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Interactions between nuclear genes and a foreign mitochondrial genome in the redbelly dace *Chrosomus eos*

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ABSTRACT

Given the coevolution process occurring between nuclear and mitochondrial genomes, the effects of introgressive hybridization remain puzzling. In this study, we take advantage of the natural co-occurrence of two biotypes bearing a similar nuclear genome (*Chrosomus eos*) but harbouring mitochondria from different species (wild type: *C. eos*; cybrids: *Chrosomus neogaeus*) to determine the extent of phenotype changes linked to divergence in the mitochondrial genome. Changes were assessed through differences in gene expression, enzymatic activity, proteomic and swimming activity. Our data demonstrate that complex IV activity was significantly higher in cybrids compared to wild type. This difference could result from one variable amino acid on the *COX3* mitochondrial subunit and/or from a tremendous change in the proteome. We also show that cybrids present a higher swimming performance than wild type. Ultimately, our results demonstrate that the absence of coevolution for a period of almost ten million years between nuclear and mitochondrial genomes does not appear to be necessarily deleterious but could even have beneficial effects. Indeed, the capture of foreign mitochondria could be an efficient way to circumvent the selection process of genomic coevolution, allowing the rapid accumulation of new mutations in *C. eos* cybrids.

1. Introduction

Mitochondria play a vital role in cell metabolism. Although the mitochondrial genome (mtDNA) of animals only encodes a few proteins, they are critically positioned in the energy producing complexes of the respiratory chain (Andersson et al., 1998; Garesse and Vallejo, 2001). Of the thirteen mtDNA-encoded proteins, seven are subunits of NADH-ubiquinone oxidoreductase (complex I; *ND1–ND6* and *ND4L*), one is a subunit of ubiquinone-cytochrome c oxidoreductase (complex III; *CYTB*), three are subunits of cytochrome c oxidase (complex IV; *COX1–COX3*, forming the functional core of the enzyme) and two are subunits of ATP synthase (complex V; *ATP6* and *ATP8*). All the components of complex II (succinate dehydrogenase) are nuclear encoded. Consequently, all other complexes require highly specific interactions between nuclear and mitochondrial subunits to function effectively, suggesting that a strong coevolution of genomes must occur (Blier et al., 2001; Rand et al., 2004).

Studies of cybrids with nuclear DNA (ncDNA) and mtDNA derived from different species provide support for the coevolution hypothesis (Kenyon and Moraes, 1997; McKenzie and Trounce, 2000; James and Ballard, 2003; McKenzie et al., 2003). Artificial cybrids obtained by the experimental introduction of mtDNA from progressively more divergent species into *Mus musculus domesticus* mtDNA-less (ρ0) cells revealed that cell viability and mitochondrial metabolism decreased as the genetic distance between mtDNA and ncDNA increased (McKenzie et al., 2003).

While most studies involve cybrids created *in vitro* (Kenyon and Moraes, 1997; McKenzie et al., 2003, 2004; Cannon et al., 2011) or obtained by directed backcrossing (Sackton et al., 2003; Ellison and Burton, 2006; Harrison and Burton, 2006; Pichaud et al., 2012), some cybrid organisms spontaneously appear in natural environments (Boratynski et al., 2011; Angers et al., 2012; Toews and Brelsford, 2012). Unlike artificially obtained cybrids, natural cybrids must deal with their foreign mitochondria to survive and reproduce in their environment. Moreover, interspecific transfer of mitochondria may induce a modification of phenotype and confer adaptability to new environmental conditions (Glémet, 1997; Toews et al., 2014). Indeed, even though the absence of coevolution between genomes is expected to decrease individual performances, several studies demonstrate that this can

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sometimes present advantageous effects (Pichaud et al., 2012; Toews et al., 2014). A particularly interesting example is the introgression of the Myrtle warbler (*Setophaga coronata*) mitochondria in Audubon's warbler (*Setophaga auduboni*) (Brelsford et al., 2011; Mila et al., 2011). For the latter birds, mitochondrial introgression permanently alters their seasonal migratory behaviour so that residents become migratory (Toews and Brelsford, 2012). According to Toews et al. (2014), this phenomenon results from mitochondria that are more efficient, and better adapted for the energetic demands of long-distance migration.

A spontaneous naturally occurring cybrid derived from the redbelly dace *Chrosomus eos* (Cyprinidae, Pisces) is found in the freshwater lakes of North America. This cybrid originates from the crossfertilization of *C. eos–neogaeus* × *eos* triploid females by *C. eos* males (Fig. 1), effectively producing fish bearing diploid *C. eos* nuclei and *Chrosomus neogaeus* mitochondria (Dawley et al., 1987; Goddard et al., 1998; Binet and Angers, 2005; Angers and Schlosser, 2007; Angers et al., 2012). Based on previous estimates from comparisons of the *cox1* gene region (Angers and Schlosser, 2007) and of restriction fragment length polymorphism (RFLP) of the complete mitochondrial genome (Goddard et al., 1989), *C. neogaeus* mtDNA differs by 9.6% compared to *C. eos*. This implies that species have been separated for almost ten million years. In addition, methylome as well as proteome are strongly different between allopatric *C. eos* wild type and cybrids (Angers et al., 2012). Given these important divergences, modification of key mitochondrial function in cybrids, such as ATP production, might be expected. According to Mee and Taylor (2012), *C. eos* wild type is found mainly in the southern part of the distribution area whereas cybrids, as well as *C. neogaeus*, occur predominately in the north. This peculiar distribution suggests that cybrids might be able to support colder conditions than wild type. In the southern part of Quebec (Canada), a region where their distribution overlaps, wild type and cybrids are found in close proximity (Angers and Schlosser, 2007), thereby allowing direct comparison of the different biotypes under similar environmental and evolutionary constraints.

This study aims at assessing the influence of foreign mitochondria on *C. eos* phenotype. To do so, we first compared the enzymatic activity of wild type and cybrids where *C. eos* nuclear genome is in association with *C. eos* and *C. neogaeus* mtDNA respectively. We measured the maximal activity of metabolic enzymes involved in aerobic metabolism (complexes I, II, II + III and IV of the mitochondrial respiratory chain) and anaerobic metabolism (lactate dehydrogenase) in white muscle. Afterwards, the source responsible for variation in enzymatic activity was investigated by analyses of gene expression, gene sequences and proteome reprogramming. To this end, qRT-PCR, sequencing and two-dimensional gel electrophoresis were respectively used. Finally, we assess whether the *C. neogaeus* mitochondria of cybrids could impact function at a higher level of biological organization by determining individual swimming performance (U_{crit}).

Since *C. eos* cybrids are able to deal with their foreign mitochondria and because the absence of coevolution between mitochondrial and nuclear genomes can sometimes present beneficial effects, we first predict that respiratory chain complex I, complex III and complex IV have higher activities in *C. eos* cybrids than in wild type. Secondly, we hypothesize that higher activities of complexes I, III and IV in cybrids improve the efficiency of mitochondrial metabolism, which in turn should improve the swimming capacity (U_{crit}) of these fish.

2. Material and method

2.1. The biological model

The cybrids used in this study occur naturally and are derived from the *C. eos–neogaeus* hybrids (Dawley et al., 1987; Goddard et al., 1998). Hybridization between *C. eos* males and *C. neogaeus* females results in the *C. eos–neogaeus* diploid hybrids that reproduce clonally *via* gynogenesis (Goddard et al., 1998; Binet and Angers, 2005). Occasionally, the nuclear genome of the *C. eos* sperm can be incorporated into the egg's diploid genome leading to the formation of triploid hybrids *C. eos–neogaeus* × *eos*. During gametogenesis of triploid hybrids, the unmatched set of chromosomes is discarded and meiosis occurs on the *C. eos* diploid genome, producing haploid gametes (Goddard et al., 1998). Fertilization of such eggs by a *C. eos* male results in the formation of individuals with a pure *C. eos* nuclear diploid genome but a female inherited *C. neogaeus* mitochondrial genome (Binet and Angers, 2005) (Fig. 1).

2.2. Sampling and biotype identification

Fish were sampled in the Laurentian region (Quebec, Canada) in six lakes located in close proximity of each other (Table 1). All fish were captured at the same period of the year, in the summer when lake temperature was around 24 °C. Except for individuals used for swimming performance (U_{crit}) tests, all fish were euthanized in the field by eugenol overdose and immediately placed on dry ice. They were then brought back to the laboratory and stored at −80 °C until analyses.

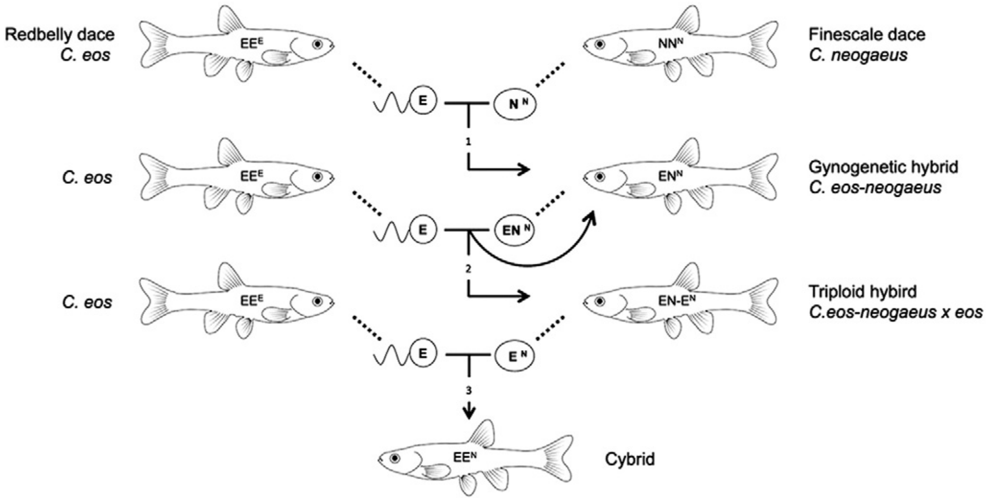

Fig. 1. Expected mechanism leading to the formation of natural cybrids. 1—Gynogenetic hybrids resulting from hybridization between *Chrosomus neogaeus* females and *C. eos* males. 2—Triploid hybrids arise when nuclear genome of *C. eos* sperm is incorporated into diploid eggs of *C. eos–neogaeus*. 3—Cybrids are formed when haploid eggs of triploid hybrids are fertilized by *C. eos* sperm. E and N refer to the nuclear genome of *C. eos* and *C. neogaeus* respectively, superscripts represent mitochondrial genome.