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SYMPOSIUM

Effects of the Mitochondrial and Nuclear Genomes on Nonshivering Thermogenesis in a Wild Derived Rodent

Pierre Bize,[1,*,†] Imogen Lowe,[*] Mikko Lehto Hürlimann[†] and Gerald Heckel[‡]

*School of Biological Sciences, University of Aberdeen, Zoology Building, AB24 2TZ Aberdeen, UK; [†]Department of Ecology and Evolution, University of Lausanne, CH-1015 Lausanne, Switzerland; [‡]Computational and Molecular Population Genetics, Institute of Ecology and Evolution, University of Bern, Hochschulstrasse 6, CH-3012 Bern, Switzerland

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[1]E-mail: pierre.bize@abdn.ac.uk

Synopsis A key adaptation of mammals to their environment is their ability to maintain a constant high body temperature, even at rest, under a wide range of ambient temperatures. In cold climates, this is achieved by an adaptive production of endogenous heat, known as nonshivering thermogenesis (NST), in the brown adipose tissue (BAT). This organ, unique to mammals, contains a very high density of mitochondria, and BAT correct functioning relies on the correct functioning of its mitochondria. Mitochondria enclose proteins encoded both in the maternally inherited mitochondrial genome and in the biparentally inherited nuclear genome, and one overlooked hypothesis is that both genomes and their interaction may shape NST. By housing under standardized conditions wild-derived common voles (*Microtus arvalis*) from two distinct evolutionary lineages (Western [W] and Central [C]), we show that W voles had greater NST than C voles. By introgressing those two lineages over at least nine generations, we then experimentally tested the influence of the nuclear and mitochondrial genomes on NST and related phenotypic traits. We found that between-lineage variation in NST and BAT size were significantly influenced by the mitochondrial and nuclear genomes, respectively, with the W mitochondrial genotype being associated with higher NST and the W nuclear genotype with a larger BAT. There were significant mito–nuclear interactions on whole animal body weight and resting metabolic rate (RMR). Hybrid voles were lighter and had higher RMR. Overall, our findings turn new light on the influence of the mitochondrial and nuclear genomes on thermogenesis and building adaptation to the environment in mammals.

Introduction

Understanding adaptation to the environment and how it is rooted in the genome(s) is one of the main goals of modern biology. In mammals, one key adaptation to cold environments is the ability to generate heat, even at rest, by mitochondria enclosed in the brown adipose tissue (BAT) (Lowell and Spiegelman 2000; Cannon and Nedergaard 2004; Oelkrug et al. 2015). This long term adaptation to cold is referred as nonshivering thermogenesis (NST) and replaces the short-term production of heat by muscular shivering in response to cold (Cannon and Nedergaard 2004). The mitochondrion is an unusual organelle since it has its own independent haploid genome that is usually inherited through the mother only, whereas the diploid nuclear genome is inherited from both parents. Therefore, mitochondrial proper functioning, and in turn NST, relies on interactions between these two genomes since the "machinery" enclosed in the mitochondrion is built from proteins encoded both in the mitochondrial genome and in the nuclear genome (Blier et al. 2001; Rand et al. 2004; Dowling et al. 2008; Ballard and Pichaud 2014). Hence, the mitochondrial and the nuclear genomes have to cooperate to be efficient at producing energy as heat and, as a consequence, variation in NST may be explained by genetic variation in the nuclear and/or mitochondrial genomes as well as by interaction between the two genomes.

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The genetic and molecular pathways accounting for heat production by mitochondria enclosed in the BAT is a "hot" research topic due to the possibility of using NST as an energy burning mechanism to treat obesity (Nedergaard and Cannon 2010). As a result, there are frequent publications providing refinement in our understanding of NST regulation and production (Villarroya and Vidal-Puig 2013; Sambeat et al. 2017). The overarching mechanisms of NST regulation and production are nevertheless well accepted and rely on the activation of BAT in response to cold by the release of norepinephrine from the sympathetic nervous system, which triggers the oxidation of glucose and fatty acids to fuel the thermogenic uncoupling protein 1 (UCP1) within the inner-membrane of BAT mitochondria during (uncoupled) mitochondrial respiration (Lowell and Spiegelman 2000; Cannon and Nedergaard 2004; Golozoubova et al. 2006). UCP1 is a nuclear encoded mitochondrial protein, and thus one first obvious source of variation in NST comes from variants in the UCP1 gene (Nishimura et al. 2017; Zheng et al. 2017). NST is nonetheless a complex trait and variation in nuclear genes regulating pathways such as sensitivity of the sympathetic nervous system (Whittle et al. 2012), fatty acid oxidation (Guerra et al. 1998), or mitochondrial biogenesis (Lelliott et al. 2006) have also been demonstrated to alter NST. Interestingly, variants in mitochondrial genes such as cytochrome *b* (Mishmar et al. 2003; Ruiz-Pesini et al. 2004; Boratyński et al. 2011, 2014) or *ATP6* (Fontanillas et al. 2005; Balloux et al. 2009) have also been suggested to shape adaptation to different thermal environments. Studies of mito(chondrial)–nuclear interaction on the phenotype are however rare in animals (Rand et al. 2004; Dowling et al. 2008; Ballard and Pichaud 2014) and in particular in mammals (Roubertoux et al. 2003; Latorre-Pellicer et al. 2016). There is only one study to date suggesting that NST may be influenced by mito–nuclear interaction. In the white-toothed shrew (*Crocidura russula*), individuals from low and high elevation populations were found to carry different mitochondrial haplotypes (Ehinger et al. 2002), and females carrying the high elevation haplotype were found to have higher NST while the reverse was true for males (Fontanillas et al. 2005). Because the Y chromosome and the mitochondrial genome are not co-transmitted, this interaction between sex and haplotype may be explained by sex-linked mito–nuclear interactions (Fontanillas et al. 2005). No study until now has evaluated the relative contribution of the nuclear and mitochondrial genomes and of mito–nuclear interactions in shaping NST.

In the present study, we investigated variation in NST in wild derived common voles (*Microtus arvalis*) from two different evolutionary lineages found in Central (C) versus Western (W) Europe (Haynes et al. 2003; Fink et al. 2004; Heckel et al. 2005; Martínková et al. 2013; Lischer et al. 2014), and that are expected thus to have evolved under and be best adapted to different climatic regimes (continental vs. oceanic). We used first a common garden experiment (Kawecki and Ebert 2004) to compare C and W voles under standardized laboratory conditions and, by doing so, test whether differences between lineages were primarily genetically based rather than induced by phenotypic changes in response to local environmental conditions. Then, we used a long-term backcrossing experiment (Ballard and Melvin 2010) to transfer the mitochondrial genome from C or W donor backgrounds to both W and C nuclear backgrounds. This approach allowed us to produce all possible combinations of mitochondrial by nuclear genome interactions ($\text{nuclear}^{\text{mitochondria}}$: W^W, W^C, C^C, C^W), and therefore to dissect the contribution of these two genomes and their interaction in shaping NST. We show that common voles from the W lineage had higher NST than voles from the C lineage and that this between-lineage variation in NST is rooted in the mitochondrial genome.

Methods

Study system

The common vole is a ca. 20–30 g rodent that shows a continuous distribution from the Atlantic coast of France to central Siberia. Examination of mitochondrial and nuclear DNA has revealed a clear phylogeographic structure in European populations, with the presence of four major evolutionary lineages in Europe: W, C, Eastern, and Italian (Haynes et al. 2003; Fink et al. 2004; Heckel et al. 2005; Martínková et al. 2013; Lischer et al. 2014). The divergence across lineages has occurred before the last glacial maximum (>18,000 years ago), with the W lineage being the oldest and most divergent lineage (Heckel et al. 2005; Lischer et al. 2014). In the present study, we investigated the importance of the nuclear and mitochondrial genomes in shaping the phenotype of voles from the W lineage and of the neighboring C lineage. Although those two lineages separated at least 40,000 generations ago (Heckel et al. 2005), they still show natural introgression at their contact zone (Sutter et al. 2013; Beysard and Heckel 2014; Beysard et al. 2015).

Common garden experiment

We tested for phenotypic differences between C and W lineages of common voles using a common garden experiment (Kawecki and Ebert 2004) where W and C voles were captured in the wild and then bred in the laboratory to compare them under standardized conditions. In spring 2012 we used Longworth live traps to capture voles in three C and three W natural populations (Fig. 1). Trapping sites were chosen according to published (Heckel et al. 2005; Braaker and Heckel 2009; Beysard et al. 2015) and unpublished (M. Beysard and G. Heckel, personal communication) information on the distribution of lineages in Switzerland and in the French Jura; we avoided sampling voles in naturally introgressed populations. We confirmed the nuclear and mitochondrial lineage identity of the study populations by examining mitochondrial and nuclear DNA in a subset of the trapped voles as described in Fink et al. (2004) and Braaker and Heckel (2009).

After their capture in the wild, voles were treated with the antiparasitic drug Eprinex® before being transferred within 24 h to an animal room at the University of Lausanne, Switzerland. The animal room was set at 22 ± 1°C, 60% relative humidity, and a 14 h:10 h light–dark cycle. Voles were individually housed in propylene cages (425 mm ×266 mm×155 mm; Eurostandard Type III) with wood sawdust as bedding and their cage environment was enriched using straw and a flower pot as roost. Water and food pellets were available *ad libitum*, with apples and seed mix added as supplements weekly. Within each lineage, wild caught voles (P generation) were reproduced by introducing one randomly chosen male for 1 week in the cage of a randomly chosen female to produce the F1 generation. The same process was repeated with F1 voles to produce the F2 generation. When offspring were 21 days old (i.e., weaning), they were individually housed in the same conditions as their parents.

Backcrossing experiment

We tested the relative contribution of the nuclear or mitochondrial genomes on the phenotype using a backcrossing experiment (Ballard and Melvin 2010) where we transferred the mitochondrial genome from W or C donor backgrounds to both C and W nuclear backgrounds. For example, because mitochondria are maternally inherited, repeated crossing of mothers and their daughters from lineage C to males from lineage W produces hybrids where the C mitochondrial genome is inserted in the W nuclear background (nuclear^{mitochondria}: W^C). We applied this approach to produce two hybrid (i.e., introgressed) lineages (W^C and C^W) and compared them to the two founder lineages (W^W and C^C). Male voles were always used twice, mating them randomly with both a pure and an introgressed female. This ensured that the males' nuclear genome contributed to both of the applicable lineages and that nuclear DNA of both lineages is standardized, preventing any confounding effects caused by genetic drift or inbreeding. Females were used until they produced daughters, which was necessary to ensure that lineages and genetic diversity continued into subsequent generations. At each generation, we reproduced at least 10 different females per experimental group (i.e., C^C, C^W, W^W, W^C). Because each backcross eliminates 50% of the nuclear genes from a given mitochondrial genome donor strain, in theory, nine successive rounds of backcrossing will replace >99.5% of the initial nuclear genes from a donor background (Ballard and Melvin 2010). Hence, we included in the analyses data on voles from generation F9 and over; the experiment stopped at generation F13.

We performed the backcrossing experiment between W voles from the Mouthe population and C voles from the Ollon population (see Fig. 1). Analysis of monthly meteorological data from 2003 to 2014 shows significant differences in the climatic

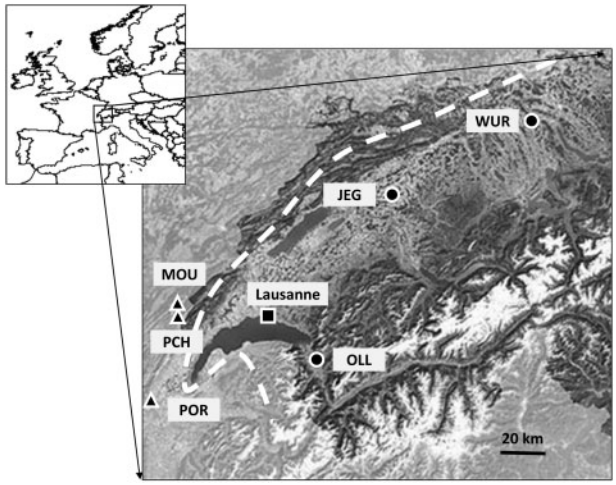

Fig. 1 Partial map of Switzerland showing the sampling sites of common vole populations from the Central (C; circles) and Western (W; triangles) evolutionary lineages. The contact zone between the C and W lineages along the Jura mountain chain is highlighted with a dashed white line. W populations were sampled in Mouthe (MOU: 46°41′08–6°08′30), Petite-Chaux (PCH: 46°41′20–6°09′30), and Por (POR: 46°09′23–5°33′47). C populations were sampled in Ollon (OLL: 46°18′00–6°58′36), Jegensdorf (JEG: 47°02′30–7°29′42), and Würenlos (WUR: 47°27′02–8°20′42). After their capture, voles were bred at the University of Lausanne (Square).

niche between the two populations, with Mouthe (W voles) being a colder place than Ollon (C voles), with (mean [95% confidence interval] minimal winter temperature: −5.5°C [−6.5, −4.6] vs. −1.9°C [−2.8, −0.9]; mean [95% CI] minimal summer temperature: 8.2°C [7.3, 9.2] vs. 13.3°C [12.4, 14.3]). Voles were bred at the University of Lausanne until the F2 generation before being relocated to the University of Aberdeen, UK. In Aberdeen, voles were kept in 48 × 15 × 13 cm M3 cages (NKP Cages, Kent, UK), at 21 ± 0.5°C and a constant 16L:8D photoperiod. Cages were enriched with straw and plastic and cardbox tubing to be used as shelter and gnawing, respectively. Water and food pellets were available *ad libitum*, with apples and seed mix added as supplements weekly. Animals were housed with litter mates of the same sex until being used for reproduction. However, when this was not possible individuals were matched with animals of the same age and experimental lineage.

Metabolic measurements

We investigated variation in resting metabolic rate (RMR) and NST capacity in wild caught voles (P generation) acclimatized for 118 ± 4.8 (mean ± SE) days to standardized laboratory conditions, in F1 and F2 voles at 51.7 ± 2.8 days of age, and in F9–F13 voles at 56.9 ± 1.5 days of age. Measurements of metabolic traits of P to F2 voles took place at the University of Lausanne and of F9–F13 at the University of Aberdeen. In Lausanne, metabolic measurements were carried out using an SMMARS-4 open flow system (Sable Systems International, Las Vegas, NV, USA) allowing the sequential measurements of oxygen consumption (VO_2; measured every 6–7.5 min) of three animals in parallel following a protocol previously described in Lehto Hürlimann et al. (2014). In Aberdeen, metabolic measurements were carried out using an open-flow Servomex respirometer system (Crowburgh, UK) allowing the continuous measurements (every 30 s) of two animals in parallel following a protocol previously described in Johnson et al. (2001). Each run lasted about 3 h, with the measures of RMR taking place in the first 2 h and of NST in the last hour. Animals were weighed (to 0.1 g) just before being put in the metabolic chamber.

RMR was defined as the lowest metabolic rate of inactive, partial post-absorptive individuals measured in a metabolic chamber set at 30°C in Lausanne (within the thermoneural zone; Devevey et al. 2008) and at 21°C in Aberdeen. One hour before starting a run, food, but not water, was withdrawn from the experimental animals to ensure that they were in a partial post-absorptive state during the measurements. RMR was measured as the mean of the lowest consecutive readings of VO_2 over a period of 12 min (two readings) in Lausanne and over a period of 5 min (10 readings) in Aberdeen.

NST was measured as the animal's peak oxygen consumption (maximum metabolic rate) in response to an injection of noradrenaline subtracted from the measure of RMR. The hormone noradrenalin is known to specifically activate the UCP1 within the BAT that, in turn, generates heat production or NST (Golozoubova et al. 2006). Animals were not anesthetized during NST measurement. Hence, the increase in VO_2 consumption following the injection of noradrenaline might be caused by a stress induced response associated to handling and injecting the animal *per se* rather than caused by the pharmacological effects of noradrenaline on UCP1 (Golozoubova et al. 2006; Cannon and Nedergaard 2011). Preliminary observations showed that the peak of oxygen consumption in response to an injection of noradrenaline is much higher than the peak of oxygen consumption induced by a sham (stress *per se*) injection (P. Bize, personal observation). Thus, our measures of peak of oxygen consumption in response to a noradrenalin injection can be safely used to compute NST measures. Because voles were not anesthetized, our measures of NST include O_2 consumption driven by BAT activation and animal activity. Of note, measures of cage activity using movement detectors indicated that voles were less active during measures of NST compared with measures of RMR (M. Lehto Hürlimann, personal observation). Voles were subcutaneously injected between the shoulder blades (near the BAT deposits), with 0.5 μg/g of 0.15 μg/μL noradrenaline solution (Golozoubova et al. 2006). NST was measured in a metabolic chamber set at a temperature of 22 ± 1°C both in Lausanne and in Aberdeen to minimize the risk of hyperthermia and death (P. Bize, personal observation).

Statistical analyses

We compared differences in body weight (to 0.1 g) between groups using data collected in the field (P generation) and at culling (P, F1–F2 in the common garden experiment, F9–F13 in the backcrossing experiment). Animals were culled usually soon after having been reproduced. In the common garden experiment, we analyzed variation in body weight and metabolic traits of both male and female adult voles. We restricted our analyses to females in the

Table 1 Description of the sample sizes used in the statistical analyses of body weight, resting metabolic rate (RMR), nonshivering thermogenesis (NST), and the size of the brown adipose tissue (BAT) of common voles in the common garden and backcrossing experimental design

			Group				
Trait	Design	Generation	C^C	W^C	C^W	W^W	Total
Body weight	Common garden	P	83			59	142
		F1–F2	118			114	232
	Backcrossing	F9–F13	59	97	74	77	307
RMR and NST	Common garden	P	23			17	40
		F1–F2	48			39	87
	Backcrossing	F9–F13	8	11	10	9	38
BAT	Backcrossing	F9–F13	28	32	33	34	127

Notes: Voles in the common garden experiment were from C (nuclear^{mitochondrial} genomes: C^C) and W (W^W) evolutionary lineages. Hybrids showing a mismatch between the mitochondrial and nuclear genomes (W^C, C^W) were generated using a back-crossing experiment (see the "Methods" section for more information).

backcrossing experiment since hybrid males were not used for reproduction.

We analyzed data from the P generation by including the effects of lineage, sex, and capture site (population ID) nested within lineage as fixed effects. We analyzed data from the common garden experiment (F1 and F2) by including lineage and sex as fixed effects, and by including father ID, mother ID, and generation as random effects. We analyzed data from the backcrossing experiment (F9–F13) by including the mitochondrial and nuclear (W vs. C) genome identities, plus their interaction, as fixed effects, and by including father ID, mother ID, and generation as random effects. Number of days of acclimation in the laboratory (P generation) or vole's age (F1 and over) were included as a fixed covariate in the statistical models when relevant. RMR and NST values were log-transformed for the statistical analyses, and for those analyses we included the log-transformed measure of body weight recorded just before putting the animal in the metabolic chamber as a fixed explanatory covariate. We measured BAT size (to 0.001 g) just after culling in a subset of non-reproducing female voles from the backcrossing experiment ($N = 127$ individuals; Table 1). BAT size values were log-transformed and for the statistical analysis we included log-transformed animal body weight at culling as a covariate. Sample sizes for the different traits and experimental groups are detailed in Table 1.

All the statistical models were run using the R cran version 3.2.1. Mixed models were run using the R package "lme4" (Bates et al. 2015). The significance of fixed effects was tested using the R package "lmerTest" and function Anova, with denominator degrees of freedom calculated using Satterthwaite's approximation (Kuznetsova et al. 2013). The different covariates included in the models are reported in the text. No model selection was performed on random effects, which were kept in all final models. Results are reported as mean ± SE. Significant results are for $P < 0.05$.

Ethical note

All animal work in Switzerland was conducted in accordance to the Vaud Veterinary Service Licence 2247.2 and in Scotland in accordance to UK Home Office Licence 70/8147 and UK Animals (Scientific Procedures) Act 1986.

Results

Common garden experiment

Voles trapped in the wild (P generation) showed no difference in body weight on their day of capture between W and C lineages (lineage: $F_{1,135} = 2.06$, $P = 0.154$), after controlling in the same model for variation in body weight explained by capture site (population ID nested in the lineage: $F_{4,135} = 2.51$, $P = 0.045$) and sexes ($F_{1,135} = 5.11$, $P = 0.025$). *Post hoc* Tukey tests showed however no significant difference between capture sites (all *P*-values >0.11). Measures of the same individuals still alive after 107.8 ± 7.6 days of housing in an animal room showed the same patterns: there was no difference in body weight between C and W voles (lineage: $F_{1,114} = 2.03$, $P = 0.157$; Fig. 2), and males were heavier than females ($F_{1,114} = 7.44$, $P = 0.007$). Voles from the P generation showed no difference in RMR between C and W lineages ($F_{1,30} = 0.51$, $P = 0.482$). However, we found higher NST in W voles compared with C voles ($F_{1,30} = 8.48$, $P = 0.007$; Fig. 1). We controlled in our analyses for the positive effect of body weight on RMR and NST (all *P*-values <0.01), and for potential effects of sex, acclimation length, and capture site on RMR and NST (all *P*-values >0.05).

Analyses of body weight and metabolic traits in C and W voles born and bred in an animal room for up to two generations (F1 and F2) showed similar results as found in the P generation. That is, we found no significant difference between C and W voles in body weight ($P = 0.445$) and RMR ($P = 0.118$), but W voles had higher NST than C

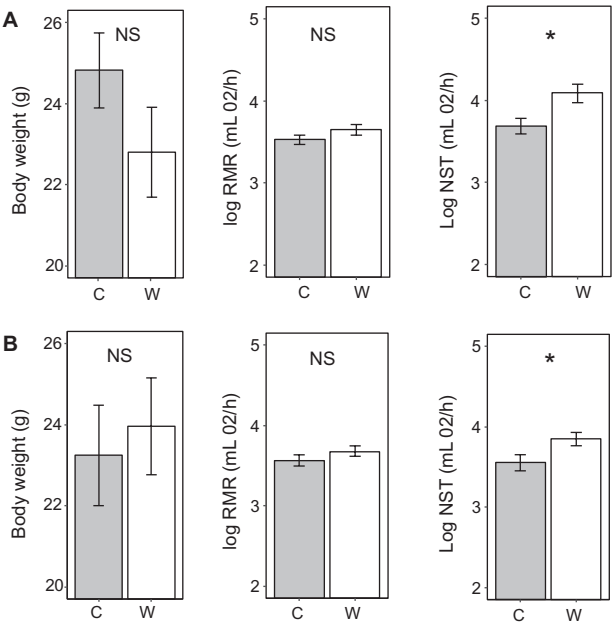

Fig. 2 Body weight, resting metabolic rate (RMR), and nonshivering thermogenesis (NST) as a function of evolutionary lineage (W, C) in (**A**) wild caught (P generation) adult common voles and in (**B**) their offspring housed under standardized conditions for two generations (F1, F2). Number of P voles analyzed for body weight, RMR, and NST are, respectively, 142, 39, and 39. Number of F1 and F2 voles analyzed for body weight, RMR, and NST are, respectively, 232, 87, and 87. Significant differences between lineages are denoted by * and non-significant differences by NS.

voles ($P = 0.005$; Table 2 and Fig. 2). We controlled in our analyses for the positive effect of body weight on RMR and NST and for potential effects of sex and age on body weight and metabolic traits (see Table 2).

Backcrossing experiment

The long-term backcrossing of voles from C and W lineages showed that the body weight of adult females was significantly influenced by interaction between the mitochondrial and nuclear genomes ($P = 0.007$; Table 3). Insertion of the W mitochondrial genome in the C nuclear background led to a significant reduction in body weight of C^W hybrid females (*post hoc* test: $P = 0.014$), whereas no significant reduction in body weight was observed in W^C hybrid females ($P = 0.18$; Fig. 3). In this model, voles' age and age squared were entered as covariates to account for quadratic changes in body weight with age ($P < 0.014$; Table 3). Variation in BAT weight was significantly explained by the nuclear genome ($P = 0.027$; Table 3), and not by mitochondrial genome ($P = 0.93$; Table 3) or the interaction between the nuclear and mitochondrial genomes ($P = 0.36$; this interaction was dropped from the final model in Table 3). Females with a W nuclear genome had a heavier BAT than females with a C nuclear genome independently of their mitochondrial genome (Fig. 3). In this analysis, we controlled for the fact that BAT weight increased with the whole organism body weight ($P < 0.001$; Table 3) and decreased linearly with age ($P < 0.001$; Table 3).

Female RMR was significantly influenced by interaction between the mitochondrial and nuclear genomes ($P = 0.032$; Table 3), with insertion of the W mitochondrial genome in the C nuclear background leading to a significant increase in RMR of C^W hybrid females compared with C^C females (*post hoc* test: $P = 0.042$; Fig. 3). Female NST was significantly influenced by the mitochondrial genome ($P = 0.005$; Table 3), and not by the nuclear genome ($P = 0.261$; Table 3) or by the interaction between the nuclear and mitochondrial genomes ($P = 0.431$; this interaction was dropped from the final model in Table 3 and Fig. 3). Females with a W mitochondrial genome had higher NST whatever their nuclear background (Fig. 3).

Discussion

This study aimed at investigating the sources of variation in NST (but also RMR, body mass, and BAT size) between two evolutionary lineages of common voles that have separated at least 40,000 generations ago (Heckel et al. 2005) and that are distributed in two different regions of Europe, with the W and C lineage being found, respectively, in W and C Europe. We used first a common garden experiment (Kawecki and Ebert 2004) to demonstrate that W voles had higher NST than C voles. We then performed a long-term backcrossing experiment (Ballard and Melvin 2010) to swap the mitochondrial and nuclear genomes between lineages, and in so doing tested the relative contribution of each genome and their interaction in shaping NST. It revealed that between-lineage variation in NST was significantly influenced by the mitochondrial genome: voles with a W mitochondrial genome had higher NST (i.e., W^W and C^W voles had higher NST than C^C and W^C voles). Our backcrossing experiment also showed a significant influence of the nuclear genome on between-lineage variation in BAT size, voles with a W nuclear genome having larger BAT, and significant mito–nuclear interactions on variation in body weight and RMR. Overall, our findings turn new light on the influence of the mitochondrial and nuclear genomes on NST and in shaping adaptation to the environment in mammals.

Table 2 Results of linear mixed models describing the influence of the evolutionary lineage (C vs. W) on phenotypic traits in adult common voles housed for up to two generation in a common garden environment

Fixed effect	Estimate	SE	DF_{num}	DF_{den}	*F*-value	*P*-value
Body weight (g)						
$V_{residuals}$=23.12 [18.43, 28.54] (*N*=232 voles), V_{mother}=4.79 [0.79, 10.90] (*N*=78 mothers), V_{father}=0 [0, 2.61] (*N*=81 father), $V_{generation}$=1.54 [0, 14.33] (*N*=2 generations)						
Lineage [W]	0.710	0.923	1	52.0	0.59	0.445
Sex [m]	5.474	0.683	1	218.0	64.28	<0.001
Age	0.068	0.014	1	199.2	22.28	<0.001
Age^2	−1.11E−04	3.88E−05	1	211.4	8.19	0.005
RMR (log-transformed)						
$V_{residuals}$=0.043 [0.029, 0.060] (*N*=87 voles), V_{mother}=0 [0, 0.010] (*N*=31 mothers), V_{father}=0.003 [0, 0.017] (*N*=32 father), $V_{generation}$=0.005 [0, 0.052] (*N*=2 generations)						
Lineage [W]	0.089	0.054	1	17.9	2.70	0.118
Sex [m]	−0.106	0.055	1	81.0	3.72	0.057
Age	−0.001	0.001	1	65.0	0.30	0.587
log(body weight)	0.593	0.122	1	77.2	22.57	<0.001
Nonshivering thermogenesis (NST) (log-transformed)						
$V_{residuals}$=0.214 [0.152, 0.275] (*N*=87 voles), V_{mother}=0 [0, 0.39] (*N*=31 mothers), V_{father}=0 [0, 0.030] (*N*=32 father), $V_{generation}$=0 [0, 0.028] (*N*=2 generations)						
Lineage [W]	0.297	0.103	1	82.0	8.31	0.005
Sex [m]	−0.012	0.119	1	82.0	0.01	0.922
Age	−0.003	0.002	1	82.0	2.26	0.136
log(body weight)	1.483	0.259	1	82.0	32.81	<0.001

Voles' sex, age, and log-transformed body weight were included as fixed effects when relevant. Mother identity, father identity, and generation were included as random effects. The partition of variance (95% CI) and the sample size are given for each model.

The main seat of heat production during NST is within the mitochondria contained in the BAT (Lowell and Spiegelman 2000; Cannon and Nedergaard 2004; Oelkrug et al. 2015), and most of the proteins enclosed in the mitochondria and responsible for mitochondrial respiration and NST are encoded in the nuclear genome (Calvo et al. 2016). Hence, predictably, most of the genetic variants so far associated with variation in NST have been found in the nuclear genome (e.g., Guerra et al. 1998; Lelliott et al. 2006; Whittle et al. 2012; Nishimura et al. 2017; Zheng et al. 2017, but see Fontanillas et al. 2005). The finding of the present study highlighting a significant influence only due to the mitochondrial genome on between-lineage difference in NST is surprizing. This result is even more striking since variation in BAT size was found to be related to the nuclear genome. Our results on NST are nonetheless concordant with findings in first generation interspecific hybrid carnivorous mice (*Onychomys* sp.) (Shipley et al. 2016) and first generation crosses between wild and random-bred laboratory house mice (*Mus domesticus*) (Richardson et al. 1994) showing strong maternal effects, potentially caused by mitochondrial effects, on animal RMR and NST, respectively (see also Boratyński et al. [2016] for sex-specific effects on RMR in introgressed voles). At least two different scenarios could account for effects of the mitochondrial genome on NST.

First, mito–nuclear interaction may distort the inheritance of nuclear genes across generations, favoring the co-inheritance of mito–nuclear genes with strong positive epistatic interactions or preventing the co-inheritance of epistatic mito–nuclear genes with incompatibilities which is a general drawback of backcrossing experiments (Trounce et al. 1994; Ballard and Melvin 2010). In this study, we analyzed hybrid animals that underwent at least nine rounds of introgression and for which, in theory, 99.8% of their initial nuclear genome had been replaced by the donor genome. Yet, it still leaves a concordance of up to 0.2% between the initial nuclear and mitochondrial genomes. Therefore, we

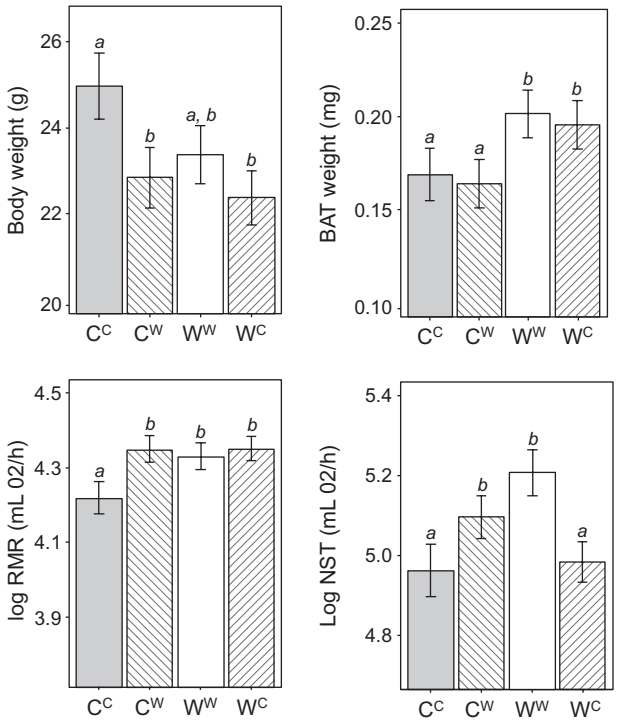

Fig. 3 Total body weight, weight of brown adipose tissue (BAT), RMR, and NST as a function of nuclear and mitochondrial genomes background (nuclear^{mitochondrial}) of adult female common voles from a long-term (>F9) backcrossing experiment. Number of voles analyzed for body weight, BAT, RMR, and NST are, respectively, 307, 127, 38, and 38. Letters represent results of *post hoc* tests, where different letters are attributed to significantly different groups.

cannot fully exclude that between group differences in NST were explained by nuclear genes co-segregating with the mitochondrial genome (Trounce et al. 1994) and having strong epistatic effects on NST. If true, this scenario nonetheless still supports the idea that the mitochondrial genome is an important player and influences NST, but that effects of the mitochondrial genome on NST are indirect and rely on epistatic interactions with one or more (unidentified) co-segregating nuclear genes with major effects on NST.

Alternatively, assuming a random segregation of the nuclear genes influencing NST, variation in the mitochondrial genome may have direct consequences on NST through retrograde signaling from mitochondria to the nucleus (Butow and Avadhani 2004; Nam et al. 2017). Accordingly, it has been recently demonstrated that a reduction in mitochondrial respiratory capacity in laboratory mouse BAT activates retrograde signaling pathways that downregulate thermogenic nuclear gene expression, and in turn BAT function and NST capacity (Nam et al. 2017). This scenario points out mitochondria as master regulators of the phenotype (Galluzzi et al. 2012; Horan et al. 2013). It posits that variants in the mitochondrial genome can be associated with subtle changes in mitochondrial respiratory capacity that can have cascading effects on the nuclear genome that can in turn induce patent changes at the whole organism level such as NST. To tease apart those two scenarios, work is now needed using microinjection or cybrid cells to fully break down the co-segregation and inheritance of mitochondrial and nuclear genomes, and thus to provide an unambiguous test of effects of the mitochondrial and nuclear genomes on mitochondrial respiratory capacity and nuclear gene expression. Future studies incorporating measures of BAT gene expression, protein content, and mitochondrial function are needed to provide a full picture of the proximate mechanisms leading to the difference in NST between groups.

Mitochondrial energy transduction depends on respiratory supercomplexes made of proteins encoded both in the nuclear and in the mitochondrial genomes (Blier et al. 2001; Rand et al. 2004), and epistatic interactions between those two genomes at the protein level could affect suites of phenotypic traits that rely on energy (Ballard and Melvin 2010; Ballard and Pichaud 2014). A mismatch between these two genomes has been suggested as an importance speciation force (Gershoni et al. 2009) that can account for the formation of reproductive barriers at contact zones between closely related but phylogenetically distinct lineages if this mismatch leads to a breakdown in mito–nuclear interactions (Burton et al. 2006; Barreto and Burton 2013). It predicts the occurrence of significant mito–nuclear interaction on phenotypic traits, and in particular impaired phenotypic values in hybrid (introgressed) individuals. Studies on secondary contact zones between W and C voles showed that those two evolutionary lineages are naturally hybridizing but that there is a strong selection against male hybrids in the wild (Sutter et al. 2013; Beysard and Heckel 2014; Beysard et al. 2015). Furthermore, since W voles are losing ground to C voles in the contact zone, it has also been suggested that hybridization may be more detrimental to the W lineage (Beysard and Heckel 2014; Beysard et al. 2015). In this study, we were able to hybridize C and W voles over more than 10 generations, thus providing no evidence of strong mito–nuclear incompatibilities (Burton and Barreto 2012) between those two evolutionary lineages of common voles. Our results support nonetheless the existence of mito–nuclear effects on the phenotype of female voles, with hybrid voles being lighter and having a higher RMR. Those effects were

Table 3 Results of linear mixed models describing the influence of the mitochondrial (mtDNA) and nuclear genomes (nDNA) and their interaction (mtDNA×nDNA) on phenotypic traits in adult female voles

Fixed effect	Estimate	SE	DF_{num}	DF_{den}	*F*-value	*P*-value
Body weight (g)						
$V_{residuals}$=15.41 [12.38, 19.29] (*N*=307 voles), V_{mother}=2.85 [0, 7.05] (*N*=159 mothers), V_{father}=2.18 [0, 5.96] (*N*=94 fathers), $V_{generation}$=0.27 [0, 2.20] (*N*=5 generations)						
mtDNA [W]	−2.126	0.852	1	97.3	0.98	0.325
nDNA [W]	−2.598	0.856	1	74.4	2.63	0.109
mtDNA×nDNA	3.132	1.133	1	95.1	7.64	0.007
Age	0.090	0.022	1	298.0	17.30	<0.001
Age^2	0.000	0.000	1	299.1	6.08	0.014
BAT weight (g) (log-transformed)						
$V_{residuals}$=0.076 [0.047, 0.124] (*N*=127 voles), V_{mother}=0.045 [0, 0.092] (*N*=97 mothers), V_{father}=0.007 [0, 0.056] (*N*=68 fathers), $V_{generation}$=0 [0, 0.014] (*N* = 5 generations)						
mtDNA [W]	−0.001	0.068	1	57.7	0.01	0.930
nDNA [W]	0.166	0.070	1	58.7	5.17	0.027
log(body weight)	2.222	0.150	1	119.5	214.92	<0.001
Age	−0.006	0.001	1	118.8	84.20	<0.001
RMR (log-transformed)						
$V_{residuals}$=0.004 [0.001, 0.015] (*N*=38 voles), V_{father}=0.009 [0, 0.021] (*N*=30), $V_{generation}$=0 [0, 0.004] (*N*=5)						
mtDNA [W]	0.130	0.046	1	4.6	3.25	0.136
nDNA [W]	0.132	0.055	1	19.8	1.72	0.205
mtDNA×nDNA	−0.150	0.061	1	10.5	6.10	0.032
log(body weight)	0.545	0.106	1	8.6	26.67	0.001
Nonshivering thermongenesis (NST) (log-transformed)						
$V_{residuals}$=0.028 [0.017, 0.041] (*N*=38 voles), V_{father}=0 [0, 0.018] (*N*=30 father), $V_{generation}$=0 [0, 0.006] (*N*=5)						
mtDNA [W]	0.254	0.254	1	33.0	8.91	0.005
nDNA [W]	0.037	0.037	1	33.0	1.31	0.261
log(body weight)	0.025	0.025	1	33	0.89	0.352

Data are from a long-term backcrossing experiment (more than eight generations) between voles from W and C evoluationary lineages. Voles' age and body weight were included as fixed covariates when relevant. Non-significant interactions were dropped from the final statistical model. Mother identity, father identity, and generation were included as random effects when relevant. The partition of variance (95% CI) and the sample size are given for each model.

stronger in C^W than W^C hybrid voles and thus, if anything, hybridization was more detrimental to the C lineage. There are two important caveats to our results. First, due to time and animal room constraints, we restricted our analyses to hybrid females. Yet, the mitochondrial genome is inherited solely from the mother and males represent an evolutionary dead-end (Gemmell et al. 2004), which predicts the occurrence of stronger negative mito–nuclear interactions in males than females (Gemmell et al. 2004; Innocenti et al. 2011). Second, the co-evolutionary process between mitochondrial and nuclear genes is likely to be sensitive to environmental factors (Arnqvist et al. 2010; Hoekstra et al. 2013; Baris et al. 2016), with different mito–nuclear combinations providing (metabolic) local adaptation to alternative environments. Hence, insertion of mitochondrial variants in alternative nuclear backgrounds may have negative, neutral, or even positive effects depending on the environment under which mito–nuclear interactions are tested (Arnqvist et al. 2010; Hoekstra et al. 2013; Baris et al. 2016). Therefore, work including males and testing differences between lineages among more than one environmental conditions is now needed to gain a full appraisal of the fitness consequences of mito–nuclear interaction in the common vole.

In conclusion, our study shows a strong association between the mitochondrial genome and NST in wild derived voles, as well as strong effects of mito–nuclear interactions on body weight and RMR. Our results support therefore the growing hypothesis that the mitochondrial genome is not an evolutionary bystander but can contribute to building adaptation to the environment, and in particular to the thermal environment of mammals (Blier et al. 2001; Rand et al. 2004; Dowling et al. 2008; Ballard and Melvin 2010; Ballard and Pichaud 2014).

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References

Arnqvist G, Dowling DK, Eady P, Gay L, Tregenza T, Tuda M, Hosken DJ. 2010. The genetic architecture of metabolic rate: environment specific epistasis between mitochondiral and nuclear genes in an insect. Evolution 64:3354–63.

Ballard JWO, Melvin RG. 2010. Linking the mitochondrial genotype to the organismal phenotype. Mol Ecol 19:1523–39.

Ballard JWO, Pichaud N. 2014. Mitochondrial DNA: more than an evolutionary bystander. Funct Ecol 28:218–31.

Balloux F, Handley L-JL, Jombart T, Liu H, Manica A. 2009. Climate shaped the worldwide distribution of human mitochondrial DNA sequence variation. Proc R Soc Lond B Biol Sci 276:3447–55.

Baris TZ, Blier PU, Pichaud N, Crawford DL, Oleksiak MF. 2016. Gene by environmental interactions affecting oxidative phosphorylation and thermal sensitivity. Am J Physiol Regul Integr Comp Physiol 311:R157–65.

Barreto FS, Burton RS. 2013. Elevated oxidative damage is correlated with reduced fitness in interpopulation hybrids of a marine copepod. Proc R Soc Lond B Biol Sci 280:20131521.

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

Beysard M, Heckel G. 2014. Structure and dynamics of hybrid zones at different stages of speciation in the common vole (*Microtus arvalis*). Mol Ecol 23:673–87.

Beysard M, Krebs-Wheaton R, Heckel G. 2015. Tracing reinforcement through asymmetrical partner preference in the European common vole *Microtus arvalis*. BMC Evol Biol 15:170.

Blier PU, Dufresne F, Burton RS. 2001. Natural selection and the evolution of mtDNA-encoded peptides: evidence for intergenomic co-adaptation. Trends Genet 17:400–6.

Boratyński Z, Alves PC, Berto S, Koskela E, Mappes T, Melo-Ferreira J. 2011. Introgression of mitochondrial DNA among *Myodes* voles: consequences for energetics? BMC Evol Biol 11:355.

Boratyński Z, Ketola T, Koskela E, Mappes T. 2016. The sex specific genetic variation of energetics in bank voles, consequences of introgression? Evol Biol 43:37–47.

Boratyński Z, Melo-Ferreira J, Alves PC, Berto S, Koskela E, Pentikäinen OT, Tarroso P, Ylilauri M, Mappes T. 2014. Molecular and ecological signs of mitochondrial adaptation: consequences for introgression? Heredity 113:277.

Braaker S, Heckel G. 2009. Transalpine colonisation and partial phylogeographic erosion by dispersal in the common vole (*Microtus arvalis*). Mol Ecol 18:2518–31.

Burton RS, Barreto FS. 2012. A disproportionate role for mtDNA in Dobzhansky–Muller incompatibilities? Mol Ecol 21:4942–57.

Burton RS, Ellison CK, Harrison JS. 2006. The sorry state of F2 hybrids: consequences of rapid mitochondrial DNA evolution in allopatric populations. Am Nat 168:S14–24.

Butow RA, Avadhani NG. 2004. Mitochondrial signaling: the retrograde response. Mol Cell 14:1–15.

Calvo SE, Clauser KR, Mootha VK. 2016. MitoCarta2.0: an updated inventory of mammalian mitochondrial proteins. Nucleic Acids Res 44:D1251–7.

Cannon B, Nedergaard J. 2004. Brown adipose tissue: function and physiological significance. Physiol Rev 84: 277–359.

Cannon B, Nedergaard J. 2011. Nonshivering thermogenesis and its adequate measurement in metabolic studies. J Exp Biol 214:242–53.

Devevey G, Niculita-Hirzel H, Biollaz F, Yvon C, Chapuisat M, Christe P. 2008. Developmental, metabolic and immunological costs of flea infestation in the common vole. Funct Ecol 22:1091–8.

Dowling DK, Friberg U, Lindell J. 2008. Evolutionary implications of non-neutral mitochondrial genetic variation. Trends Ecol Evol 23:546–54.

Ehinger M, Fontanillas P, Petit E, Perrin N. 2002. Mitochondrial DNA variation along an altitudinal gradient in the greater white-toothed shrew, *Crocidura russula*. Mol Ecol 11:939–45.

Fink S, Excoffier L, Heckel G. 2004. Mitochondrial gene diversity in the common vole *Microtus arvalis* shaped by

historical divergence and local adaptations. Mol Ecol 13:3501–14.
Fontanillas P, Depraz A, Giorgi MS, Perrin N. 2005. Non-shivering thermogenesis capacity associated to mitochondrial DNA haplotypes and gender in the greater white-toothed shrew, *Crocidura russula*. Mol Ecol 14:661–70.
Galluzzi L, Kepp O, Kroemer G. 2012. Mitochondria: master regulators of danger signalling. Nat Rev Mol Cell Biol 13:780.
Gemmell NJ, Metcalf VJ, Allendorf FW. 2004. Mother's curse: the effect of mtDNA on individual fitness and population viability. Trends Ecol Evol 19:238–44.
Gershoni M, Templeton AR, Mishmar D. 2009. Mitochondrial bioenergetics as a major motive force of speciation. BioEssays 31:642–50.
Golozoubova V, Cannon B, Nedergaard J. 2006. UCP1 is essential for adaptive adrenergic nonshivering thermogenesis. Am J Physiol 291:E350–7.
Guerra C, Koza RA, Walsh K, Kurtz DM, Wood PA, Kozak LP. 1998. Abnormal nonshivering thermogenesis in mice with inherited defects of fatty acid oxidation. J Clin Invest 102:1724–31.
Haynes S, Jaarola M, Searle JB. 2003. Phylogeography of the common vole (*Microtus arvalis*) with particular emphasis on the colonization of the Orkney archipelago. Mol Ecol 12:951–6.
Heckel G, Burri R, Fink S, Desmet JF, Excoffier L. 2005. Genetic structure and colonization processes in European populations of the common vole, *Microtus arvalis*. Evolution 59:2231–42.
Hoekstra LA, Siddiq MA, Montooth KL. 2013. Pleiotropic effects of a mitochondrial–nuclear incompatibility depend upon the accelerating effect of temperature in *Drosophila*. Genetics 195:1129–39.
Horan MP, Gemmell NJ, Wolff JN. 2013. From evolutionary bystander to master manipulator: the emerging roles for the mitochondrial genome as a modulator of nuclear gene expression. Eur J Hum Genet 21:1335–7.
Innocenti P, Morrow EH, Dowling DK. 2011. Experimental evidence supports a sex-specific selective sieve in mitochondrial genome evolution. Science 332:845–8.
Johnson MS, Thomson SC, Speakman JR. 2001. Limits to sustained energy intake II. Inter-relationships between resting metabolic rate, life-history traits and morphology in *Mus musculus*. J Exp Biol 204:1937–46.
Kawecki TJ, Ebert D. 2004. Conceptual issues in local adaptation. Ecol Lett 7:1225–41.
Kuznetsova A, Brockhoff PB, Christensen RHB. 2013. lmerTest: tests for random and fixed effects for linear mixed effect models (lmer objects of lme4 package) (http://cran.r-project.org/package=lmerTest).
Latorre-Pellicer A, Moreno-Loshuertos R, Lechuga-Vieco AV, Sánchez-Cabo F, Torroja C, Acín-Pérez R, Calvo E, Aix E, González-Guerra A, Logan A, et al. 2016. Mitochondrial and nuclear DNA matching shapes metabolism and healthy ageing. Nature 535:561–5.
Lehto Hürlimann M, Stier A, Scholly O, Criscuolo F, Bize P. 2014. Short- and long-term effects of litter size manipulation in a small wild-derived rodent. Biol Lett 10:20131096.
Lelliott CJ, Medina-Gomez G, Petrovic N, Kis A, Feldmann HM, Bjursell M, Parker N, Curtis K, Campbell M, Hu P, et al. 2006. Ablation of PGC-1β results in defective mitochondrial activity, thermogenesis, hepatic function, and cardiac performance. PLOS Biol 4:e369.
Lischer HEL, Excoffier L, Heckel G. 2014. Ignoring heterozygous sites biases phylogenomic estimates of divergence times: implications for the evolutionary history of *Microtus* voles. Mol Biol Evol 31:817–31.
Lowell BB, Spiegelman BM. 2000. Towards a molecular understanding of adaptive thermogenesis. Nature 404:652–60.
Martínková N, Barnett R, Cucchi T, Struchen R, Pascal M, Pascal M, Fischer MC, Higham T, Brace S, Ho SYW, et al. 2013. Divergent evolutionary processes associated with colonization of offshore islands. Mol Ecol 22:5205–20.
Mishmar D, Ruiz-Pesini E, Golik P, Macaulay V, Clark AG, Hosseini S, Brandon M, Easley K, Chen E, Brown MD, et al. 2003. Natural selection shaped regional mtDNA variation in humans. Proc Natl Acad Sci U S A 100:171–6.
Nam M, Akie TE, Sanosaka M, Craige SM, Kant S, Keaney JF Jr, Cooper MP. 2017. Mitochondrial retrograde signaling connects respiratory capacity to thermogenic gene expression. Sci Rep 7:2013.
Nedergaard J, Cannon B. 2010. The changed metabolic world with human brown adipose tissue: therapeutic visions. Cell Metab 11:268–72.
Nishimura T, Katsumura T, Motoi M, Oota H, Watanuki S. 2017. Experimental evidence reveals the UCP1 genotype changes the oxygen consumption attributed to non-shivering thermogenesis in humans. Sci Rep 7:5570.
Oelkrug R, Polymeropoulos ET, Jastroch M. 2015. Brown adipose tissue: physiological function and evolutionary significance. J Comp Physiol B 185:587–606.
Rand DM, Haney RA, Fry AJ. 2004. Cytonuclear coevolution: the genomics of cooperation. Trends Ecol Evol 19:645–53.
Richardson CS, Dohm MR, Garland T. 1994. Metabolism and thermoregulation in crosses between wild and random-bred laboratory house mice (*Mus domesticus*). Physiol Zool 67:944–75.
Roubertoux PL, Sluyter F, Carlier M, Marcet B, Maarouf-Veray F, Chérif C, Marican C, Arrechi P, Godin F, Jamon M, et al. 2003. Mitochondrial DNA modifies cognition in interaction with the nuclear genome and age in mice. Nat Genet 35:65–9.
Ruiz-Pesini E, Mishmar D, Brandon M, Procaccio V, Wallace DC. 2004. Effects of purifying and adaptive selection on regional variation in human mtDNA. Science 303:223–6.
Sambeat A, Gulyaeva O, Dempersmier J, Sul HS. 2017. Epigenetic regulation of the thermogenic adipose program. Trends Endocrinol Metab 28:19–31.
Shipley JR, Campbell P, Searle JB, Pasch B. 2016. Asymmetric energetic costs in reciprocal-cross hybrids between carnivorous mice (*Onychomys*). J Exp Biol 219:3803–9.
Sutter A, Beysard M, Heckel G. 2013. Sex-specific clines support incipient speciation in a common European mammal. Heredity 110:398–404.
Trounce I, Neill S, Wallace DC. 1994. Cytoplasmic transfer of the mtDNA nt 8993 T → G (ATP6) point mutation associated with Leigh syndrome into mtDNA-less cells demonstrates cosegregation with a decrease in state III respiration and ADP/O ratio. Proc Natl Acad Sci U S A 91:8334–8.
Villarroya F, Vidal-Puig A. 2013. Beyond the sympathetic tone: the new brown fat activators. Cell Metab 17:638–43.

Whittle AJ, Carobbio S, Martins L, Slawik M, Hondares E, Vázquez MJ, Morgan D, Csikasz RI, Gallego R, Rodriguez-Cuenca S, et al. 2012. BMP8B increases brown adipose tissue thermogenesis through both central and peripheral actions. Cell 149:871–85.

Zheng Q, Lin J, Huang J, Zhang H, Zhang R, Zhang X, Cao C, Hambly C, Qin G, Yao J, et al. 2017. Reconstitution of *UCP1* using CRISPR/Cas9 in the white adipose tissue of pigs decreases fat deposition and improves thermogenic capacity. Proc Natl Acad Sci U S A 114:E9474–82.