell size (higher surface to volume ratio) which could alleviate oxygen limitation at elevated temperatures. On the other hand, the presence of triploids in colder environments might be linked with a better tolerance to cold, either through nucleotypic effects, or heterosis, or both. The merging of divergent gene combinations has been considered as key in promoting rapid adaptation to novel environments, conferring allopolyploids with advantages in times of climatic instability (Van de Peer et al. 2021). Clones that endure colder temperatures could sustain normal physiological functions and start their reproductive cycle earlier during spring giving them an advantage over less tolerant clones.

This study explores how cold tolerance of *Daphnia* clones may be linked to thermal niche, ploidy and its phenotypic correlates, and how plastic responses to acclimation temperature influence cold tolerance. Specifically, we measured acute cold tolerance of North American *D. pulex* clones differing in ploidy and geographic origin acclimated to 16 and 24°C. Chill coma (sometime defined as CTmin) is commonly measured in insects and has been defined as the temperature at which the insects lose the ability to walk, i.e. loss of coordination and is a proxy of cold tolerance (for a review of the concepts of chill coma and CTmin, see Hazell and Bale 2011). To our knowledge, no data on CTmin has been recorded in *Daphnia*. We here propose a new protocol to measure acute cold tolerance in *Daphnia* and small aquatic organisms.

We hypothesized that clonal and ploidy related distribution patterns as well as ability to acclimate to different temperatures are linked to differences in cold tolerance. We predict that polyploids and cold acclimated individuals will have better tolerance to cold shock.

Material and methods

Daphnia rearing

Several diploid and triploid clones of *Daphnia pulex* from temperate and subarctic North American regions were reared for at least three generations in standardized conditions before the experiments to eliminate potential maternal effects (six subarctic triploid clones, four subarctic diploid clones, eight temperate diploid clones). The ploidy of the clones has been determined by flow cytometry in previous studies (Vergilino et al. 2009).

Daphnia were reared in two environmental test chambers at 16 and 24°C, on a 12:12 h photoperiod and a light intensity of 3000 lux. *Daphnia* were raised in glass jars containing 300 ml of FLAMES culture medium (Celis-Salgado et al. 2008). Each clone was raised in triplicates

(three jars per clone per temperature). Animals were fed 2.0 × 10^5 cells ml^{-1} of *Raphidocelis subcapitata* (formerly known as *Pseudokirchneriella subcapitata* or *Selenastrum capricornotum*) three times a week. Populations in each jar were controlled to keep between 10 and 30 individuals per jar. One third of the medium was renewed every two weeks.

Cold tolerance test

Cold tolerance was tested by performing a critical thermal minimum (CTmin) challenge. CTmin is here defined as the lower temperature that prevents locomotion, resulting in the inability of the animal to escape danger or move to a place where conditions are viable. CTmin tests were performed on randomly selected female *Daphnia* of different reproductive stages. Selection of individuals was made to ensure that the whole size range was represented in the test sample for each clone. The clones used, their origin, ploidy, and sample size for each rearing temperature are summarized in Table 1 and a description of the experimental design is represented on Fig. 1. A total of 543 daphnids (268 acclimated at 16°C and 275 at 24°C respectively) from 18 clones were tested for cold tolerance. A minimum of 15 individuals per clone were used for the assays. Prior to the experiment, *Daphnia* reared at 24°C were placed for 14 h at 16°C to partially acclimate them at the temperature of cold-acclimated *Daphnia*. CTmin were performed using a cooling bath circulator filled with a mix of ethylene glycol – distilled water (30/70, v/v). *Daphnia* were individually placed inside 650 µl tubes containing 300 µl of a solution of 0.5 mg ml^{-1} bovine serum albumin in FLAMES culture medium. Tubes were randomly selected so that *Daphnia* from the same jar would not all be placed on the same row of tubes. The bovine serum albumin increased the surface tension of the solution and kept individual *Daphnia* out of the surface. A thermocouple sensor was placed in one tube to evaluate the temperature inside the tubes. Tubes were placed in the cooling bath at 16°C for 10 min to let the individuals acclimate to test conditions. Cooling bath temperature was then manually lowered using the following temperature ramp: between 16 and 10°C: direct decrease (−0.43°C min^{-1}), between 10 and 5°C: −0.15°C min^{-1}, between 5 and −5°C: −0.13°C min^{-1}. Because of the supercooling process, the medium stayed in a liquid state below 0°C, allowing the *Daphnia* to continue moving. A minority of tubes (approximately 5%) spontaneously froze during the experiments and were not accounted in further statistical analyses. One CTmin experiment lasted approximately 2 h.

Daphnia were filmed using a Panasonic Lumix DC-G9 camera, equipped with a Panasonic Leica DG Macro Elmarit 45 mm F2.8 ASPH MEGA O.I.S lens. Videos were shot at 30 frames per second and 1920 × 1080 pixels quality. A second camera was used to record the screen of the thermocouple. Lighting was kept constant throughout all tests. Videos of *Daphnia* and thermocouple screen were synchronized in post processing using DaVinci Resolve 17 (Blackmagic Design) before visual analysis. The temperature at which individuals became immobilized for over 60 s was recorded as CTmin.

Body size and cell area

Body size and cell area measures were carried out right after CTmin test (Fig. 2). Most individuals were alive during the measurements because the chill coma is a reversible state. ToupView software was used for all microscopic measures (ToupTek Photonics Co.). Measures were done with a microscope (Leica DMLB) equipped with a digital camera (model OMAX A35100U3, Omax Microscope) at 2.5 × 10 and 10 × 10 magnification respectively. Body size was measured as the distance between the anterior border of the eye and the tip of the shell spine. Cell size was measured on the carapace at 100× magnification. Cell imprints are easily visualized in this tissue and correspond to well-defined

Table 1. Clones, ploidy, climate, origin and sample size for each temperature for each *Daphnia pulex* clone used for cold tolerance experiments.

Clone	Ploidy	Climate	Location	Latitude	Longitude	n 16°C	n 24°C
A07	Triploid	Subarctic	Churchill, MB, Canada	58°46'03.504″	93°58'30.72″	16	15
C102			Churchill, MB, Canada	58° 46'03.504″	93°58'30.72″	15	17
IQ12			Kuujjuarapik, NU, Canada	55°16'36.012″	77°46'35.0394″	16	3
R202			Kuujjuarapik, NU, Canada	55°16'36.012″	77°46'35.0394″	14	19
SAS-16-17			Kuujjuarapik, NU, Canada	55°16'36.012″	77°46'35.0394″	15	15
C144			Churchill, MB, Canada	58°46'03.504″	93°58'30.72″	15	15
C44	Diploid	Subarctic	Churchill, MB, Canada	58°46'03.504″	93°58' 30.72″	14	15
R206			Kuujjuarapik, NU, Canada	55°16'036.012″	77°46'35.0394″	22	17
R210			Kuujjuarapik, NU, Canada	55°16' 36.012″	77°46'35.0394″	15	14
WP2			Kuujjuarapik, NU, Canada	55°16'36.012	77°46'35.0394″	13	15
Stukely	Diploid	Temperate	Cantons-de-l'Est, QC, Canada	45°17'12.7674	72°25'12.504″	14	19
BUS15			Urbana, IL, USA	40°07'47.604	88°12'23.4″	16	15
Deeplake			MI, USA	42°37'05.4114	85°27'32.4″	15	17
KAP-53			Danville, IL, USA	40°10'58.008	87°38'46.3194″	14	16
Longlake			MI, USA	42°33'05.9034	85°22'40.0794″	13	17
NFL68			Webster, IN, USA	39°54'12.6354	84°56'29.4″	16	16
SPS100			Homer, IL, USA	40°45'14.184	73°58'55.56″	15	15
Ste-Luce			Sainte-Luce, QC, Canada	48°32'51.252	68°23'38.4″	10	15

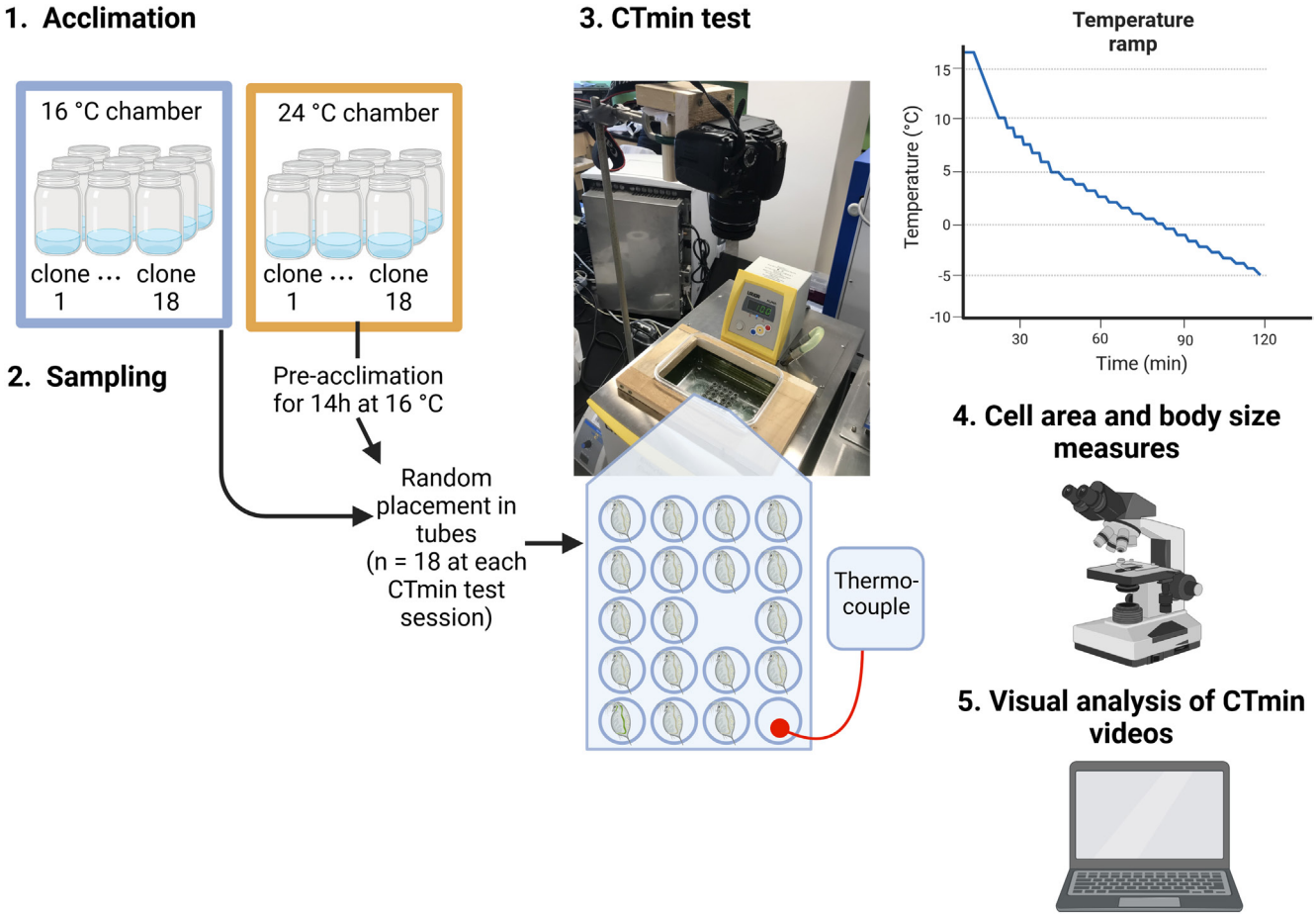

Figure 1. Description of the experimental design carried out for CTmin experiments. Acclimation: 18 clones per chamber, 3 jars per clone. Start of experiments after three generations in the same conditions. Sampling: all development stages (males and ephippia-bearing females excluded), whole body size range. CTmin test: repeated more than 30 times until desired sample size reached.

cells. To measure cell area, the average of ten cells was calculated for each individual. Cells located on the posterior and anterior ventral side of the individual were measured. When they were not visible, cells from the center of the carapace were measured.

Statistical analyses

To find out which variables have a significant effect on cold tolerance, body size or cell area, linear mixed models were used (R package 'lme4', Bates et al. 2015) and 'lmerTest', Kuznetsova et al. 2017). Our experimental design was hierarchical, with each clone being replicated in three jars at each temperature and several individuals were sampled per jar. Clones and jar were computed as random factors. The same random effect structure was used for all models. Cell area and body size were standardized (mean = 0, SD = 1) to allow for a better interpretation of parameter estimates (see Schielzeth 2010 for more details). The assumptions of homoscedasticity, independence and normality of residuals were validated graphically. Models used can be found in the Supporting information. All statistical analyses were performed using R ver 4.2.2 (www.r-project.org).

Results

There was no significant interaction between ploidy and acclimation temperature on CTmin ($F_{1,86} = 2.32$; $p = 0.131$). Triploid *Daphnia* had significantly lower CTmin than diploid *Daphnia* ($F_{1,15.7} = 7.11$; $p = 0.017$; $\beta = -0.68$°C 95% CI [−1.11, −0.25]) (Fig. 3A). Warm acclimation significantly increased CTmin ($F_{1,86.2} = 49.46$; $p < 0.001$; $\beta = 0.66$°C [0.40, 0.93]) (Fig. 3B). Linear mixed models examining the effects of geographic origin of clones (temperate and subarctic areas) and acclimation temperature revealed no significant interaction between the two factors ($F_{2,86.5} = 1.82$; $p = 0.168$) and a close to significant effect of geographic origin on CTmin ($F_{2,16.4} = 3.33$; $p = 0.061$). When the effects were considered separately for the two acclimation temperatures, a highly significant effect of geographic origin on CTmin was detected at 16°C ($F_2 = 15.69$, $p < 0.001$) but not at 24°C ($F_2 = 2.23$, $p = 0.109$). Multiple comparisons tests at 16°C using emmeans indicated that triploids from the subarctic had significantly lower CTmin than both subartic and temperate diploids (Fig. 4, Supporting information).

We found no evidence that the effect of body size on CTmin varied according to temperature of acclimation

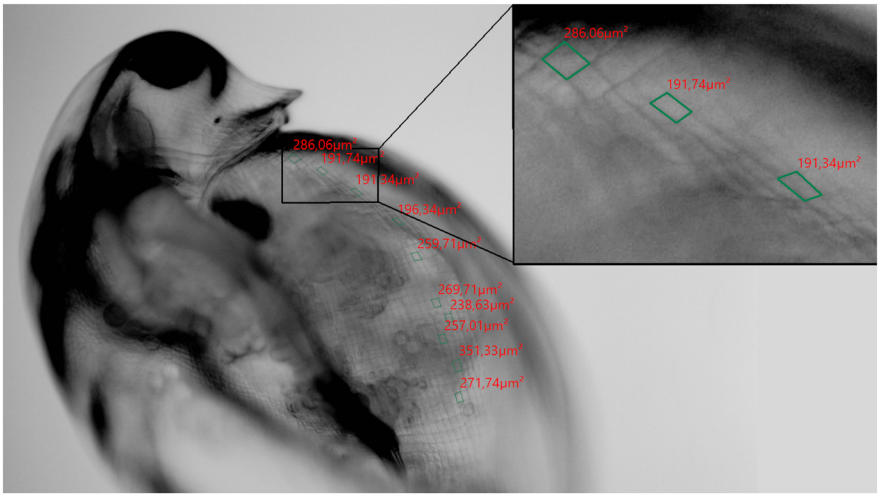

Figure 2. *Daphnia pulex* individual observed under 10 × 10 magnification. Cell areas are highlighted in red.

($F_{1,523}=0.02$; $p=0.873$). There was no significant relationship between body size and CTmin ($F_{1,525}=2.0$; $p=0.16$). There was no evidence that the effect of cell area on CTmin varied according to temperature of acclimation ($F_{1,500}=0.41$, $p=0.521$). There was no significant relationship between cell size and CTmin ($F_{1,519}=1.53$, $p=0.217$).

The effect of ploidy on body size did not differ significantly between rearing temperatures ($F_{1,83.4}=0.962$, $p=0.330$). Ploidy had a significant effect on body size ($F_{1,14.7}=6.270$, $p=0.025$) as well as rearing temperature ($F_{1,83.4}=7.895$, $p=0.006$). Body size was smaller in warm-acclimated individuals and triploids had a larger body size than diploids.

The effect of body size on cell area differed depending on rearing temperature ($F_{1,520}=9.1$; $p=0.003$; Table 2). Cell size did not change with temperature of acclimation in small individuals but was larger at 24°C than at 16°C in larger individuals (Fig. 5). The increase in cell size with increasing body size was significantly higher at 24°C than at 16°C in diploids ($t_{521}=-2.78$; $p=0.029$; Table 3). It was also higher at 24°C in triploids however the difference of slope estimates was not significant ($t_{515}=-1.75$; $p=0.300$; Table 3). Triploids had larger cells than diploids regardless of body size and rearing temperature (Table 2, Fig. 5).

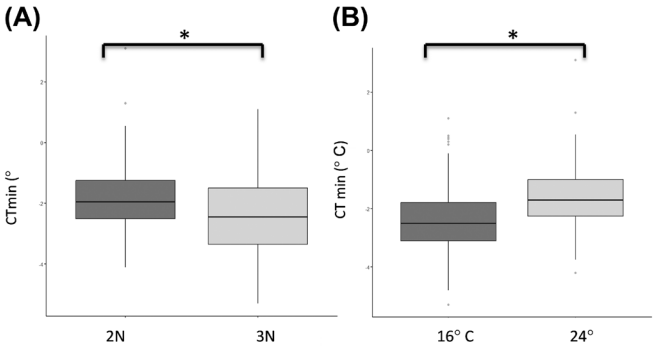

Figure 3. Effects of (A) ploidy and (B) temperature on CTmin. Means and their 95% CIs are shown. * indicates significant differences at the 0.05 level.

Discussion

This study aimed to examine the extent to which cold tolerance could explain the distribution patterns of clones of the North American *D. pulex* complex by comparing responses to cold shock and testing the hypothesis that polyploids have lower CTmin. We found that triploid *Daphnia* outperformed diploids at cold-shock tests. We discuss below the implication of these results for the distribution of polyploid organisms.

Our study is one of the first to examine CTmin in *Daphnia*. This measure is most often used to determine the lower temperature limits of insects. Our results showed that *Daphnia* acclimated to low temperatures had lower CTmin than those raised at higher temperatures. The increase in

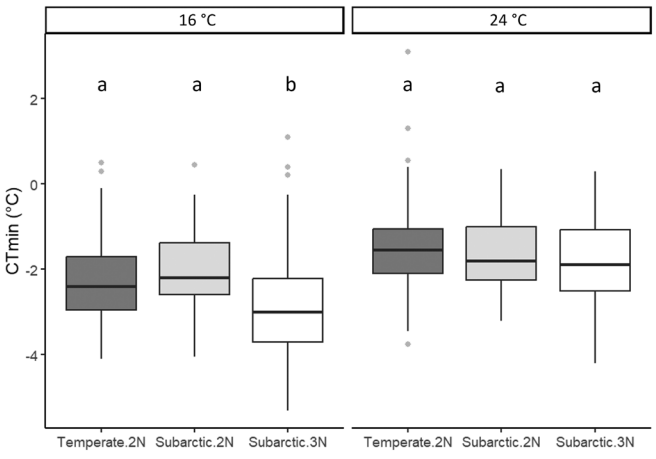

Figure 4. CTmin for *Daphnia* differing in climatic zone and ploidy levels and raised under two acclimation temperatures. Means and their 95% CIs are shown. Different letters indicate significant differences at the 0.05 level.

Table 2. Type III analysis of variance table for the linear mixed model explaining cellarea of *Daphnia pulex* as a function of rearing temperature and ploidy corrected for body size. The F-statistics are calculated based on Satterthwaite's degrees of freedom. Significant terms ($p < 0.05$) are shown in bold.

Model term	Num df	Den df	F-value	p-value
Body size (Tind)	1	522.75	223	< 0.001
Rearing temperature (Tacc)	1	82.97	15.3	< 0.001
Ploidy	1	15.58	29.9	< 0.001
Tind × Tacc	1	520.43	9.1	<0.003
Tind × Ploidy	1	522.75	0.5	0.477
Tacc × Ploidy	1	82.97	0.1	0.66
Tind × Tacc × Ploidy	1	520.43	0.009	0.926

cold tolerance after cold acclimation or rapid cold hardening is a widely observed mechanism in ectotherms (porcelain crab: Ronges et al. (2012); *Drosophila*: Colinet et al. (2012), Enriquez and Colinet (2019), Tarapacki et al. (2021)). Cold acclimation leads to a vast array of physiological changes such as the remodeling of membrane fatty acid composition, the production of cryoprotectants, the upregulation of various proteins or changes in the activities of metabolic enzymes (Ronges et al. 2012, Overgaard and MacMillan 2017, Enriquez and Colinet 2019). Our results thus indicate that our experimental protocol is sensitive enough to detect differences in cold tolerance induced by temperature acclimation in *Daphnia*. Contrary to aquatic ectotherms, terrestrial ectotherms are more likely to encounter sub-zero temperatures and supercooling of body liquids to cope with acute cold (Sinclair et al. 2015, Berman et al. 2021). In ectotherms, CTmin reflect minimum temperatures in both terrestrial and aquatic environments (Addo-Bediako et al. 2000, Sunday et al. 2011). High elevation tropical insects were found to be more resistant to cold than low elevation ones (Shah et al. 2017). Temperate insects did not show the same relationship with elevation and had near freezing tolerance (Shah et al. 2017). The authors reported that ice crystals would often terminate the experiments (Shah et al. 2017). Unexpectedly, we found that *Daphnia* could tolerate temperatures (−7°C) well below freezing. The ecological relevance of CTmin is not clear as *Daphnia* produce resting eggs when environmental conditions start to deteriorate (e.g. low food availability, temperature decrease). Winter survival has been reported in some lakes (Koch et al. 2009, Mariash et al. 2017), suggesting that the capacity to overwinter as adults may be advantageous in environments where the growing season is short. Mechanisms allowing *Daphnia* to resist such low temperatures should be examined.

We found that triploid *Daphnia* had lower CTmin than diploid clones, a trait that would be advantageous for them to thrive in subarctic and arctic environments. In addition, polyploid clones had lower CTmin than sympatric diploid clones, suggesting that some characteristics associated with polyploidy were important for cold adaptation. Polyploids had larger cells and body size than diploids but these two parameters were not significantly associated with an increased

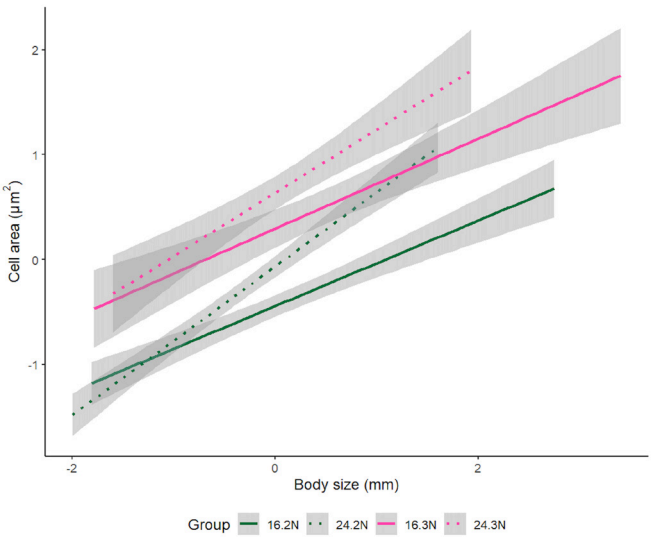

Figure 5. Relationship between cell area and body size at 16 and 24°C. . See Table 3 for intercept and slope estimates at each temperature and ploidy.

cold tolerance in our study. This suggests that nucleotypic effects alone may not be major determinants of higher cold tolerance and that other factors such as genetic ones may be at play. Positive effects of body size on cold tolerance have been found in bumblebees (Oyen et al. 2016, Gonzalez et al. 2022) but not in ants (Baudier and O'Donnell, 2018) or tropical gobies (Di Santo and Lobel 2017). Leiva et al. (2019) report that ectotherm species with larger cells and bodies performed better at cold tolerance tests. CTmin was especially enhanced in air-breathing species during long-term trials as opposed to short-term trials as is the case in our study. It would be interesting to revisit the effects of cell size on cold tolerance in *Daphnia* using a longer assay as well as using a higher number of individuals with the same body size ranges. In our study, we selected diploid and triploid individuals with a wide range in body sizes, which could have limited our ability to disentangle the effects of cell size from the effects of body size. Bigger cells could allow resisting longer periods of cold stress by reducing energetic costs and providing higher energetic reserves without any significant consequences in the ability to resist to cold shocks. Hence, the larger cells and body sizes of polyploids may be beneficial when competing with diploid clones in cold arctic environments with lower food resources.

A few studies pointed to an advantage of polyploidy at low temperatures in animals. Faster development rates have been reported for polyploids at lower temperature in *Daphnia* (Dufresne and Hebert 1998) and tadpoles (Hermaniuk et al. 2016), consistent with a higher metabolic rate in cold-acclimated polyploids. Warm acclimated triploid zebrafish had higher metabolic rates than diploids at acute cold temperature (Hermaniuk et al. 2021). They interpreted this higher metabolic rate as advantageous for maintaining performance of the animal at low temperatures and suggested that this improvement was linked to cell size. No advantage of triploidy

Table 3. Intercept and slope estimates for cell area as a function of body size for each group of ploidy and acclimation temperature. Comparison of slope estimates between temperatures for each ploidy.

Group	Intercept	95% CI	df	Slope	95% CI	df	t	df	p
2N 16°C	−0.489	[−0.670; −0.308]	34.1	0.464	[0.356; 0.573]	504	−2.78	521	0.029
2N 24°C	−0.106	[−0.288; 0.076]	35.2	0.692	[0.574; 0.811]	497			
3N 16°C	0.252	[−0.006; 0.511]	36.2	0.418	[0.279; 0.558]	507	−1.74	515	0.3
3N 24°C	0.557	[−0.006; 0.823]	36.3	0.633	[0.435; 0.831]	520			

was found in Atlantic salmon in terms of metabolic capacity or swimming performance at low temperatures (Riseth et al. 2020). In plants, few studies have experimentally compared resistance to cold stress in polyploids and results vary depending on the study system. Decanter et al. (2020) found that octoploid *Saxifraga* had higher frost tolerance than hexaploid *Saxifraga*. Freezing resistance in response to decreasing temperatures differed between ecotypes of tetraploid and diploid populations of *Arabidopsis* from the Tatra Mountains (Kapplenig et al. 2022). A recent study on kiwifuits revealed that leaf semi-lethal temperatures were lower in polyploid than in diploid kiwifruits (Yang et al. 2024). The authors hypothesized that the enhanced cold tolerance might be due to an increased expression of genes related to freeze tolerance and antioxidant enzymes and osmoregulatory substances that might reduce the freeze tolerance of cells and enable polyploid kiwis to inhabit higher altitudes than diploid species. Unlike animals, plants are stationary organisms that cannot escape away from the adverse environment. The fact that increased cold tolerance is often observed in these two groups suggest that polyploidy may be important to cope with environmental stress.

Which phenotypic differences led our triploid *Daphnia* to resist cold shock better than diploids? The combined effects of hybridity and genome doubling on growth vigor have been reported in many polyploid species (Te Beest et al. 2012). For example, heterosis conferred by epigenetic modifications of circadian clock has been shown to contribute to increased morphological vigor of allopolyploid plants (Chen 2010). Genome doubling increases the number of gene copies, which can lead to an increased expression of certain genes (Doyle and Coate 2019), some of which could be helpful to tolerate cold shock. Exposure to cold stress in diploid and autotetraploid individuals of the alpine *Ranunculus kuepferi* induced different gene expression profiles (Syngelaki et al. 2012). Diploids changed more gene set pathways than tetraploids and suppressed pathways involved in ion/cation homeostasis whereas tetraploids mostly activated gene set pathways related to cell wall and plasma membrane suggesting greater cold tolerance in the high altitude tetraploid cytotype (Syngelaki et al. 2012). In polyploid fish, the expression of some genes such as heat shock genes, that play an important role in the response to cold stress in ectotherms (Hayward et al, 2014, Štětina et al. 2015, Jiang et al. 2021, Reid et al. 2022). Because of the delay between stress and HSP expression and the rate of temperature decrease, it is uncertain whether *Daphnia* were able to overexpress HSPs during the trial. It is however possible that due to their higher gene copy number, triploids keep higher baseline levels of genes involved in cold shock response, such as HSPs.

Another physiological aspect potentially involved in adaptation and acclimation to cold temperatures concerns lipids and membrane fatty acids. Some cases of overwintering *Daphnia* have been observed in clones from subarctic and boreal lakes and these populations were characterized by high body fat content, high fatty acid concentrations and a higher retention of polyunsaturated fatty acids, mainly stearidonic acid (Mariash et al. 2017). According to this study, high lipid reserves constitute a source of energy to survive periods of low food availability and probably help to tolerate cold stress. Higher body fat accumulation in cold-acclimated triploids, which could be favored by their larger cells, could help them better tolerate cold stress. Fatty acid composition of cell membranes also has an impact on ectotherm cold tolerance through changes in membrane fluidity (Overgaard et al. 2005, Waagner et al. 2013, Hayward et al. 2014, Enriquez and Colinet 2019, Trenti et al. 2022) which is consistent with the homeoviscous adaptation hypothesis (Ernst et al. 2016). Comparisons of fatty acid profiles of cold-acclimated diploid and triploid *Daphnia* clones found that triploid *Daphnia* tended to accumulate the highest amount of eicosapentaenoic acid (EPA) in their tissues (Pecl 2023), suggesting it could partly explain the higher cold tolerance of triploid clones.

This study is the first to provide CTmin estimates in *Daphnia* clones differing in ploidy level and geographic origin. We show that triploids were more resistant to cold shock than diploids and suggests that this might explain their differential distribution pattern. A large number of polyploid species of insects, amphibians and ray-finned fish in areas are known to occupy areas that are colder and have greater annual temperature fluctuations than those occupied by their diploid counterparts (David 2022). The ability of polyploids to thrive in cold climates may contribute to their persistence and diversification in high-latitude or high-altitude ecosystems.

Acknowledgements – We thank Alain Caron for his help with statistical analyses.
Funding – This work was supported by NSERC Discovery Grants to FD (RGPIN-2018-06349) and PUB (RGPIN- 2019–05992).

Author contributions

Ivan Pecl: Conceptualization (equal); Formal analysis (lead); Investigation (equal); Methodology (lead); Writing – original draft (equal); Writing – review and editing (equal).

Pierre U. Blier: Methodology (equal); Resources (equal); Supervision (equal); Writing – review and editing (equal). **France Dufresne**: Conceptualization (equal); Data curation (equal); Formal analysis (equal); Funding acquisition (equal); Investigation (equal); Methodology (equal); Project administration (equal); Resources (equal); Supervision, Writing – original draft (supporting); Writing – review and editing (le).

Data availability statement

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.3tx95x6qp (Pecl et al. 2024).

Supporting information

The Supporting information associated with this article is available with the online version.

References

Addo-Bediako, A., Chown, S. L. and Gaston, K. J. 2000. Thermal tolerance, climatic variability and latitude. – Proc. R. Soc. B 267: 739–745.

Bates, D., Mächler, M., Bolker, B. and Walker, S. 2015. Fitting linear mixed-effects models using lme4. – J. Stat. Soft. 67: 1–48.

Baudier, K. and O'Donnell, S. 2018. Complex body size differences in thermal tolerance among army ant workers (*Eciton burchellii parvispinum*). – J. Therm. Biol. 78: 277–280.

Beaton, M. J. and Hebert, P. D. N. 1988. Geographical parthenogenesis and polyploidy in *Daphnia pulex*. – Am. Nat. 132: 837–845.

Beaudreau, N., Massamba-N'Siala, G., Belzile, C., Calosi, P. and Dufresne, F. 2021. Life-history traits display strong associations to genome size in annelids. – Hydrobiologia 848: 799–810.

Berman, D., Bulakhova, N., Korosov, A. and Ganyushina, N. 2021. Cold resistance and wintering of the common viper (*Vipera berus*, Reptilia, Viperidae) on Kizhi island, Karelia. – Biol. Bull. 48: 1022–1029.

Bernier, S. B. 2020. Polyploïdie et tolérance thermique chez les microcrustacés du genre *Daphnia*. Mémoire de maîtrise. – Univ. du Québec à Rimouski.

Brochmann, C., Brysting, A. K., Alsos, I. G., Borgen, L., Grundt, H. H., Scheen, A.-C. and Elven, R. 2004. Polyploidy in arctic plants. – Biol. J. Linn. Soc. 82: 521–536.

Celis-Salgado, M. P., Cairns, A., Kim, N. and Yan, N. D. 2008. The FLAMES medium: a new, soft-water culture and bioassay medium for *Cladocera*. – SIL Proc. 1922–2010 30: 265–271.

Chen, Z. J. 2010. Molecular mechanisms of polyploidy and hybrid vigor. – Trends Plant Sci. 15: 57–71.

Colinet, H., Larvor, V., Laparie, M. and Renault, D. 2012. Exploring the plastic response to cold acclimation through metabolomics. – Funct. Ecol. 26: 711–722.

Comai, L. 2005. The advantages and disadvantages of being polyploid. – Nat. Rev. Genet. 6: 836–846.

David, K. T. 2022. Global gradients in the distribution of animal polyploids. – Proc. Natl Acad. Sci. USA 119: e2214070119.

Decanter, L., Colling, G., Elvinger, N., Heimarsson, S. and Matthies, D. 2020. Ecological niche differences between two polyploid cytotypes of *Saxifraga rosacea*. – Am. J. Bot. 107: 423–435.

Di Santo, V. and Lobel, P. S. 2017. Body size and thermal tolerance in tropical gobies. – J. Exp. Mar. Biol. Ecol. 487: 11–17.

Doyle, J. J. and Coate, J. E. 2019. Polyploidy, the nucleotype, and novelty: the impact of genome doubling on the biology of the cell. – Int. J. Plant Sci. 180: 1–52.

Doyle, J. J. and Coate, J. E. 2020. Autopolyploidy: an epigenetic macromutation. – Appl. Plant Sci. 107: 1097–1100.

Dufresne, F. and Hebert, P. D. N. 1994. Hybridization and origins of polyploidy. – Proc. R. Soc. B 258: 141–146.

Dufresne, F. and Hebert, P. D. N. 1997. Pleistocene glaciations and polyphyletic origins of polyploidy in an arctic cladoceran. – Proc. R. Soc. B 264: 201–206.

Dufresne, F. and Hebert, P. D. N. 1998. Temperature-related differences in life-history characteristics between diploid and polyploid clones of the *Daphnia pulex* complex. – Ecoscience 5: 433–437.

Edgeloe, J. M., Severn-Ellis, A. A., Bayer, P. E., Mehravi, S., Breed, M. F., Krauss, J. S. L., Batley, G. A., Kendrick, E. and Sinclair, A. 2022. Extensive polyploid clonality was a successful strategy for seagrass to expand into a newly submerged environment. – Proc. R. Soc. B 289: 20220538.

Ehrendorfer, F. 1979. Polyploidy and distribution. – In: Lewis, W. H. (ed.), Basic life sciences. Springer, pp. 45–60.

Enriquez, T. and Colinet, H. 2019. Cold acclimation triggers lipidomic and metabolic adjustments in the spotted wing drosophila *Drosophila suzukii* (Matsumara). – Am. J. Physiol. 316: R751–R763.

Ernst, R., Ejsing, C. S. and Antonny, B. 2016. Homeoviscous adaptation and the regulation of membrane lipids. – J. Mol. Biol. 428: 4776–4791.

Glazier, D. S. 2022. How metabolic rate relates to cell size. – Biology 11: 1106.

Gonzalez, V. H., Oyen, K., Aguilar, M. L., Herrera, A., Martin, R. D. and Ospina, R. 2022. High thermal tolerance in high-elevation species and laboratory-reared colonies of tropical bumble bees. – Ecol. Evol. 12: e9560.

Gregory, T. R. 2001. The bigger the C-value, the larger the cell: genome size and red blood cell size in vertebrates. – Blood Cells Mol. Dis. 27: 830–843.

Hayward, S. A., Manso, B. and Cossins, A. R. 2014. Molecular basis of chill resistance adaptations in poikilothermic animals. – J. Exp. Biol. 217: 6–15.

Hazell, S. P. and Bale, J. S. 2011. Low temperature thresholds: are chill coma and CTmin synonymous? – J. Insect Physiol. 57: 1085–1089.

Hegarty, M. J. and Hiscock, S. J. 2008. Genomic clues to the evolutionary success of polyploid plants. – Curr. Biol. 18: R435–R444.

Hermaniuk, A., Rybacki, M. and Taylor, J. R. E. 2016. Low temperature and polyploidy result in larger cell and body size in an ectothermic vertebrate. – Physiol. Biochem. Zool. 89: 118–129.

Hermaniuk, A., van de Pol, I. L. E. and Verberk, W. C. E. P. 2021. Are acute and acclimated thermal effects on metabolic rate modulated by cell size? A comparison between diploid and triploid zebrafish larvae. – J. Exp. Biol. 224: 10.

Hessen, D. O. and Persson, J. 2009. Genome size as a determinant of growth and life-history traits in crustaceans. – Biol. J. Linn. Soc. 98: 393–399.

Jiang, F., Chang, G., Li, Z., Abouzaid, M., Du, X., Hull, J. J., Ma, W. and Lin, Y. 2021. The HSP/co-chaperone network in environmental cold adaptation of *Chilo suppressalis*. – Int. J. Biol. Macromol. 187: 780–788.

Kapplenig, D., Bertel, C., Arc, E., Villscheider, R., Ralser, M., Kol, F., Wos, G., Hülbers, K., Kranner, I. and Neuner, G. 2022. Repeated colonization of alpine habitats by *Arabidopsis arenosa* viewed through freezing resistance and ice management strategies. – Plant Biol. 24: 939–949.
Koch, U., Von Elert, E. and Straile, D. 2009. Food quality triggers the reproductive mode in the cyclical parthenogen *Daphnia* (Cladocera). – Oecologia 159: 317–324.
Kuznetsova, A., Brockhoff, P. B. and Christensen, R. H. 2017. lmerTest package: tests in linear mixed effects models. – J. Stat. Soft. 82: 1–26.
Leiva, F. P., Calosi, P. and Verberk, W. C. E. P. 2019. Scaling of thermal tolerance with body mass and genome size in ectotherms: a comparison between water-and air-breathers. – Philos. Trans. R. Soc. B 374: 14–14.
Lorch, S., Zeuss, D., Brandl, R. and Brändle, M. 2016. Chromosome numbers in three species groups of freshwater flatworms increase with increasing latitude. – Ecol. Evol. 6: 1420–1429.
Madlung, A., Tyagi, A. P., Watson, B., Jiang, H., Kagochi, T., Doerge, R. W., Martienssen, R. and Comai, L. 2005. Genomic changes in synthetic *Arabidopsis* polyploids. – Plant J. 41: 221–230.
Mariash, H. L., Cusson, M. and Rautio, M. 2017. Fall composition of storage lipids is associated with the overwintering strategy of *Daphnia*. – Lipids 52: 83–91.
Mata, J. K., Martin, S. L. and Smith, T. W. 2023. Global biodiversity data suggest allopolyploid plants do not occupy larger ranges or harsher conditions compared with their progenitors. – Ecol. Evol. 13: e10231.
Morgan, C., Zhang, H., Henry, C. E., Franklin, F. C. H. and Bomblies, K. 2020. Derived alleles of two axis proteins affect meiotic traits in autotetraploid *Arabidopsis arenosa*. – Proc. Natl Acad. Sci. USA 117: 8980–8988.
Overgaard, J. and MacMillan, H. A. 2017. The integrative physiology of insect chill tolerance. – Annu. Rev. Physiol. 79: 187–208.
Overgaard, J., Sørensen, J. G., Petersen, S. O., Loeschcke,V. and Holmstrup, M. 2005. Changes in membrane lipid composition following rapid cold hardening in *Drosophila melanogaster*. – J. Insect Physiol. 51: 1173–1182.
Overgaard, J. L., Gerber, L. and Andersen, M. K. 2021. Osmoregulatory capacity at low temperature is critical for insect cold tolerance. – Curr. Opin. Insect Sci. 47: 38–45.
Oyen, K. J., Giri, S. and Dillon, M. E. 2016. Altitudinal variation in bumble bee (*Bombus*) critical thermal limits. – J. Therm. Biol. 59: 52–57.
Pecl, I. 2023. Déterminants physiologiques de la tolérance aux extrêmes de température chez le microcrustacé, *Daphnia pulex*. Mémoire de maîtrise. – Univ. du Québec à Rimouski.
Pecl, I., Blier, P. U. and Dufresne, F. 2024. Data from: Polyploidy confers better cold tolerance in *Daphnia*. – Dryad Digital Repository, https://doi.org/10.5061/dryad.3tx95x6qp.
Reid, C. H., Patrick, P. H., Rytwinski, T., Taylor, J. J., Willmore, W. G., Reesor, B. and Cooke, S. J. 2022. An updated review of cold shock and cold stress in fish. – J. Fish Biol. 100: 1102–1137.
Rice, A., Šmarda, P., Novosolov, M., Drori, M., Glick, L., Sabath, N., Meiri, S., Belmaker, J. and Mayrose, I. 2019. The global biogeography of polyploid plants. – Nat. Ecol. Evol. 3: 265–273.
Riseth, E. N., Fraser, T. W. K., Sambraus, F., Stien, L. H. and Hvas, M. 2020. Is it advantageous for Atlantic salmon to be triploid at lower temperatures? – J. Therm. Biol. 89: 102548.
Ronges, D., Walsh, J. P., Sinclair, B. J. and Stillman, J. H. 2012. Changes in extreme cold tolerance, membrane composition and cardiac transcriptome during the first day of thermal acclimation in the porcelain crab *Petrolisthes cinctipes*. – J. Exp. Biol. 215: 1824–1836.
Schielzeth, H. 2010. Simple means to improve the interpretability of regression coefficients. – Methods Ecol. Evol. 1: 103–113.
Shah, A. A., Funk, W. C. and Ghalambor, C. K. 2017. Thermal acclimation ability varies in temperate and tropical aquatic insects from different elevations. – Integr. Comp. Biol. 57: 977–987.
Sinclair, B. J., Coello Alvarado, L. E. C. and Ferguson, L. V. 2015. An invitation to measure insect cold tolerance: methods, approaches and workflow. – J. Therm. Biol. 53: 180–197.
Štětina, T., Koštál, V. and Korbelová, J. 2015. The role of inducible Hsp70, and other heat shock proteins, in adaptive complex of cold tolerance of the fruit fly (*Drosophila melanogaster*). – PLoS One 10: e0128976.
Sunday, J. M., Bates, A. E. and Dulvy, N. K. 2011. Global analysis of thermal tolerance and latitude in ectotherms. – Proc. R. Soc. B 278: 1823–1830.
Syngelaki, E., Paetzold, C. and Hörandl, E. 2012. Gene expression profiles suggest a better cold acclimation of polyploids in the alpine species *Ranunculus kuepferi* (Ranunculaceae). – Genes 18: 1818.
Tarapacki, P., Jørgensen, L. B., Sørensen, J. G., Andersen, M. K., Colin, H. and Overgaard, J. 2021. Acclimation, duration and intensity of cold exposure determine the rate of cold stress accumulation and mortality in *Drosophila suzukii*. – J. Insects Physiol 135: 1–10.
Te Beest, M., Le Roux, J. J., Richardson, D. M., Brysting, A. K., Suda, J., Kubesová, M. and Pysek, P. 2012. The more the better? The role of polyploidy in facilitating plant invasions. – Ann. Bot. 109: 19–45.
Tilquin, A. and Kokko, H. 2016. What does the geography of parthenogenesis teach us about sex? – Philos. Trans. R. Soc. B 371: 1–16. https://doi.org/10.1098/rstb.2015.0538
Trenti, F., Sandron, T., Guella, G. and Lencioni, V. 2022. Insect cold-tolerance and lipidome: membrane lipid composition of two chironomid species differently adapted to cold. – Cryobiology 106: 84–90.
Van de Peer, Y., Mizrachi, E. and Marchal, K. 2017. The evolutionary significance of polyploidy. – Nat. Rev. Genet. 18: 411–424.
Van de Peer, Y., Ashman, T.-L., Soltis, P. S. and Soltis, D. E. 2021. Polyploidy: an evolutionary and ecological force in stressful times. – Plant Cells 33: 11–26.
Vergilino, R., Belzile, C. and Dufresne, F. 2009. Genome size evolution and polyploidy in the *Daphnia pulex* complex (Cladocera: Daphniidae). – Biol. J. Linn. Soc. 97: 68–79.
Waagner, D., Bouvrais, H., Ipsen, J. H. and Holmstrup, M. 2013. Linking membrane physical properties and low temperature tolerance in arthropods. – Cryobiology 67: 383–385.
Yang, S.-H., Zhang, Y.-J., Zhang, Y., Peng, J.-C., Liu, C.-Y., Li, D.-W. and Guo, W. 2024. Divergence in cold tolerance promotes niche differentiation between diploid and polyploid kiwifruits along an altitudinal gradient in southwest China. – Oikos 2024: e10181.
Zhu, Y., Xiong, W., Xu, Y., Zhang, P., Zhang, J. and Luo, Y. 2021. Comparison of metabolic scaling between triploid and diploid common carp. – J. Comp. Physiol. B 191: 711–719.