**Endopolyploidy as a potential driver of animal ecology and evolution**

Maurine Neiman[1], Margaret J. Beaton[2], Dag O. Hessen[3], Punidan D. Jeyasingh[4], Lawrence J. Weider[5]

*[1]Department of Biology, University of Iowa, Iowa City, IA, 52242, USA; maurine-neiman@uiowa.edu*

*[2]Biology Department, Mount Allison University, Sackville, E4L 1G7, CANADA; mbeaton@mta.ca*

*[3]Department of Biosciences, University of Oslo, Box 1066 Blindern, 0316 Oslo, NORWAY; d.o.hessen@bio.uio.no*

*[4]Department of Integrative Biology, Oklahoma State University, Stillwater, OK, 74078, USA; puni.jeyasingh@okstate.edu*

*[5]Department of Biology, Program in Ecology and Evolutionary Biology, University of Oklahoma, Norman, OK, 73019, USA; ljweider@ou.edu*

Abstract

Endopolyploidy – the existence of higher-ploidy cells within organisms that are otherwise of a lower ploidy level (generally diploid) – was discovered decades ago, but remains poorly studied relative to other genomic phenomena, especially in animals. Our synthetic review suggests that endopolyploidy is more common in animals than often recognized and likely influences a number of fitness-related and ecologically important traits. In particular, we argue that endopolyploidy is likely to play a central role in key traits such as gene expression, body and cell size, and growth rate, and in a variety of cell types, including those responsible for tissue regeneration, nutrient storage, and inducible anti-predator defenses. We also summarize evidence for intraspecific genetic variation in endopolyploid levels and make the case that the existence of this variation suggests that endopolyploid levels are likely to be heritable and thus a potential target for natural selection. We then discuss why, in light of evident benefits of endopolyploidy, animals remain primarily diploid. We conclude by highlighting key areas for future research such as comprehensive evaluation of the heritability of endopolyploidy and the adaptive scope of endopolyploid-related traits, the extent to which endopolyploid induction incurs costs, and characterization of the relationships between environmental variability and endopolyploid levels.

Key words: Chromosomal evolution, genome evolution, endomitosis, endoreduplication, endoreplication, phenotypic plasticity, ploidy level, polyploid, polyteny, somatic polyploidy

Contents:



## I. Introduction

Genome size and structure often varies among and within eukaryotic species (Gregory, 2005; Parfrey, Lahr & Katz, 2008). From evolutionary and ecological perspectives, this variation is significant because genomic features can influence growth rate, life cycle, metabolism, morphology, and development (Gregory, 2005; Lynch, 2007; Parfrey *et al*., 2008; Hessen, Daufresne & Leinaas, 2013) and might also play a key role in divergence and speciation (Hessen *et al*., 2013; Seehausen *et al*., 2014). Genome duplication (polyploidy) is widely acknowledged as one of the most important sources of spontaneous genomic variation that can catalyze phenotypic change and diversification (Soltis *et al*., 2014; Vanneste *et al*., 2014; Selmecki *et al*., 2015). Here, we make the case that ploidy-level elevation *within* an individual (endopolyploidy) might itself confer important evolutionary and ecological consequences, with a particular focus on animals.

Ploidy elevation, defined as an increase in the number of chromosome sets per cell relative to the ancestral (usually diploid) state, is one of the most common and important means

by which large-scale genomic variation is generated. Ploidy level can profoundly influence molecular evolution, gene expression, and cellular or organismal phenotype (reviewed in King, Seppälä & Neiman, 2012; Mayfield-Jones *et al*., 2013; Neiman, Kay & Krist, 2013a), and ploidy elevation is thought to play an important role in the remarkably successful radiations of taxa such as angiosperms (Soltis *et al*., 2009; *Amborella* Genome Project, 2013) and teleost fishes (Santini *et al*., 2009). Despite the evident biological importance of ploidy level, there is no consensus on the causes and consequences of ploidy level changes (Parisod, Holderegger & Brochmann, 2010; Mable, Alexandrou & Taylor, 2011; Albertin & Marullo, 2012; Leslie, 2014).

Ploidy is typically viewed as an organism-level trait. Although most multicellular eukaryotes are diploid, it is increasingly clear that ploidy level variation is common across and even within many plant and animal populations (Barlow, 1978; Mable *et al*., 2011). Less recognized, especially in animals, is the fact that ploidy level variation is also common *within* individuals (reviewed in Parfrey *et al*., 2008): even though the germline and most of the other cells of any particular organism may be diploid (or triploid, tetraploid, etc.), certain tissues or a subset of cells will very often feature a higher ploidy level than represented by the ploidy of the organism as a whole. This phenomenon, known as endopolyploidy, is common in the embryonic tissues of animals (trophoblast cells) (Lee, Davidson & Duronio, 2009), and occurs in a variety of other juvenile and adult animal tissues (Lee *et al*., 2009; Edgar, Zielke & Gutierrez, 2014). Endopolyploidy has also attracted attention as a central player in tumor development (Dewhurst *et al*., 2014; Leslie, 2014).

The functional role of endopolyploidy is far from settled, but we will contend that it

should not be dismissed as some cellular peculiarity of little evolutionary or ecological relevance to animal populations. In particular, we will make the case that endopolyploidy is likely to be a key contributor to a variety of ecologically important traits. More broadly, we will argue that endopolyploidy is not only widespread, but also more important to animal evolution and ecology than generally appreciated.

Critical insights into the evolutionary and ecological significance of endopolyploidy will be revealed by: (i) determining the types of taxa and tissues that are typically associated with endopolyploidy; (ii) identifying the cellular and organismal traits that are influenced by endopolyploidy; and (iii), determining whether there is genetic variation in endopolyploidy levels and/or inducibility that is visible to selection. We note that despite earlier papers discussing the prevalence and highlighting the potential evolutionary and ecological relevance of endopolyploidy (e.g., Nagl, 1976, 1978), there still do not exist enough data to allow rigorous quantitative analyses. In this review, we synthesize recent insights and discoveries that both illuminate the phenomenon of endopolyploidy and are consistent with the possibility that endopolyploidy might have adaptive functions. Our ultimate motivation is to inspire new studies directed towards revealing the ecological and evolutionary implications of endopolyploidy.

## II. What is endopolyploidy and how does it occur?

To be clear, the term endopolyploidy (or endoreplication) has been used in the literature in both broad and narrow contexts (in a manner similar to the use of the term heritability). As broadly defined, endopolyploidy describes somatic cells with nuclei containing more than two times the haploid DNA amount. This broad description does not preclude cells with under- or over-

replication of specific genomic segments and includes both polyteny and the more narrowly delineated endopolyploidy, which are the result of endocycling and endomitosis, respectively. The increase in nuclear DNA amounts for all forms of the expansive endopolyploidy condition is achieved during the S phase of altered cell cycles. Endocycling (polyteny) is the form of endoreplication whereby chromosome strands are duplicated but mitosis is entirely bypassed, leaving chromosome numbers unchanged (Edgar *et al*., 2014). By contrast, cells undergoing endomitosis (endopolyploidy) fail to complete the late mitotic stages of telophase and/or cytokinesis, resulting in duplicated chromosomes as discrete units within the same nucleus or in separate nuclei and, typically, complete (unbiased) nuclear replication within a cell (Lee *et al*., 2009). The number of endoreplication cycles (as endomitoses or endocycles) then determines the ploidy level.

It is important to be clear about the differences between endopolyploidy and the related but distinct phenomenon of polyploidy, which is defined as a condition where the ploidy level of the majority of the cells in an organism (including the germline) is greater than diploid. Most importantly, while endopolyploid cells arise from cells with lower ploidy via endoreplication, the polyploid cells in polyploid organisms are generated from other polyploid cells by standard mitotic processes. Endopolyploidy also differs from polyploidy by occurring within an otherwise lower-ploidy organism and by its tissue-specific nature (cf. Comai, 2005). Despite these differences, the many clear parallels between polyploidy and endopolyploidy mean that there is obvious potential for insights generated from the study of polyploid organisms to apply to endopolyploidy as well.

Protocols to detect and quantify endopolyploidy include flow cytometry (e.g., Korpelainen *et al.*, 1997) and a variety of densitometric methods (e.g., Rasch & Wyngaard, 2008). Flow cytometry typically involves the automated measurement of large numbers of fluorescently labeled cells. The primary advantages of flow cytometry are speed and the high number of nuclei that can be processed at one time. Where flow cytometry falls short is with respect to resolution, meaning that a flow cytometry approach is relatively likely to miss cells that represent only a minor fraction of the population. DNA densitometry involves employing microscopy and image analysis software on tissues subjected to the Feulgen reaction to quantify the intensity of the nuclear stain for tissue-specific cells (see Hardie, Gregory & Hebert, 2002 and Rasch, 2004 for relatively recent reviews of the protocol). While DNA densitometry is time consuming, it is otherwise superior to flow cytometry in its ability to provide detailed ploidy maps for individual tissues and detect ploidy levels that are rare within an organism (typically the highest ploidy levels).

The developmental genetic mechanisms underlying endocycles and endomitosis are still not fully understood and have been studied in detail only in a few model organisms (reviewed in Edgar *et al*., 2014). Nevertheless, it is evident that endopolyploid tissues are more sensitive to environmental stimuli such as nutrients and temperature than mitotic tissues (Wilson & Roach, 2002). A good example of the sensitivity of induction of endopolyploidy to environmental conditions was provided by Britton and Edgar (1998), who studied how starvation affected proliferation in mitotic and endoreplicating cells in first-instar *Drosophila* larvae. They found that while mitotic cells continued to proliferate in a nutrition-independent manner, most endoreplicating cells instead entered a quiescent state under starvation, reinitiating division only

when the starved larvae were again provided nutrients. Similar nutrient-dependent endocycle responses have been observed in the ovarian nurse cells of *Drosophila* (Drummond-Barbosa & Spradling, 2001), mollusk neurons (Yamagishi *et al.*, 2011), and the silk gland cells of silkworms (Zhang *et al*., 2012). A recent study by Li *et al*. (2015) revealed that endomitotic DNA synthesis in silk gland cells of silkworms fluctuated periodically, increasing during intermolt stages when larvae feed and experiencing inhibition during molting periods when larvae do not feed, also suggesting a close link between endopolyploidy and nutrition. A mechanistic underpinning for this relationship is suggested by the evidence for covariation between expression of cell cycle-related genes and synthesis of endomitotic DNA and the discovery that key growth hormones such as ecdysone contribute to the regulation of endomitotic DNA synthesis (Li *et al.,* 2015). Effects of temperature on endoreplication and the degree of endopolyploidy have been reported from dung flies (Blanckenhorn & Llaurens, 2005), *Drosophila* (Jalal *et al*., 2015), and *Daphnia* (Jalal *et al*., 2013). In all three of these examples, individuals raised at lower temperatures exhibited a higher proportion of polyploid cells. This demonstration of a connection between endopolyploidy and temperature is consistent with earlier observations that polyploidy is more prevalent at low temperatures (e.g., Dufresne & Hebert, 1995; Otto & Whitton, 2000; Brochmann *et al*., 2004) and can be induced experimentally by changes in temperature (e.g., Leggatt & Iwama, 2003), though the extent to which the temperature responses in endopolyploidy parallel those of induced polyploidy remains an open question. Together, this growing body of literature highlights the potential for an important role of endopolyploidy in phenotypic plasticity.

## III. Where does endopolyploidy occur?

Endopolyploidy has been documented in a diverse set of plant, fungal, and animal taxa (i.e., Nagl, 1978; Brodskiĭ & Uryvaeva, 1985; Yin, Gater & Karrer, 2010). One could argue that "endopolyploidy" also exists in unicellular organisms, such as some bacteria, given the documentation of extensive and variable polyploidization (i.e., multiple genome copies) in different subfunctional regions of the cytoplasm of the relatively large (600 μm in length) single cell bacterium *Epulopiscium* spp., a symbiont found in surgeonfish (Mendell *et al*., 2008). We acknowledge that such examples from unicellular organisms do not fit neatly into the standard definition of endopolyploidy (i.e., variation in ploidy level among cells or tissues within an organism). Even so, it is worth considering the ecological and evolutionary mechanisms that influence this type of genomic variation within unicellular organisms and the extent to which these mechanisms are similar or different than the mechanisms that operate at the multicellular level.

The evolution of endopolyploidy in eukaryotes may be quite ancient. In particular, evidence for fundamental mechanistic similarities of endocycles across plant, fungal, and animal taxa (e.g., down-regulation of cyclin-dependent kinase (CDK) while maintaining S-phase CDK; Edgar *et al*., 2014) suggests that endopolyploidy might have first evolved in eukaryotes as long as 800 million years ago (Edgar *et al*., 2014; but see discussion below of the likelihood of the independent evolution of distinct molecular mechanisms leading to endopolyploidy).

Our survey of the animal taxa and the type and function of tissues in which endopolyploidy has been observed demonstrates that endopolyploidy is widespread across invertebrate (e.g., insects, crustaceans, annelids, mollusks) and vertebrate (e.g., fishes, birds,

mammals) groups and occurs in many animal phyla and in a variety of tissues (Table 1). While we do not intend this survey to provide a comprehensive report of the recorded instances of endopolyploidy in animals, it does illustrate the taxonomic and functional expanse of the readily available literature on the topic. In particular, our survey suggests that while substantial information exists on endopolyploid levels in arthropods and mollusks and in selected tissues in chordates, knowledge regarding the extent of endopolyploidy for many tissues and many animal groups is limited to just one species or a few related taxa (Table 1).

Despite the remarkable diversity of taxa and tissues that feature endopolyploidy, the cellular mechanisms that lead to endopolyploidy are broadly similar, featuring either alternating S phases and G phases in the absence of mitosis or an abbreviated mitosis without completion of cytokinesis (Lee *et al.*, 2009; Edgar *et al*., 2014). At face value, these patterns might suggest that the specific mechanisms underlying endopolyploidy are ancient and highly conserved,, although the phylogenetic distribution of the various distinct molecular mechanisms leading to endopolyploidy suggests that endopolyploidy has evolved independently on multiple occasions in different taxa and different tissue types through evolutionary time (Anisimov, 2005; Anisimov & Zyumchenko, 2012; Edgar *et al*., 2014).

As has been previously shown in plants (Nagl, 1978; Barow & Meister, 2003; Edgar *et al*., 2014), endopolyploid levels in animals also feature taxon- and tissue-specific variation (Table 1). In at least some invertebrates, a large fraction of somatic cells may be polyploid (Scholes *et al*., 2014), although the degree of endopolyploidy can itself be influenced by internal (e.g., age, nutritional status) and external (e.g., temperature) environmental factors (e.g., Beaton

& Hebert, 1997; Korpelainen, Ketola & Hietala, 1997; Yamagishi *et al.*, 2011; Jalal *et al*., 2013). Among vertebrates, hepatocytes and cardiomyocytes can be mono- or binucleate, but the highest recorded level for either tissue in these animals is 32C (Table 1). The insect fat body, which, similar to the vertebrate liver, performs multiple functions related to metabolism and storage, also exhibits low to moderate endopolyploid levels for a hemipteran (i.e., a maximum of 128C; Nagl, 1978). By contrast, mammalian trophoblast cells can exhibit ploidy levels of 64-4096C (Nagl, 1978; Anatskaya, Vinogradov & Kudryavtsev, 1994; Vinogradov, Anatskaya & Kudryavtsev, 2001; Anatskaya & Vinogradov, 2004). In arthropods (e.g., hymenopterans), endopolyploid levels can reach 512C across tissues such as Malpighian tubules, small intestine, and thoracic gland (Nagl, 1978; Yamagishi *et al.*, 2011), and salivary glands routinely achieve endopolyploid levels of 1024C or more (Nagl, 1978). The neurons of mollusks feature remarkable ploidy variation, from a modest 32C in the land snail *Triodopsis divesta* to an astounding 200000C in the gigantic neurons of the sea hare *Aplysia californica* (Lasek & Dower, 1971; Mandrioli *et al*., 2010). The highest endopolyploid level that has been recorded in any animal is >500000C, reported from the silk-producing glands of the silk moth *Bombyx mori* (Perdrix-Gillot, 1979; Gregory & Hebert, 1999). In general, the maximal tissue-specific ploidy level achieved via endopolyploidy appears to be developmentally programmed (Edgar *et al*., 2014), but it is still not clear what governs maximal endopolyploid levels in different tissues and taxa.

IV. Is endopolyploidy heritable?

Individual-level heritable phenotypic variation (i.e., either broad-sense heritability, $H^2$, or narrow-sense heritability, $h^2$, > 0) is the raw material for evolution by natural selection, raising

the questions of 1) whether there exists among-individual variation in endopolyploidy levels and/or induction thresholds, and 2) whether this variation is heritable. While there are relatively few studies of endopolyploidy that use the quantitative genetics approach required to estimate heritability, both of these questions have been addressed indirectly by the multiple studies that provide empirical evidence for consistent intraspecific differences in levels of endopolyploidy among distinct lineages and genotypes (Beaton & Hebert, 1997; Korpelainen *et al*., 1997; Cheniclet *et al*., 2005; Gegas *et al*., 2014). In other words, these studies demonstrate a critical component of heritability: that phenotypic differences in endopolyploid levels are reliably transmitted to offspring.

Some of the best examples of such intraspecific variation in animals are provided by the freshwater microcrustacean *Daphnia* (Fig. 1; also see a similar example from other *Daphnia* species in Beaton & Hebert, 1989). For example, Korpelainen *et al*. (1997) found that the percentage of 2C, 4C, and 8C cells ranged from ~63-80%, ~18-32%, and ~2-5% of all cells, respectively, among *Daphnia* genotypes isolated from 13 different Finnish rockpool populations. Similarly, Beaton and Hebert (1997) noted extensive interspecific variation in the number of polyploid cells located in the head/helmet region of 20 daphniid species as well as substantial intraspecific variation in this trait among genotypes within species. The existence of both genotype- and species-specific endopolyploid levels in *Daphnia* suggests that endopolyploid levels have at least a partial genetic basis and thus are potentially heritable.

As is typical for studies on any aspect of endopolyploidy, there is a larger body of evidence from plants than from animals in support of the possibility that endopolyploid

phenotypes can be heritable. One clear example is provided by Cheniclet *et al*. (2005), who examined across-line levels of endopolyploidy in the pericarp of the fruit of tomato (*Solanum lycopersicum*). This study revealed extensive significant across-line variation (i.e., genetic variation) for the extent of expression of the endopolyploid phenotype as well as strong positive correlations between endopolyploid levels and cell diameter and fruit weight in *S. lycopersicon*. Intraspecific genetic variability in tissue-specific endopolyploidy has also been demonstrated in other plant taxa (e.g., accessions of *Arabidopsis thaliana*; Gegas *et al*., 2014). Altogether, there is a growing body of data indicating that the intraspecific variation required for endopolyploid levels to be heritable exists. The critical next step towards evaluating whether natural selection plays a role in maintaining variation in endopolyploid levels across tissues and taxa – determining whether endopolyploidy levels and induction thresholds can evolve via selection on endopolyploid-associated phenotypes – remains to be empirically addressed.

## V. Why endopolyploidy occurs: evolutionary and ecological drivers

Here, we synthesize concepts and data to address the extent to which endopolyploidy is likely to influence evolutionary and ecological processes, and in particular, evaluate whether endopolyploidy might serve an adaptive function (Table 1, Fig. 1). Most of the examples that we discuss invoke or assume associations between endopolyploidy and two fundamentally important cell-level characteristics, (1) levels of gene expression, and (2), cell size. Because these cellular traits comprise plausible links between endopolyploidy and organism-level traits (e.g., body size, growth rate) that are likely themselves to often influence fitness-related phenotypes in animals, we then summarize and synthesize the data that allow us to address these potential links between endopolyploidy and organismal biology. In particular, we focus on whether and to what extent

endopolyploidy influences gene expression and cell size and whether these functional connections may have evolutionary and/or ecological consequences, particularly with respect to organ or organismal growth. Finally, we ask why, in light of apparent evolutionary and ecological advantages of endopolyploidy, most cells in most animals remain diploid.

*a. Does endopolyploidy increase the level of gene expression?*

It is commonly assumed that endopolyploidy functions to generate the extra gene copies needed to produce the RNA required to sustain key fitness-enhancing anabolic (e.g., protein synthesis) and/or catabolic (e.g., energy metabolism) processes. This predicted functional connection between endopolyploidy and levels of gene expression is nearly always followed by the caveat that whether endopolyploidy in fact influences transcription remains unclear (Edgar & Orr-Weaver, 2001; Leiva-Neto *et al.*, 2004; John & Qi, 2008; Lee *et al*., 2009; Bourdon *et al*., 2010; Chevalier *et al*., 2011; Mayfield-Jones *et al*., 2013; Sher *et al.*, 2013), to the extent that Bourdon *et al*. (2010) concluded that the hypothesis that a major functional role of endopolyploidy is to increase gene expression had yet to be adequately tested. Subsequent research by Bourdon *et al*. (2012) in tomato showed that ribosomal RNA, RNA polymerase II, and gene transcript levels increase with nuclear ploidy level, providing direct evidence for a positive relationship between endopolyploidy and gene expression in a vascular plant model system. Determining whether these results extend to animals requires similar rigorous assessments in animal systems.

A promising starting point for addressing questions regarding a functional role for endopolyploidy as a mechanism to increase gene expression and protein production in animals is provided by silk-producing arthropods such as spiders, silk moths, and some caddisflies

(Trichoptera). These animals are ideal models to explore such links because 1) their silk-producing glands typically consist of polyploid cells (Sehnal & Sutherland, 2008), 2), silk is a very conspicuous protein product that clearly may be the target of selection, and 3), there exist species that only produce silk during a single life stage as well as species that use silk throughout their life cycle, enabling powerful across-taxa comparisons. The common occurrence of endopolyploidy in animal silk (and venom) glands led Gregory and Shorthouse (2003; also see Rasch & Connelly, 2005) to suggest that there very likely is an association between high protein output and endopolyploidy in such glands, and that a comparison among species with different silk-spinning habits would be rewarding in this context. One of the more striking examples of a positive endopolyploid level-silk production relationship is provided by the silk moth *Bombyx mori*, whose silk-producing glands feature endopolyploid levels exceeding 500000C (Perdrix-Gillot, 1979; Gregory & Hebert, 1999), likely linked to intensive artificial selection for silk yield (Perdrix-Gillot, 1979). Recent evidence that the genes involved in silk production in *B. mori* have experienced rapid evolution since these moths were domesticated (Xia *et al*., 2009), coupled with the likely possibility of a causal endopolyploidy-silk production connection, provide another line of evidence that tissue-specific endopolyploid levels are evolvable.

*b. Does endopolyploidy increase cell size?*

There is often (e.g., Melaragno, Mehrotra & Coleman, 1993; Cheniclet *et al*., 2005; Gonzalez *et al*., 2010; Bourdon *et al*., 2010; recently reviewed in De Veylder, Larkin & Schnittger, 2011; Edgar *et al*., 2014) but not always (Fankhauser, 1945; Bourdon *et al*., 2010; De Veylder *et al*., 2011) a positive association between nuclear ploidy level and cell size in both plants and animals. While the precise mechanisms that link endopolyploidy to increased cell size remain

unclear (John & Qi, 2008; Bourdon *et al*., 2010; De Veylder *et al*., 2011), one possibility is that the increased DNA content in the nuclei of polyploid cells results in increased nuclear volume, which itself then induces increased cell volume ("karyoplasmic ratio"; Cavalier-Smith, 1982; Olmo, 1983; Sugimoto-Shirasu & Roberts, 2003; Cheniclet *et al*., 2005; Bourdon *et al*., 2010; Gonzalez *et al.*, 2010). This hypothesis has found recent direct support in a study of the relationship between endopolyploidy, cell size, and nuclear size in tomato (Bourdon *et al*., 2012). A contrary view is expressed by John and Qi (2008; also see e.g., Massonnet *et al.*, 2011; Gegas *et al*., 2014), who argue that recent evidence that increases in cell size are required for the initiation of endoreplication suggests that at least in some instances, endopolyploidy might be more accurately considered an effect rather than a cause of increased cell size.

Regardless of the mechanisms connecting endopolyploidy to cell size, it is evident that increased cell size can affect traits that might influence organismal ecology and/or fitness (Olmo, 1983; Szaro & Tompkins, 1987). These connections between cell size and phenotype are often mediated by relationships between cell size, cell number, and/or body size, which themselves are quite different in plants than in animals (Sugimoto-Shirasu & Roberts, 2003). For example, while polyploid plants frequently have both larger cells and larger bodies than diploid counterparts, the relatively large cells that characterize polyploid vs. diploid forms of particular animals often (Day & Lawrence, 2000; e.g., Fankhauser, 1945; Santamaria, 1983; Henery, Bard & Kaufman, 1992) but not always (e.g., Hessen *et al*., 2013) lead to larger body sizes. An excellent example of the complex consequences of ploidy elevation in animals is provided by polyploid salamanders, which have larger but fewer cells than diploid counterparts (e.g., Fankhauser, 1945). This loss of cell number does appear to confer costs related to organ complexity:

polyploid salamanders have fewer neurons and simpler brains than their diploid counterparts (Roth, Blanke & Wake, 1995; also see Roth et al. 1994). Vernon and Butsch (1957) even argued that these differences in neuron number and brain structure could underlie the inferior performance of tetraploid vs. diploid salamanders in a maze running experiment.

*c. Endopolyploidy and growth*

It is evident that endopolyploidy has the potential to affect traits (e.g., gene expression levels, cell and body size, organ complexity, behavior) that might confer ecological and/or fitness consequences. In particular, the connections between endopolyploidy and traits that directly or indirectly influence gene expression and cell and/or body size suggest that an important evolutionary and ecological function of endopolyploidy might be to facilitate organ or organismal growth in conditions where early maturation, large size, or rapid growth/regeneration are favored (Cavalier-Smith, 1978; Melaragno *et al*., 1993; Anatskaya *et al*., 1994; e.g., Scholes & Paige, 2011, Losick, Fox & Spradling, 2013).

Animals can grow either by increasing their cell number or by increasing their cell size. For organisms with fixed cell numbers (e.g., nematodes), growth is largely attributed to the increased cell size associated with endopolyploidy (Flemming *et al*., 2000; Edgar & Orr-Weaver, 2001; Lozano *et al*., 2006). While this form of whole-body growth is thought to be relatively uncommon (Day & Lawrence, 2000), it is probably more widespread than hitherto recognized because it has been observed in a diverse set of invertebrate taxa (e.g., appendicularians, Ganot & Thompson, 2002; copepods, Rasch & Wyngaard, 2008). Under environmental conditions that inhibit cell division (e.g., desiccation, UV-B irradiation), increases in cell size that are correlated

with endopolyploidy might even provide a mechanism by which organ/organism size can be maintained in the absence of cell division (Sugimoto-Shirasu & Roberts, 2003; De Veylder *et al*., 2011; Gegas *et al*., 2014).

The silk-producing moth *Ephestia küehniella* provides a striking example of how endopolyploidy can regulate growth of specific organs during ontogeny. Between the first and second larval instars, the cells comprising the Malphigian tubules and silk glands increase in volume by factors of ~1800 and 3100, respectively, via repeated endocycles. By the final larval instar, the Malphigian tubules have reached 1024C, while the silk glands have attained up to 8192C (Buntrock *et al*., 2012). Another line of evidence connecting endopolyploidy and organ growth in *E. küehniella* is provided by evidence that the size of the scales covering *E. küehniella* wings is positively associated with the endopolyploid level of the epidermal cell beneath the scale: 8C cells tend to be found below relatively small scales, and the largest scales often are coupled with 32C cells (Kühn, 1965, as cited in Nagl, 1978).

Two recent studies of inter- and intra-individual variation in endopolyploidy in several ant species illustrate how endopolyploidy may be related both to body size and organ function (Scholes, Suarez & Paige, 2013; Scholes *et al*., 2014). Scholes *et al*. (2013) found that body size is positively related to endopolyploidy, such that larger workers have relatively high levels of endopolyploidy across a variety of tissues. The authors discovered that abdominal tissues had the highest endopolyploid levels of all, inspiring Scholes *et al*. (2014) to characterize endopolyploid levels in various organs of the giant ant *Dinoponera australis*. This study revealed significantly higher levels of endopolyploidy in organs involved in digestion (e.g., foregut/crop, mid-gut) and

417 exocrine function (e.g., Dufour's gland – pheromone production) relative to tissues/organs in
418 either the head or the thorax, which themselves did not differ significantly from one another in
419 mean endopolyploid levels. The one exception to this pattern was the mandibular gland of the
420 exocrine system. Although this gland resides in the head, it also exhibited high endopolyploid
421 levels, indicating that there is an elevated level of endopolyploidy for the exocrine system even
422 when tissue source is taken into account. Scholes *et al*. (2014) interpreted this result as
423 representing a possible connection between elevated endopolyploidy in tissues that require high
424 cellular metabolism and specialized function (also see Anatskaya *et al*., 1994).
425
426 *d.* Daphnia *as a model system linking endopolyploidy, evolution, and ecology.*
427 *Daphnia* species are well suited as an animal model for studies of endopolyploidy because of
428 widespread tissue involvement (Fig. 1) and the diversity of associated functions. The
429 increasingly prominent role of *Daphnia* as a model organism for functional genomics (Colbourne
430 *et al*., 2011) allows for a thorough evaluation of gene regulation, expression, and dosage effects
431 at the tissue (or cellular) level. Polyploid cell numbers for a given tissue appear to be established
432 by the first instar (Beaton & Hebert, 1999), pointing to embryogenesis as the transitional period
433 for the development of endopolyploidy in *Daphnia*.
434
435 The drivers of maximum ploidy levels in each *Daphnia* tissue seem to vary. For example,
436 epipodites, key ion regulatory tissues (Kikuchi, 1983), are entirely polyploid (Fig. 1), which may
437 reflect ontological changes in sodium uptake mechanisms (Bianchini & Wood, 2008) and/or
438 function to reduce cell-cell interactions in the tissue. Ploidy levels in the epipodites plateau soon
439 after reaching maturity, indicating tight developmental control (Beaton & Hebert, 1999).

Because the animal continues to grow throughout life but the endopolyploid level in the epipodites stays constant, the osmoregulatory load per cell may increase over time. By contrast, cells in the tissues associated with food acquisition (e.g., secretory labrum, lipid storage cells) are not entirely polyploid, but the endopolyploid cells in these tissues have the highest ploidy levels (2048C) found in the animal as a whole (Sterba, 1956, 1957; Beaton & Hebert, 1999). Again, in contrast to the epipodites, in which endopolyploid levels stabilize by maturity, the initiation and number of endomitotic cycles in tissues associated with food acquisition are linked to development, growth, and nutritional status (Beaton & Hebert, 1999).

*Daphnia* produce a variety of inducible epidermal structures (e.g., neckteeth, spines, helmets) in response to chemical signals indicating the presence of predators (Brooks, 1965). These defensive structures form as modifications of the epidermis, a primarily diploid tissue containing occasional polyploid cells at the dorsal and ventral margins (Fig. 1). Beaton and Hebert (1997) proposed a regulatory function for the polyploid epidermal cells wherein these cells modulate surrounding cell division and allow localized tissue growth via the release of an unknown mitogen (Beaton & Hebert, 1997). In a preliminary loss-of-function study, the ablation of selected cephalic polyploid cells in *D. lumholtzi* resulted in a helmet size reduction of ~20%-40% after one molt, supporting this model (Beaton, unpubl). Recently, Weiss *et al.* (2012) showed that polyploid cells in the head epidermis of several species of *Daphnia* have plasma bulges and high rates of protein synthesis. Since then, an immunohistochemistry-based study revealed that these cells serve as storage sites for dopamine, a neurohormone (L. Weiss, pers. comm.). While the mechanism of action for dopamine will depend on the receptor type upon which it acts, dopamine is known to act as modulator of stress responses in insects (Johnson &

White, 2009), lending further support to the polyploid control center model proposed by Beaton and Hebert (1997). A preliminary survey of gene expression in juvenile *D. lumholtzi* raised in the presence or absence of well-fed fish revealed that predator-induced animals (with helmets double the size of control animals at similar body sizes) exhibited a general down-regulation of mRNA transcripts relative to *Daphnia* in the predator-free treatments (McKinnon, 2013). This result hints at an alternative (though non-mutually exclusive) hypothesis for the functional role of endopolyploidy in this tissue: Because neonates, regardless of stress level, form helmets (though these helmets are much smaller than those produced by stressed adults), perhaps helmet formation is the default state and, in the absence of predator cues, enlarged head cells negatively modulate cell division. When faced with predation risk, the transcriptional activity of these polyploid cells decreases, allowing uninhibited cell division (and maximal helmet formation). Regardless of the mechanism involved, the presence of endopolyploidy in epidermal tissue appears to be critical in reducing vulnerability to predation through the production of inducible defenses in *Daphnia*.

## VI. Why aren't all animal cells polyploid?

We here have summarized evidence demonstrating that endopolyploidy is very widely distributed across animal taxa and tissues and is likely to often confer substantial advantages. Even so, and even in organisms harboring a relatively high fraction of polyploid cells, most cells remain diploid, which suggests that there may exist substantial costs associated with endopolyploidy.

One possible cost associated with endopolyploidy was suggested by Melaragno *et al*. (1993), who speculated that once a cell begins cycling endomitotically, it cannot return to the

mitotic cycle and cannot thus create additional new cells (also see Edgar & Orr-Weaver, 2001; John & Qi, 2008). This hypothesis is supported by data from *Arabidopsis* indicating that new cells are primarily produced by diploid progenitors (Galbraith, Harkins & Knapp, 1991), though exceptions have been reported in at least three invertebrate species (Beaton & Hebert, 1999; Fox, Gall & Spradling, 2010).

There is some evidence that larger genome and/or cell size can slow the rate of cell division (reviewed in Gregory, 2005), suggesting the non-mutually exclusive possibility that polyploid cells might generate costs associated with a relatively low cell division rate. In addition, the generally positive relationship between endopolyploid level and cell size (as described above) will also reduce the cell surface area to volume ratio, potentially generating constraints on the efficiency of energy, nutrient, and waste transport between cells and intercellular space (Gregory, 2005). The lack of data on the abundance and distribution of organelles and surface transport systems in endopolyploid cells compared to mitotic cells precludes any conclusive arguments about such potential costs of endopolyploidy but should be a fruitful avenue for future research.

There are also potential material costs associated with higher cellular DNA content that are themselves connected to the notable abundance of nitrogen (N) and phosphorus (P) in nucleic acids. The nucleus contains a relatively large fraction of nucleic acids, and is thus rich in P (ca. 2.5 % P per dry weight (DW)). Chromosomes are nearly 4 % P per DW and > 15 % N, while DNA and RNA are the most P-rich macromolecules in the cell, with > 5 % P of DW (Sterner & Elser, 2002). An especially large fraction of P is bound in nucleic acids in unicellular

heterotrophic eukaryotes and invertebrates (Sterner & Elser, 2002). Hessen *et al*. (2010) hypothesized that because P is often scarce in nature, reallocation of P from DNA to RNA in these organisms via genome downsizing could constitute an evolutionary response to selection favoring increased individual growth rate.

These connections between P investment in DNA vs. RNA and organismal growth rate imply that there could also be material costs of endopolyploidy related to the P allocation demanded by polyploid tissue. Indeed, Neiman *et al*., (2009) found that polyploid snails had higher per unit mass P content than diploid counterparts, indicating that higher ploidy levels might bear material costs. Furthermore, evidence for connections between organismal growth rates, P availability, and ploidy level in snails (Neiman, Kay & Krist, 2013b) and vascular plants (Šmarda *et al*., 2013) do suggest a tradeoff between the higher rate of transcription and production that could be afforded by ploidy elevation and the metabolic and/or nutrient costs associated with a higher rate of synthesis of body components. These results highlight the likelihood that ploidy elevation (and, perhaps, endopolyploidy) is more likely to confer advantages in conditions where the availability of resources (e.g., phosphorus, Hessen *et al*., 2010; Neiman *et al*., 2013a) needed to synthesize more/larger tissues is relatively high (also see Mayfield-Jones *et al*., 2013). Such mechanisms could play an important role in the evolutionary responses of populations to drastic alterations to environmental nutrient availability caused by anthropogenic activities.

## VII. Conclusions and future directions

(1) It is evident that endopolyploidy is both common and is often associated with major phenotypic consequences, though this phenomenon remains relatively understudied in animals. Some of these consequences of endopolyploidy (e.g., response to herbivory, wound healing, the induction and formation of morphological defenses) have either been documented (e.g., Beaton & Hebert, 1997; Scholes & Paige, 2011; Bainard *et al*., 2012; Losick *et al*., 2013; Scholes & Paige, 2014) or are likely to serve as potential drivers of ecological and evolutionary processes.

(2) Because relatively little empirical attention has been directed to the study of endopolyploidy in evolutionary and ecological contexts, especially in animals, critical questions regarding the importance of endopolyploidy for animal evolution and ecology remain unanswered, ranging from the evolutionary processes underlying the complex phylogenetic distribution of endopolyploidy to the molecular basis of endocycling and endomitosis. Quantification of the frequency and distribution of endopolyploidy across tissues, organisms, and different environmental conditions will allow for rigorous characterization of patterns at physiological, phylogenetic, and ecological levels. These data can be used to perform a wide variety of important tests of the evolutionary and ecological significance of polyploidy. Such tests would range from addressing whether there are phylogenetic patterns in the incidence of endopolyploidy (e.g., Anisomov & Zyumchenko, 2012) and whether there exist specific ecological "syndromes" (i.e., terrestrial, marine, freshwater) that might favor the evolution of endopolyploidy, to determining whether endopolyploidy is more prevalent in secretory tissues (e.g., Perdrix-Gillot, 1979) and/or rapidly growing tissues (e.g., Anatskaya & Vinogradov, 2002)? A powerful empirical approach in this context would be to compare sister taxa that show distinct differences in the incidence of endopolyploidy, with the goal of identifying the

555 ecological and/or evolutionary factors involved in these differences. The availability of these
556 data will catalyze formulation of hypotheses about the proximate (e.g., Edgar *et al*., 2014) and
557 ultimate (e.g., Scholes & Paige, 2014) mechanisms that underlie the induction and extent of
558 endopolyploidy.
559
560 (3) In particular, quantification of the heritability of endopolyploidy levels and
561 inducibility and evaluation of whether endopolyploid levels respond to selection on phenotypes
562 connected to endopolyploidy (e.g., cell size, protein production, organ growth rate) will provide
563 important tests of the extent to which endopolyploidy is likely to be a major player in adaptive
564 evolution. An important role for endopolyploidy as a driver of evolutionary processes will
565 require that endopolyploidy levels and inducibility thresholds are heritable and can influence
566 organismal fitness. Key research directions from an ecological perspective, which are connected
567 to but distinct from the evolutionary side of the story, include the evaluation of associations
568 between environmental variability (e.g., nutrient availability, predator presence) and
569 endopolyploidy and the extent to which endopolyploid induction incurs costs. Empirical studies
570 of whether and how particular environmental conditions can induce endopolyploidy and how the
571 induction of endopolyploidy affects ecologically relevant traits like sensitivity to nutrient
572 limitation and susceptibility to predation will provide important steps towards establishing the
573 extent to which endopolyploidy influences ecology, and vice versa, in natural animal
574 populations.
575
576 (4) Definitive answers to such fundamental questions about the evolution and ecology of
577 endopolyploidy will require an interdisciplinary approach. In particular, ecologists, geneticists,

developmental biologists, physiologists, and evolutionary biologists will need to work together to evaluate the ecological stimuli for endopolyploid induction, how endopolyploidy influences fundamental cell-, tissue-, and organism-level traits like cell and organ size, gene expression, and growth rate, and in turn, how these traits influence organismal and population ecology and evolution.

(5) Our ultimate goal would be to understand how these traits impact ecological functions and the adaptive potential of natural populations.

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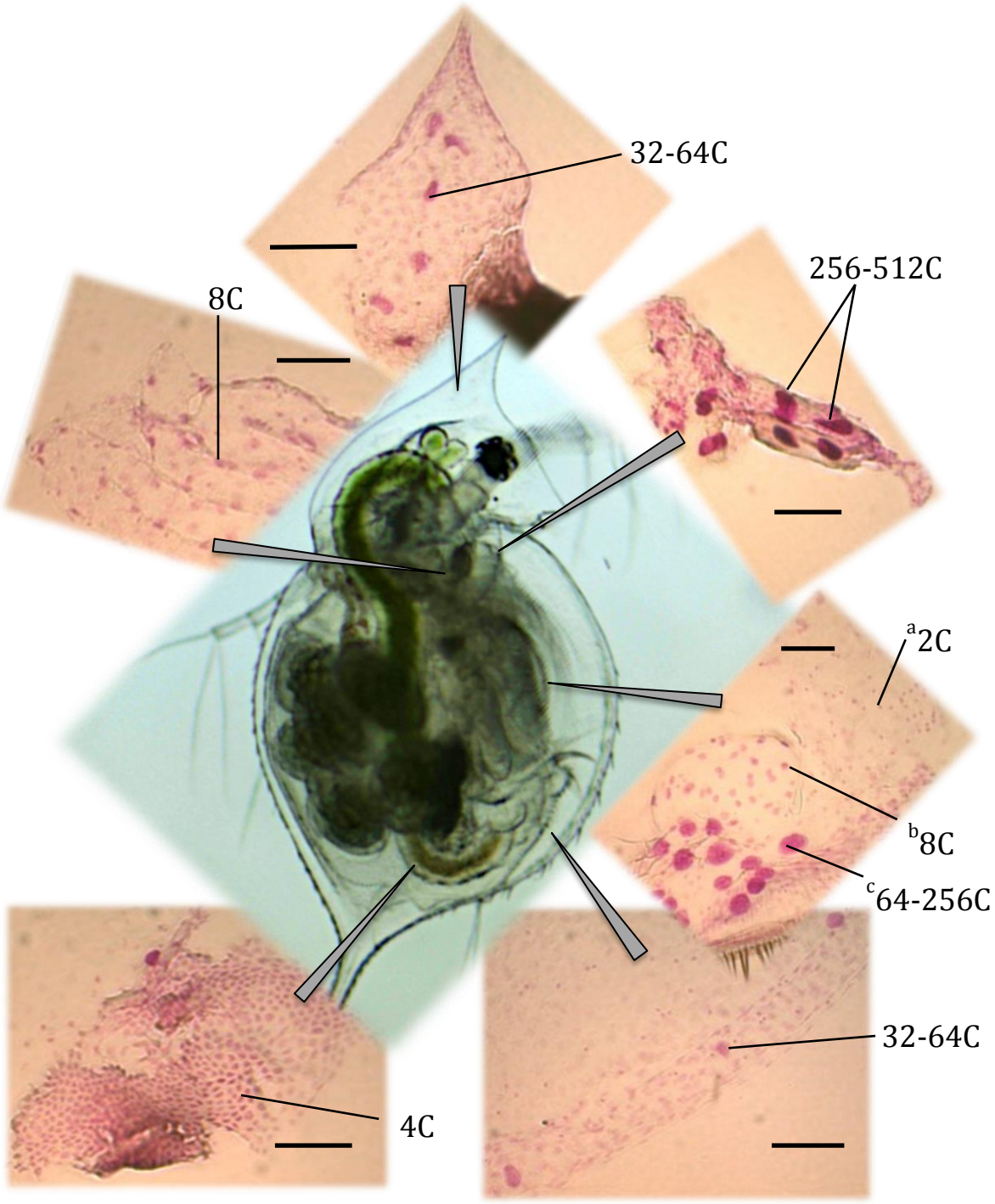
Figure Legends

Figure 1.

Endopolyploidy in adult female *Daphnia lumholtzi* from laboratory cultures. Six Feulgen-stained tissues showing ploidy level ranges: a. head epidermis; b. labrum; c. appendage with exopodite (2C), epipodite (8C), and lipid cells (64-256C); d. thoracic epidermis; e digestive tract; f. shell gland. All scale bars indicate 50 µm.

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