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RESEARCH ARTICLE

Asymmetric energetic costs in reciprocal-cross hybrids between carnivorous mice (*Onychomys*)

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ABSTRACT

Aerobic respiration is a fundamental physiological trait dependent on coordinated interactions between gene products of the mitochondrial and nuclear genomes. Mitonuclear mismatch in interspecific hybrids may contribute to reproductive isolation by inducing reduced viability (or even complete inviability) due to increased metabolic costs. However, few studies have tested for effects of mitonuclear mismatch on respiration at the whole-organism level. We explored how hybridization affects metabolic rate in closely related species of grasshopper mice (genus *Onychomys*) to better understand the role of metabolic costs in reproductive isolation. We measured metabolic rate across a range of temperatures to calculate basal metabolic rate (BMR) and cold-induced metabolic rate (MR_c) in *O. leucogaster*, *O. torridus* and *O. arenicola*, and in reciprocal F_1 hybrids between the latter two species. Within the genus, we found a negative correlation between mass-specific BMR and body mass. Although *O. arenicola* was smaller than *O. torridus*, hybrids from both directions of the cross resembled *O. arenicola* in body mass. In contrast, hybrid BMR was strongly influenced by the direction of the cross: reciprocal F_1 hybrids were different from each other but indistinguishable from the maternal species. In addition, MR_c was not significantly different between hybrids and either parental species. These patterns indicate that metabolic costs are not increased in *Onychomys* F_1 hybrids and, while exposure of incompatibilities in F_2 hybrids cannot be ruled out, suggest that mitonuclear mismatch does not act as a primary barrier to gene flow. Maternal matching of BMR is suggestive of a strong effect of mitochondrial genotype on metabolism in hybrids. Together, our findings provide insight into the metabolic consequences of hybridization, a topic that is understudied in mammals.

KEY WORDS: Mitonuclear mismatch, Metabolic rate, Interspecific hybrids, F_1 hybrid, OXPHOS, Reproductive isolation

INTRODUCTION

Aerobic respiration depends on coordinated interaction between products of the mitochondrial and nuclear genomes. The interdependence of these two genomes is among the most ancient and taxonomically pervasive examples of coevolution (Lang et al., 1999; Rand et al., 2004). Energy in the form of ATP is generated via oxidative phosphorylation (OXPHOS) within the mitochondria (Alberts et al., 2014). Four of the five OXPHOS enzyme complexes comprise proteins of both nuclear and mitochondrial origin (Ryan and Hoogenraad, 2007; McKenzie et al., 2007), and mitochondrial DNA replication, repair and transcription all depend on nuclear genes (reviewed in Rand et al., 2004). While the identity of mitochondrial genes is highly conserved across Metazoa (Saccone et al., 2006), the high substitution rate of mitochondrial DNA can drive compensatory evolution in the nuclear genome on microevolutionary time scales (Wolff et al., 2014). For example, nuclear components of the cytochrome *c* oxidase complex in primates (Osada and Akashi, 2012) and nuclear-encoded mitochondrial ribosome proteins in invertebrates exhibit accelerated evolution (Barreto and Burton, 2012). Because efficient mitochondrial function is fundamental to energy production, suboptimal mitochondrial-nuclear (mitonuclear) interactions are expected to have large negative effects on key physiological processes, including growth, development and fertility (Lane, 2011).

Interdependence, opportunity for rapid coevolution, and high fitness costs of inefficient respiration suggest that mismatches between mitochondrial and nuclear genomes have the potential to contribute to barriers to gene flow between closely related or incipient species (Gershoni et al., 2009; Johnson, 2010; Burton and Barreto, 2012; Wolff et al., 2014). This argument is consistent with the predictions of the Dobzhansky–Muller model for the evolution of postzygotic reproductive isolation, in which hybrid inviability and sterility are explained by faulty interactions between loci evolving on different genetic backgrounds (Bateson, 1909; Dobzhansky, 1937; Muller, 1942). Indeed, mitonuclear incompatibilities appear to contribute to speciation in copepods (*Tigriopus californicus*); in inter-population crosses, hybrid inviability is strongly associated with reduced efficiency of OXPHOS complexes and of nuclear-encoded mitochondrial transcription (Burton et al., 2006; Ellison and Burton, 2008, 2010). While such incompatibilities appear widespread in invertebrates (parasitoid wasps: Breeuwer and Werren, 1995; Niehuis et al., 2008; Ellison et al., 2008; nematodes: Chang et al., 2015; *Drosophila*: Meiklejohn et al., 2013) and yeast (Lee et al., 2008; Chou et al., 2010), further work is needed to assess the taxonomic scope and contribution of mitonuclear dysregulation to reproductive isolation.

In vertebrates, the effects of mitonuclear mismatch are surprisingly underexplored. The prevalence of F_1 hybrid inviability in sunfishes (Centrarchidae) is positively correlated with the rate of mitochondrial evolution in the maternal parental species (Bolnick et al., 2008). Similarly, analyses of mitochondrial function in mitonuclear hybrid cell lines in rodents (McKenzie et al., 2003) and primates (Kenyon and Moraes, 1997) indicate that mitochondrial OXPHOS efficiency decreases with increased genetic distance between the host nuclear genome and

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heterospecific mitochondria. At the organismal level, basal metabolic rate (BMR) differs among allopatric populations of the stonechat (*Saxicola torquata*), a Eurasian passerine, but is not dysregulated in interpopulation hybrids (Tieleman et al., 2009). In contrast, wild-caught chickadee hybrids (*Poecile atricappillus*×*Poecile carolinensis*; Olson et al., 2010) and lab-reared F_1 crested newt hybrids (*Triturus carnifex*×*Triturus dobrogicus*; Gvoždík, 2012) exhibit significantly increased metabolic rates relative to parental species. Given that BMR represents the minimal cost of maintenance, such increased metabolic costs in hybrids translates into less energy available for other functions such as growth or reproduction (Lane, 2011). Furthermore, metabolic inefficiency should become more pronounced in animals under stress to meet energetic demands, such as maintaining homeothermy in low ambient temperatures. To our knowledge, the contribution of mitonuclear mismatch to the origin and maintenance of species boundaries is untested in mammals. Herein, using BMR and cold-induced metabolic rate (MR_c) as indicators of mitochondrial efficiency, we tested for evidence of mitonuclear incompatibility in laboratory-reared grasshopper mice (genus *Onychomys*) and their interspecific F_1 hybrids.

Grasshopper mice are carnivorous rodents that inhabit arid to semi-arid deserts, grasslands and prairies throughout western North America. Northern grasshopper mice (*O. leucogaster*) range throughout the Interior Plains and Columbia and Great Basins (26–49 g; McCarty, 1978), whereas Southern (*O. torridus*; 20–40 g) and Chihuahuan grasshopper mice (*O. arenicola*; 20–35 g) primarily inhabit Sonoran and Chihuahuan desert, respectively (McCarty, 1975; Sullivan et al., 1986). In southwestern New Mexico, all three species come into contact. Here, *O. leucogaster* appears to segregate from its congeners based on a preference for more mesic environments (Findley et al., 1975). Although relatively cryptic based on external phenotype (Hinesley, 1979; Sullivan et al., 1986), *O. arenicola* and *O. torridus* are readily discriminated by karyotype, mitochondrial haplotypes and nuclear gene sequences (Riddle, 1995; Riddle and Honeycutt, 1990). In fact, molecular phylogenies place *O. arenicola* as sister to *O. leucogaster* (Riddle, 1995; Miller and Engstrom, 2008). Importantly, there is no evidence of historic mitochondrial introgression between any of the three species (P.C. and B.P., unpublished). F_1 hybrids between *O. arenicola* and *O. torridus* occur in the contact zone but are extremely rare (Sullivan et al., 1986). Nonetheless, the genus can be interbred in the laboratory and there is anecdotal evidence for reduced viability in hybrids (Pinter, 1971). The system provides an excellent opportunity to examine whether abnormalities in hybrid energetics contribute to reproductive isolation.

We measured metabolic rates at a range of temperatures in all three *Onychomys* species to characterize the range of metabolic rates within the genus, and used reciprocal F_1 hybrids between *O. arenicola* and *O. torridus* to explore the energetics of hybrid offspring and the genetic mechanisms mediating aerobic respiration. If mitonuclear compatibility regulates metabolism, we predicted that either hybrid BMR or hybrid MR_c would be outside the range of both parental species ('mitochondrial–nuclear coadaptation' hypothesis; Blier et al., 2001; Rand et al., 2004; Gershoni et al., 2009; Fig. 1A). Given the low frequency of hybrids in nature and relatively high mitochondrial divergence between the two species (10% for cytochrome *b*; Bradley et al., 2004; Miller and Engstrom, 2008), we hypothesized that mitonuclear incompatibility would reduce respiratory efficiency in hybrids, resulting in higher BMR or MR_c compared with that of parental species (e.g. Olson et al., 2010; Lane, 2011; Gvoždík, 2012). In other words, hybridization would carry a metabolic cost. In contrast, if mitochondrial genes are the primary determinant of metabolism, we predicted that metabolic rate would differ between hybrids from reciprocal directions of the cross and would match BMR or MR_c of the maternal species ('mitochondrial control' hypothesis; Tieleman et al., 2009; Fig. 1B). Finally, if metabolism is primarily under nuclear control, we predicted no difference in either metric between reciprocal F_1 hybrids and metabolic rates intermediate to those of both parent species ('nuclear control' hypothesis; Tieleman et al., 2009; Fig. 1C).

MATERIALS AND METHODS

Animal housing

Mice used in this study were the progeny of wild-caught *O. leucogaster* (Wied-Neuwied 1841), *O. torridus* (Coues 1874) and *O. arenicola* Mearns 1896 captured in the Animas Valley, New Mexico, USA, in June–August 2014 and transferred to animal facilities at Cornell University. Mice were housed in pairs for breeding, maintained on a 14 h:10 h light:dark cycle (21±2°C), and provided with rodent chow (LabDiet Prolab RMH 3000) and water *ad libitum*. Reciprocal F_1 hybrids were produced by crossing *O. torridus* females with *O. arenicola* males (hereafter, *t*♀×*a*♂) and *O. arenicola* females with *O. torridus* males (hereafter, *a*♀×*t*♂),

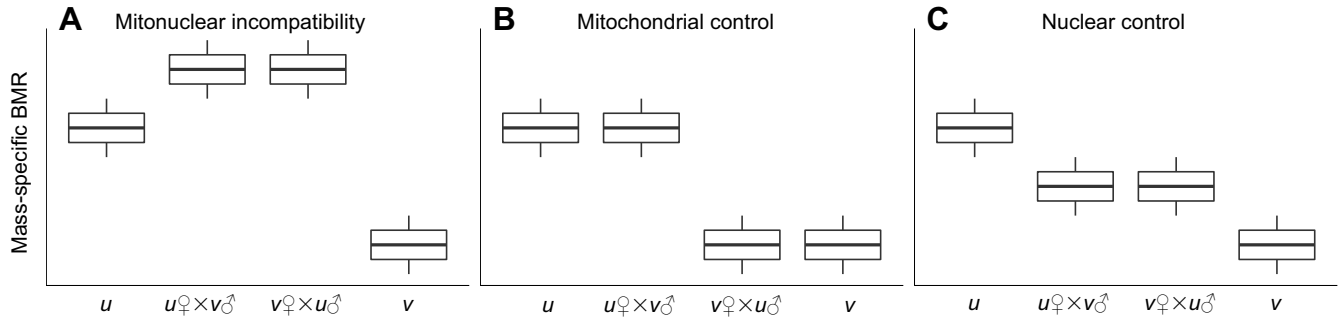

Fig. 1. Possible mechanisms that influence mitochondrial respiration in F_1 hybrids as illustrated with hypothetical results for parental species (*u* or *v*) and the two possible types of hybrid (*u*♀×*v*♂, *v*♀×*u*♂, according to the direction of the reciprocal cross). If mitonuclear incompatibility influences hybrid respiration (A), then basal metabolic rate (BMR) of hybrids should be greater than that of either parent species as a result of mismatch and reduced efficiency (Lane, 2011; Olson et al., 2010). However, if the mitochondrial genome controls respiration (B), then the hybrids should most closely resemble their maternal parent. If respiration is primarily under nuclear control (C), then we expect no difference in BMR between reciprocal F_1 hybrids and metabolic rates intermediate to those of both parent species (Tieleman et al., 2009).

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resulting in offspring with equivalent hybrid nuclear genomes (except for the sex chromosomes in males) and mitochondrial DNA from either *O. torridus* or *O. arenicola*. Pups were weaned at 30 days and housed individually until respirometry experiments were performed. All individuals used in the respirometry experiments were adults (>5 months) in non-reproductive condition. Animals were collected with approval from the New Mexico Department of Game and Fish (authorization no. 3562). The research was approved by IACUC (protocol no. 2014-0063) at Cornell University.

Respirometry

Respirometry trials were performed during the diurnal quiescent period (09:00 h–17:00 h) in December 2015. Access to food and water was restricted 3–5 h before initiation of respirometry to ensure that animals were post-absorptive (i.e. not actively digesting food). Individuals were acclimated to a starting ambient temperature (T_a) of 1–3.5°C for 2 h before measurements were collected. Measurements were taken at the initial temperature for 1 h, then T_a was increased at 5°C h^{-1}, and maintained at a stable temperature for 1 h until a final T_a of 37–38°C was reached.

We measured resting metabolic rate (RMR) using a pull-mode flow-through respirometer coupled with a climate-controlled chamber (Lighton, 2008). Excurrent gas was analyzed using a Sable Systems FoxBox field O_2/CO_2 analyzer. Samples were scrubbed of water vapor before CO_2 measurements using refurbished Drierite. Before the respirometry measurements, all Drierite was exposed to ambient air for a minimum of 2 min, thereby reducing CO_2 affinity and overall washout times (White et al., 2006). After CO_2 measurements, the gas stream was scrubbed using a combination of scrubbers including Drierite–soda lime–Ascarite before measuring O_2 concentration. Baseline measurements lasting 7 min were taken every 35 min, and after the completion of the trial. Measured versus actual flow rates were corrected using the water displacement method (i.e. measuring the time for excurrent airflow to displace water in an inverted graduated cylinder of known volume; see Lighton and Halsey, 2011). A 3 m copper coil constructed of 6.35 mm i.d. tubing was placed in-line upstream inside the respiration chamber to equilibrate the temperature between the chamber and the incurrent airstream. All connection tubing was 6.35 mm i.d. Bev-a-line IV.

Raw O_2 and CO_2 measurements were first drift-corrected (3rd degree polynomial – cubic Hermite spline) using baseline data. Using the standard temperature and pressure (STP)-compensated flow rate, oxygen consumption ($\dot{V}_{O_2}$) was calculated as:

$$\dot{V}_{O_2} = \mathrm{FR} \times (F_{I_{O_2}} - F_{E_{O_2}})/(1 - F_{I_{O_2}}) \quad (1)$$

and carbon dioxide production as:

$$\dot{V}_{CO_2} = [\mathrm{FR}(F_{E_{CO_2}} - F_{I_{CO_2}}) - F_{E_{CO_2}}(\dot{V}_{O_2})]/(1 - F_{E_{CO_2}}), \quad (2)$$

where FR is the corrected incurrent flow rate, $F_{I_{O_2}}/F_{I_{CO_2}}$ is the fractional incurrent gas concentration and $F_{E_{O_2}}/F_{E_{CO_2}}$ is the fractional excurrent gas concentration.

Statistical analyses

BMR was calculated as the lowest stable value for $\dot{V}_{O_2}$ between 30 and 32°C, within the previously published limits of the thermoneutral zone in *Onychomys* (Whitford and Conley, 1971). MR_c was calculated as the predicted RMR for each individual at 0°C. We used the RMR measurements below BMR to derive a linear equation for each individual. The starting temperature for each respirometry trial was between 1 and 3.5°C; thus, we were able to interpolate metabolic rates for all individuals at 0°C. Finally, we re-ran respirometry trials on an individual from each of the three species and one F_1 hybrid ($t♀ \times a♂$) a minimum of 5 days after the initial trial to test for repeatability of our metabolic measurements. The effect of species (*O. leucogaster*, *O. torridus*, *O. arenicola*) or reciprocal F_1 hybrid identity on body mass and on mass-specific BMR was tested with one-way ANOVA. All pairwise comparisons were evaluated with *post hoc* Tukey's HSD tests. Analyses were performed using R 3.2.2 (R Development Core Team 2015).

RESULTS

Body mass

Overall, body mass differed among *O. arenicola*, *O. leucogaster*, *O. torridus* and reciprocal F_1 hybrids between *O. arenicola* and *O. torridus* (ANOVA, $F_{4,15}$=16.48, P<0.001; Fig. 2A). Both F_1 hybrid crosses were smaller than *O. leucogaster* and *O. torridus* (Tukey HSD, P<0.01, all comparisons), yet were indistinguishable from each other and from *O. arenicola* (Tukey HSD, P=0.73, 0.78 and 1.0, respectively; Table 1).

Metabolic rates

BMR varied with body mass, explained by an exponential curve with the following relationship:

$$\mathrm{BMR} = -0.0166 \times M + 1.6829, \quad (3)$$

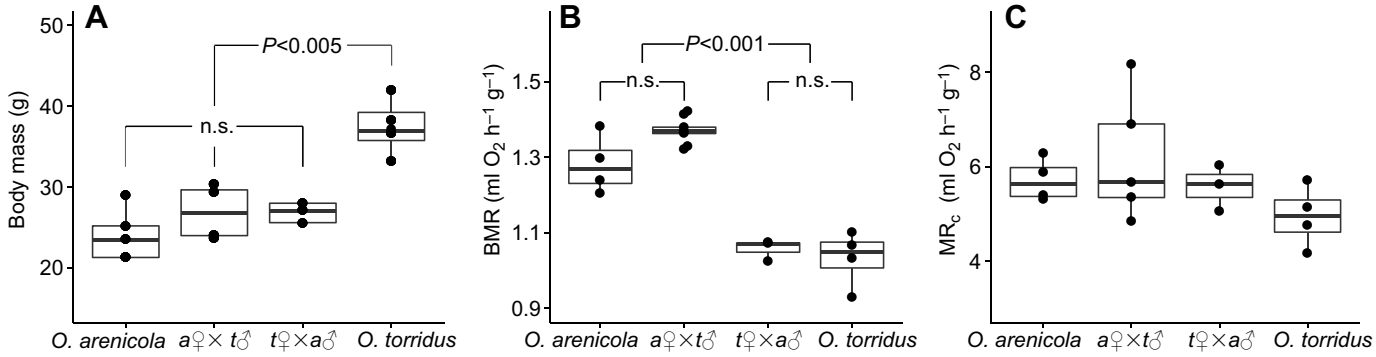

Fig. 2. Size and energetics comparison of parental and hybrid *Onychomys*. (A) Body mass, (B) mass-specific BMR and (C) mass-specific cold-induced metabolic rate (MR_c) of *Onychomys arenicola*, *Onychomys torridus* and reciprocal hybrids. The mass of *O. torridus* is significantly different from that of the other parent species, *O. arenicola*, and the reciprocal hybrid crosses (GLM, Tukey HSD, P<0.005). The BMR of each hybrid most closely resembles the BMR of the female parent species. Sample size: *O. arenicola*, N=4; *O. torridus*, N=4; $a♀ \times t♂$, N=5; $t♀ \times a♂$, N=3).

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Table 1. Body mass, whole-organism and mass-specific basal metabolic rate (BMR), and mass-specific cold-induced metabolic rate (MR_c) for 3 species and 2 hybrid crosses of *Onychomys*

Species or hybrid cross	*N*	*n*	Body mass (g)	Whole-organism BMR (ml O_2 h^{-1})	Mass-specific BMR (ml O_2 h^{-1} g^{-1})	Mass-specific MR_c (ml O_2 h^{-1} g^{-1})
O. arenicola (*a*)	4	5	26.90±3.49	33.30±3.05	1.28±0.08	5.72±0.45
a♀×*t*♂	5	5	26.92±1.21	28.43±0.81	1.37±0.03	6.18±1.34
t♀×*a*♂	3	4	24.06±3.21	32.91±3.95	1.06±0.03	5.57±0.49
O. torridus (*t*)	4	5	37.25±3.62	37.45±2.32	1.03±0.07	4.95±0.65
O. leucogaster	4	5	38.87±4.30	40.94±4.88	1.05±0.03	4.17±0.56

N, number of individuals; *n*, number of respirometry trials. Values are means±s.d.

where *M* is body mass (r^2=0.78). There was a significant overall effect of species/hybrid identity on whole-organism BMR and mass-specific BMR (ANOVA, $F_{4,15}$=6.851, P<0.01 and $F_{4,15}$=30.36, P<0.001, respectively; Fig. 2B). *Onychomys arenicola* had a higher mass-specific BMR than that of either *O. torridus* or *O. leucogaster* (Tukey HSD, both P<0.005), whereas there was no difference in mass-specific BMR between *O. leucogaster* and *O. torridus* (Tukey HSD, P=0.98; Table 1). Mass-specific BMR was significantly different between the two hybrid crosses (Tukey HSD, P<0.001) and from their paternal parental species (Tukey HSD: *t*♀×*a*♂ versus *O. arenicola*, P<0.001; *a*♀×*t*♂ versus *O. torridus*, P<0.001), but was indistinguishable from their maternal parental species (*t*♀×a♂ versus *O. torridus*, P=0.97; *a*♀×*t*♂ versus *O. arenicola*, P=0.15; Fig. 2B). This pattern of maternal matching in hybrids is consistent with the predictions of the mitochondrial control hypothesis (Fig. 1B).

We also found a significant overall effect of species/hybrid identity on mass-specific MR_c ($F_{4,15}$=3.715, P=0.03) but not on whole-organism MR_c (ANOVA, $F_{4,15}$=1.474, P=0.26; Fig. 2C). Mass-specific MR_c was greater in *a*♀×*t*♂ hybrids than in *O. leucogaster* (Tukey HSD, P=0.02; Table 1), but was not different for any other pairwise comparison (all P>0.2; Fig. 2C).

Metabolic rate measurements showed strong repeatability for all individuals when compared with the original measurements ($F_{1,6}$=714.89, P<0.001, R=0.90; Lessells and Boag, 1987).

DISCUSSION

We tested for evidence of mitonuclear incompatibility in the laboratory-reared F_1 hybrid progeny of two species of grasshopper mice from a contact zone where hybrids occur at very low frequencies. Mice from reciprocal hybrid crosses differed from each other in mass-specific BMR but not in body mass or MR_c. Hybrid values for these phenotypes were within the range of variation found within parental species and the genus as a whole. Thus, we did not find consistent evidence of respiratory inefficiency with either whole-organism or mass-specific metabolic rates. This suggests that mitonuclear incompatibilities are not expressed in *Onychomys* F_1 hybrids. In addition, our results suggest that hybrid metabolic rate is maternally inherited, with no detectable influence of the F_1 nuclear genome. These findings provide evidence that the mitochondrial genome is a primary regulator of energy metabolism in *Onychomys*. In contrast, body mass of both hybrids was indistinguishable from that of the smaller parental species, *O. arenicola*. This result was also unexpected, as it is suggestive of a dominant inheritance pattern for a trait that prior studies in wild rodents have found to be determined by either additive inheritance (e.g. Sadowska et al., 2005) or maternal effects (e.g. Nespolo et al., 2003, 2005). We discuss the BMR and body mass results in turn.

No evidence for metabolic costs in F_1 hybrids

The potential for rapid coevolution between mitochondrial and nuclear genomes and the essential role of mitochondria in energy production is expected to yield reduced fitness when the two genomes are mismatched in hybrids (Gershoni et al., 2009; Johnson, 2010; Burton and Barreto, 2012). If nuclear genes that contribute to mitonuclear dysregulation are dominant-acting in hybrids, then incompatibilities should be exposed in F_1 animals. Indeed, this seems to be the case in crested newt hybrids (*Triturus* species), in which BMR of F_1 hybrids is significantly elevated relative to that of both parent species, consistent with reduced metabolic efficiency due to mitonuclear mismatch (Gvoždík, 2012). In contrast, F_1 *Onychomys* hybrids appear to suffer no metabolic deficits, with individuals exhibiting equal or lower metabolic rates than expected based on body mass. This result suggests that mitonuclear incompatibility does not explain the rarity of natural hybrids between *O. torridus* and *O. arenicola*. There are, however, two important caveats to this conclusion.

First, we cannot rule out effects on hybrid metabolism beyond the F_1 generation. Notably, the best-documented cases of mitonuclear incompatibility involve the exposure of recessive-acting incompatibilities in the nuclear genome. In both copepods (*T. californicus*) and parasitoid wasps (*Nasonia* species), F_1 hybrids are normal and inviability associated with mitonuclear dysfunction is not expressed until the F_2 generation (Burton et al., 2006; Niehuis et al., 2008; Ellison et al., 2008; Ellison and Burton, 2008). Future experiments using hybrids with either *O. torridus* or *O. arenicola* mitochondrial DNA on an F_2 background will be required to determine whether mitonuclear-associated hybrid breakdown occurs in *Onychomys*.

Second, despite the lack of evidence for metabolic inefficiency when the organism is subjected to low temperatures, it is possible that exercise-induced stress (e.g. summit metabolism, $\dot{V}_{O_2,max}$)

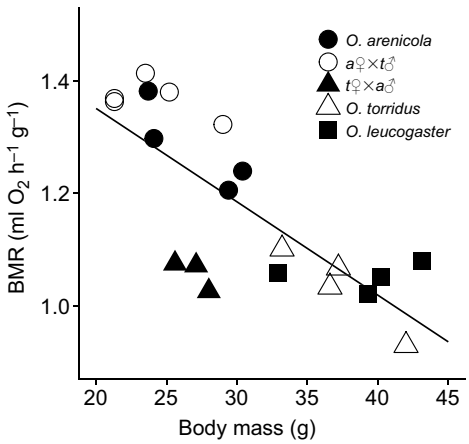

Fig. 3. Mass-specific BMR versus body mass in the genus *Onychomys*. The reciprocal F_1 hybrids each resemble their female parent in mass-specific BMR but the smaller parental species, *O. arenicola*, in body mass. *Onychomys leucogaster* is included for comparison. Sample size: *O. arenicola*, *N*=4; *O. leucogaster*, *N*=4; *O. torridus*, *N*=4; *a*♀×*t*♂, *N*=5; *t*♀×*a*♂, *N*=3.

would uncover respiratory defects in F_1 hybrids. For example, while gross metabolic function is normal in *Mus musculus domesticus*-derived lab mice with mitochondria from a closely related congener, *Mus spretus* (McKenzie et al., 2004), these mitochondrial hybrids (cybrids) tire faster than controls in a running to exhaustion test (Nagao et al., 1998). A 2-fold excess in lactate production indicative of inefficient cellular metabolism may explain cybrid physical performance deficits (McKenzie et al., 2004). Given that mitochondrial divergence between *O. torridus* and *O. arenicola* is higher than that between *M. m. domesticus* and *M. spretus* (Suzuki et al., 2004), testing physiological performance limits in *Onychomys* hybrids would be of considerable interest.

Evidence for mitochondrial effects on BMR

Understanding the genetic architecture of bioenergetic traits is a central focus in evolutionary physiology (Hayes and Garland, 1995; Storz et al., 2015). The pattern of maternally matched BMR in reciprocal F_1 hybrids reported here is consistent with mitochondrial control of energy metabolism in grasshopper mice (Fig. 1B; Tieleman et al., 2009). Given the importance of a large number of nuclear genes to mitochondrial function, this is a surprising result. In the absence of evidence for metabolic costs in hybrids, we would expect hybrid BMR to fall between that of the two parent species (Fig. 1C), as seen in most inter-population crosses in stonechats (Tieleman et al., 2009). Indeed, we are aware of only one other report of a similar signal of mitochondrial determination of BMR: analysis of a suite of metabolic parameters, including BMR, in the offspring of laboratory and wild house mice (*M. m. domesticus*) showed a significant effect of cross direction on BMR and other metabolic parameters (Richardson et al., 1994).

An alternative interpretation is that asymmetric BMR in *Onychomys* hybrids is due to maternal effects (the impact of maternal genotype or phenotype on offspring phenotype, Wolf and Wade, 2009; see also Lynch and Walsh, 1998). While the current experiment cannot discriminate between mitochondrial and maternal effects, quantitative genetic analyses of metabolic traits in other endotherms have not detected effects of maternal environment on BMR (Nespolo et al., 2003, 2005; Sadowska et al., 2005; Tieleman et al., 2009). Finally, phenotypes that depend on the direction of a cross are consistent with parent-of-origin effects, characteristic of imprinted genes. Although we are not aware of any direct connection between imprinted genes and BMR, several imprinted genes are highly expressed in the hypothalamus, a brain region critical to thermoregulation and energy homeostasis (Wilkinson et al., 2007). Ultimately, separating the proposed contribution of mitochondrial genotype to hybrid metabolism from that of maternal or parent-of-origin effects will require production of reciprocal cybrid lines: mice with *O. arenicola* mitochondria on an *O. torridus* background and vice versa.

Effects of hybridization on body mass

In contrast to hybrid mass-specific BMR, hybrid body mass was independent of the direction of the reciprocal cross: all hybrids resembled *O. arenicola*, the smaller of the two parent species. This result was surprising for two reasons. First, additive effects of many genes typically explain the heritable component of adult body mass in rodents (Cheverud et al., 1996; Gray et al., 2015), an inheritance pattern that should produce intermediate phenotypes in the offspring of a cross between different-sized parents. Non-additive effects on body mass, when present, are either maternal (e.g. Nespolo et al., 2005) or parent-of-origin dependent (e.g. Wolf et al., 2008). In both cases, offspring body mass depends on the direction of the reciprocal cross. Body mass in hybrid grasshopper mice did not match either of these genetic scenarios; the pattern we observed was consistent with dominant effects of *O. arenicola* nuclear genes. Second, regardless of the underlying genetic mechanism, the combination of invariant body mass and mitochondrially matched BMR resulted in hybrids with mass-specific BMR differing by greater than 40% in the reciprocal crosses. Specifically, BMR in hybrids with *O. torridus* mitochondria was low relative to body mass (Fig. 3). From a strictly energetic perspective, this metabolic phenotype could be advantageous under food-limited conditions but might negatively impact fitness when maximal energy expenditure is required (e.g. gestation and lactation; Derting, 1989; Koteja, 2000; Burton et al., 2011; Sadowska et al., 2013). Determining whether these trade-offs exist in $t♀ \times a♂$ hybrids will require manipulation of food intake in reproductive and non-reproductive animals (e.g. Derting, 1989).

Conclusions

We found that mitochondrial and metabolic divergence between two species of grasshopper mice does not result in inefficient basal metabolism in F_1 hybrids. Thus, while mitonuclear mismatch may affect hybrid phenotypes or generations (e.g. F_2) not studied here, our current results suggest that mitonuclear incompatibility does not explain the rarity of *Onychomys* hybrids in nature. Instead, our results can be interpreted as a strong effect of mitochondrial, but not nuclear, genotype on hybrid metabolic phenotype. Given the rarity of tests for metabolic costs of hybridization in endotherms, this study represents an important first step towards understanding the importance of mitonuclear incompatibilities to the evolution of reproductive isolation in mammals relative to other taxonomic groups. Future studies that focus on mitonuclear interactions in natural hybrid systems will help reveal the prevalence of incompatibilities in contributing to reproductive isolation in wild populations.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

J.R.S. and B.P. conceived the study; J.B.S. and B.P. organized permits/logistics; J.R.S. and B.P. designed and performed the experiments; J.R.S., P.C. and B.P. analyzed the data; J.R.S., P.C., J.B.S. and B.P. wrote the manuscript.

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Data availability

Data are available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.22k3c (Shipley et al., 2016).

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