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Exploring the consequences of mitochondrial differences arising through hybridization of sunfish

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ABSTRACT

Previous studies have shown evidence of genomic incompatibility and mitochondrial enzyme dysfunction in hybrids of bluegill (*Lepomis macrochirus* Rafinesque) and pumpkinseed (*Lepomis gibbosus* Linnaeus) sunfish (Davies et al., 2012 Physiol. Biochem. Zool. 85, 321–331). We assessed if these differences in mitochondria had an impact on metabolic processes that depend on mitochondrial function, specifically hypoxia tolerance and recovery from burst exercise. Bluegill, pumpkinseed, and their hybrids showed no difference in the critical oxygen tension (P_{crit}) and no differences in tissue metabolites measured after exposure to 10% O_2 for 30 min. In contrast, loss of equilibrium (LOE) measurements showed that hybrids had reduced hypoxia tolerance and lacked the size-dependence in hypoxia tolerance seen in the parental species. However, we found no evidence of systematic differences in metabolite levels in fish after LOE. Furthermore, there were abundant glycogen reserves at the point of loss of equilibrium. The three genotypes did not differ in metabolite status at rest, showed an equal disruption at exhaustion, and similar metabolic profiles throughout recovery. Thus, we found no evidence of a mitochondria dysfunction in hybrids, and mitochondrial differences and oxidative metabolism did not explain the variation in hypoxia tolerance seen in the hybrid and two parental species.

1. Introduction

Hybridization between species is typically prevented by pre-zygotic and post-zygotic isolating mechanisms, however hybrids can occur naturally. Many hybrids suffer from hybrid breakdown — a loss of fitness compounded through successive generations — which is likely due to incompatibilities between the two parental genomes (Breeuwer and Werren, 1995). The effects on F1 hybrids however are often unpredictable; hybrids can display hybrid vigor — increased fitness in certain traits relative to the parental species (Livesay, 1930; Manwell et al., 1963; McDaniel and Grimwood, 1971) — but more commonly display defects. In many cases, defects in hybrids are linked to incompatibilities between nuclear and mitochondrial genomes (Liepins and Hennen, 1977; Edmands and Burton, 1999; Sackton et al., 2003; Ellison and Burton, 2006, 2008a; Ellison et al., 2008; Lee et al., 2008; Niehuis et al., 2008). While a hybrid's nuclear DNA is inherited from both parents (and therefore both species), its mitochondrial DNA is inherited from only one parent (in most cases, the mother). This can lead to genetic incompatibility, depending on the extent of mitochondrial genome differences between the two parentals (Ellison and Burton, 2008b). Nuclear-mitochondrial incompatibility has the greatest effect on oxidative phosphorylation (OXPHOS). Complexes I, III and IV of the electron transport system have subunits encoded by both nuclear and mitochondrial genomes. The coordination between these two genomes is thus critically important for aerobic energy production, and incompatibilities between the two genomes can lead to impaired catalytic activity of enzyme complexes (Sackton et al., 2003; Willett and Burton, 2003), reduction in OXPHOS efficiency, or production of reactive oxygen species (Lane, 2011).

Sunfish of the *Lepomis* genus are a popular model for studying the consequences of hybridization in vertebrates; although they last shared a common ancestor 14.6 million years ago, many species readily hybridize with each other (Near et al., 2005). When crosses are made between *Lepomis* species, hybrids from species with greater evolutionary distance between parentals show more pronounced defects than hybrids between more closely related species (Bolnick and Near, 2005). Bluegill (*Lepomis macrochirus*) and pumpkinseed (*Lepomis gibbosus*) sunfish, common in lakes throughout North America, hybridize naturally where their niches overlap. A molecular genetic analysis of bluegill-pumpkinseed hybrids in Lake Opinicon revealed that all hybrids sampled were unidirectional F1 hybrids resulting from crosses between bluegill males and pumpkinseed females (Konkle and Philipp, 1992). The unidirectionality of this hybridization is thought to be due to the reproductive strategies of bluegills and an asymmetry in sexual discrimination between the two species. Previous work suggests that bluegill-pumpkinseed hybrids may suffer from nuclear-mitochondrial incompatibilities (Davies et al., 2012), specifically through reduced activity of OXPHOS Complexes III and IV.

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The two sunfish parental species differ both in ecological niches and metabolic traits (Mittelbach, 1984; Osenberg et al., 1992). Bluegill are less tolerant of hypoxia than pumpkinseeds (Farwell et al., 2006). As a result of the distinctive metabolic properties of parentals and the suggestion that hybrids may have compromised mitochondrial function, we compared the three genetic backgrounds in terms of the metabolic phenotype. We compared the groups in terms of the relative hypoxia tolerance and the capacity to recover from burst exercise. We hypothesized that the hybrids would have a reduced hypoxia tolerance due to the apparent mitochondrial defects. We also hypothesized that, since recovery from burst exercise is a mitochondrial-dependent aerobic process (Moyes et al., 1992), hybrids may show a reduced rate of recovery.

2. Materials and methods

2.1. Fish capture and holding

Bluegill (*L. macrochirus*), pumpkinseed (*L. gibbosus*) and bluegill-pumpkinseed hybrids were caught with hook and line from Lake Opinicon at Queen's University Biological Station (44°35′N, 76°20′W). Fish were transferred to the Biology Aquatic Facility at Queen's University and held in an aerated 800 L flow-through tank at 15–20 °C. They were fed daily with commercial feed. Fish masses were as follows (mean ± sd): bluegill (144 ± 39 g); hybrid (185 ± 32 g), pumpkinseed (163 ± 39 g). There were no significant differences between groups ($p = 0.12$), and this size range has little impact on mitochondrial enzymes (Davies and Moyes, 2007). This research was approved by the Queen's University Animal Care Committee (protocol: Moyes-2008-034).

2.2. P_{crit} and respiration rates

Fish (85–160 g) were transferred to individual 5.1 L plexiglass test chambers and held overnight for 18–22 h at 25 °C. During this period, tanks were supplied with aerated, flow-through water and a small pump to internally circulate water. After this period, flow-through and aeration were shut off and the tanks were sealed. Dissolved oxygen concentration was monitored with FOXY oxygen probes (Ocean Optics, Dunedin, FL, USA), with measurements taken every 6 s. Standard metabolic rate was calculated from the rate of decrease in oxygen concentration over a 20 min period starting after the first 10 min. During this period, the rate of respiration was linear, and oxygen concentration declined by no more than 20%.

Fish were left in the chambers to self-deplete oxygen until the water reached 0% saturation. P_{crit} was calculated using a critical points calculator (P_{crit} calculator, Queen's University, Kingston, ON, Canada) that identifies the critical partial pressure of oxygen (PO_2) at which the fish shifts from regulation of oxygen consumption to conformation of oxygen consumption.

2.3. Loss of equilibrium

Fish (75–150 g) were transferred to individual 5.1 L plexiglass test chambers and held overnight for 18–22 h at 25 °C. During this period, tanks were supplied with aerated, flow-through water and a small pump to internally circulate water. To begin the trial, aeration and flow-through were shut off. A mix of nitrogen and air was bubbled into the tank to decrease the dissolved oxygen concentration to 9–10% within 10 min. Dissolved oxygen concentration was monitored with FOXY oxygen probes. Fish were monitored for signs of disequilibrium, with loss of equilibrium (LOE) defined as the time at which a fish could no longer maintain an upright position. Following LOE, fish were lethally anesthetized in a solution of tricaine menthane sulfonate (0.4 g/L) and sodium bicarbonate (0.8 g/L). Brain, heart and white muscle were collected and immediately frozen in liquid nitrogen.

2.4. Hypoxia treatment

Fish were transferred to the same 5.1 L plexiglass test chambers used for LOE experiments and held for 18–22 h. During this period, tanks were supplied with aerated, flow-through water and a small pump to internally circulate water. To begin the hypoxia exposure, aeration and flow-through were shut off. A mix of nitrogen and air was bubbled into the tank to decrease the dissolved oxygen concentration to 9–10% of air saturation within 10 min. Dissolved oxygen concentration was monitored with FOXY oxygen probes. This oxygen concentration was maintained for 30 min, after which fish were lethally anesthetized in a solution of tricaine menthane sulfonate (0.4 g/L) and sodium bicarbonate (0.8 g/L). Brain, heart and white muscle were collected and immediately frozen in liquid nitrogen.

2.5. Exercise and recovery treatment

Fish were transferred to an 800 L tank and immediately chased by hand. Fish were deemed exhausted when they failed to respond to touch and could not maintain equilibrium in the water column. At this point the exhausted fish was either anesthetized (time = 0) or transferred to an aerated 5.1 L tank and allowed to recover. At determined times, anesthetic was added directly to the tank, and the fish was euthanized and sampled.

2.6. Metabolite assays

Frozen white muscle, brain and heart tissues were broken into small pieces in liquid nitrogen using a mortar and pestle. Tissue (500–1000 mg) was transferred to a small test tube, combined with 10 vol. of ice cold 3.5% perchloric acid, and then homogenized at medium speed for 10 s using a Polytron homogenizer. An aliquot of the homogenate was removed for glycogen measurements. The remaining homogenate was centrifuged at 15,000 *g* for 5 min. The supernatant (1 mL) was removed and neutralized (pH 6.8–7.2) with 0.1 mL saturated Tris (tris(hydroxymethyl)aminomethane), 0.1 mL 2 M KCl, 0.1 mL 2 M KOH, 1 μg phenol red. The neutralized metabolite extracts were centrifuged (2 min at 14,000 *g*) and supernatants were frozen (−80 °C) until metabolite determination. Assays were performed using a Spectromax plate spectrometer (Molecular Devices, Sunnyvale, CA, USA), using the absorbance at 340 nm determined at equilibrium, usually for 20–30 min.

2.6.1. Lactate

Lactate concentration was measured following the addition of 50 μL neutralized metabolite extract to 250 μL of assay mix containing 0.2 M hydrazine, 0.5 M glycine, 1 mM ethylenediaminetetraacetic acid (EDTA) and 2 mM NAD^+. The reaction was started by an addition of lactate dehydrogenase (LDH) at 1 U/well.

2.6.2. ATP

ATP concentration was measured following the addition of 50 μL neutralized metabolite extract to 250 μL of assay mix containing 50 mM Tris, 5 mM $MgCl_2$, 5 mM glucose, 2 mM NAD^+, and 0.4 U/mL glucose-6-phosphate dehydrogenase (G6PDH). The reaction was started with an addition of 1 U yeast hexokinase (HK).

2.6.3. ADP

ADP concentration was measured following the addition of 50 μL neutralized metabolite extract to 250 μL of assay mix containing 50 mM Hepes, 5 mM $MgCl_2$, 1 mM phosphoenol pyruvate (PEP), 2 mM NADH, and 0.25 U LDH. The reaction was started with an addition of 1 U pyruvate kinase (PK).