Effects of the mitochondrial and nuclear genomes on nonshivering thermogenesis in a wild derived rodent

Pierre Bize[1,2], Imogen Lowe[1], Mikko Lehto Hürlimann[2], Gerald Heckel[3]

[1]School of Biological Sciences, University of Aberdeen, Zoology Building, AB24 2TZ Aberdeen, United Kingdom

[2]Department of Ecology and Evolution, University of Lausanne, Biophore, CH-1015 Lausanne, Switzerland

[3]Institute of Ecology and Evolution, University of Bern, Baltzerstrasse 6, CH-3012, Bern, Switzerland.

E-mail: pierre.bize@abdn.ac.uk

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Abstract. 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 therefore one overlooked hypothesis is that both genomes and their interaction may shape NST. By housing under standardised conditions wild-derived common voles (*Microtus arvalis*) from two distinct evolutionary lineages (Western and Central), we show that Western voles had greater NST than Central voles. By introgressing those two lineages over at least 9 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 Western mitochondrial genotype being associated with higher NST and the Western nuclear genotype with a larger BAT. There were significant mito-nuclear interactions on whole animal body weight and resting metabolic rate. Hybrid voles were lighter and had higher resting metabolic rate. 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) (Cannon and Nedergaard 2004; Lowell and Spiegelman 2000; Oelkrug and others 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 (Ballard and Pichaud 2014; Blier and others 2001; Dowling and others 2008; Rand and others 2004). 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.

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 (Sambeat and others 2017; Villarroya and Vidal-Puig 2013). 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 (Cannon and Nedergaard 2004; Golozoubova and others 2006; Lowell and Spiegelman 2000). 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 and others 2017; Zheng and others 2017). NST is nonetheless a complex trait and variation in nuclear genes regulating pathways such as sensitivity of the sympathetic nervous system (Whittle and others 2012), fatty acid oxidation (Guerra and others 1998) or mitochondrial biogenesis (Lelliott and others 2006) have also been demonstrated to alter NST. Interestingly, variants in mitochondrial genes such as cytochrome *b* (Boratyński and others 2011; Boratyński and others 2014; Mishmar and others 2003; Ruiz-Pesini and others 2004) or *ATP6* (Balloux and others 2009; Fontanillas and others 2005) 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 (Ballard and Pichaud 2014; Dowling and others 2008; Rand and others 2004) and in particular in mammals (Latorre-Pellicer and others 2016; Roubertoux and others 2003). 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 and others 2002), and females carrying the high elevation haplotype were found to have higher NST while the reverse was true for males (Fontanillas and others 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 and others 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 (Fink and others 2004; Haynes and others 2003; Heckel and others 2005; Lischer and others 2014; Martínková and others 2013), and that are expected thus to have evolved under and be best adapted to different climatic regimes (continental versus oceanic). We used first a common garden experiment (Kawecki and Ebert 2004) to compare C and W voles under standardised 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 (nuclear^{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. 20-30g 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: Western, Central, Eastern and Italian (Fink and others 2004; Haynes and others 2003; Heckel and others 2005; Lischer and others 2014; Martínková and others 2013). The divergence across lineages has occurred before the last glacial maximum (>

18,000 years ago), with the Western lineage being the oldest and most divergent lineage (Heckel and others 2005; Lischer and others 2014). In the present study, we investigated the importance of the nuclear and mitochondrial genomes in shaping the phenotype of voles from the Western (W) lineage and of the neighbouring Central (C) lineage. Although those two lineages separated at least 40,000 generations ago (Heckel and others 2005), they still show natural introgression at their contact zone (Beysard and Heckel 2014; Beysard and others 2015; Sutter and others 2013).

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 standardised conditions. In spring 2012 we used Longworth live traps to capture voles in three C and three W natural populations (Figure 1). Trapping sites were chosen according to published (Beysard and others 2015; Braaker and Heckel 2009; Heckel and others 2005) and unpublished (M. Beysard & G. Heckel, pers. com.) 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 (Braaker and Heckel 2009; Fink and others 2004).

After their capture in the wild, voles were treated with the antiparasitic drug Eprinex® before being transferred within 24 hours to an animal room at the University of Lausanne, Switzerland. The animal room was set at 22 ± 1°C, 60% relative humidity and a 14h:10h light-dark cycle. Voles were individually housed in propylene cages (425 mm x 266 mm x 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 one 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 standardised, 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, 9 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 Figure 1). Analysis of monthly meteorological data from 2003 to 2014 shows significant differences in the climatic 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°C ± 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 nonshivering thermogenesis capacity (NST) in wild caught voles (P generation) acclimatized for 118 ± 4.8 (mean ± s.e.) days to standardised laboratory conditions, in F1 and F2 voles at 51.7 ± 2.8 days of age, and in F9 to 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 to F13 at the University of Aberdeen. In Lausanne, metabolic measurements were carried out using an SM-MARS-4 open flow system (Sable Systems International, Las Vegas, USA) allowing the sequential measurements of oxygen consumption (VO_2; measured every 6 to 7.5 minutes) of three animals in parallel following a protocol previously described in (Lehto Hürlimann and others 2014). In Aberdeen, metabolic measurements were carried out using an open-flow Servomex respirometer system

(Crowburgh, UK) allowing the continuous measurements (every 30 seconds) of two animals in parallel following a protocol previously described in (Johnson and others 2001). Each run lasted about 3 hours, with the measures of RMR taking place in the first 2 hours 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 and others 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 (2 readings) in Lausanne and over a period of 5min (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 uncoupling protein 1 (UCP1) within the brown adipose tissue that, in turn, generates heat production or NST (Golozoubova and others 2006). Animals were not anesthetised 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 (Cannon and Nedergaard 2011; Golozoubova and others 2006). 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 (PB, pers. obs.). 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 anesthetised, 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 to measures of RMR (MLH, pers. obs.). 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 and others 2006). NST was measured in a metabolic chamber set at a temperature of 22 ± 1°C both in Lausanne and in Aberdeen to minimise the risk of hyperthermia and death (PB, pers. obs.).

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 analysed variation in body weight and metabolic traits of both male and female adult voles. We restricted our analyses to females in the backcrossing experiment since hybrid males were not used for reproduction.

We analysed data from the P generation by including the effects of lineage, sex and capture site (population ID) nested within lineage as fixed effects. We analysed 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 analysed data from the backcrossing experiment (F9 to F13) by including the mitochondrial and nuclear (W versus 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 0.001g) 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 (R Core Team 2016). Mixed models were run using the R package 'lme4' (Bates and others 2015). The significance of fixed effects were tested using the R package 'lmerTest' and function Anova, with denominator degrees of freedom calculated using Satterthwaite's approximation (Kuznetsova and others 2016). 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:

261 $F_{1,114}$ = 2.03, P = 0.157; Figure 2), and males were heavier than females ($F_{1,114}$ = 7.44, P =
262 0.007). Voles from the P generation showed no difference in RMR between C and W lineages
263 ($F_{1,30}$ = 0.51, P = 0.482). However, we found higher NST in W voles compared to C voles
264 ($F_{1,30}$ = 8.48, P = 0.007; Figure 1). We controlled in our analyses for the positive effect of body
265 weight on RMR and NST (all P-values < 0.01), and for potential effects of sex, acclimation
266 length and capture site on RMR and NST (all P-values > 0.05).

267 Analyses of body weight and metabolic traits in C and W voles born and bred in an
268 animal room for up to 2 generations (F1 and F2) showed similar results as found in the P
269 generation. That is, we found no significant difference between C and W voles in body weight
270 (P = 0.445) and RMR (P = 0.118), but W voles had higher NST than C voles (P = 0.005; Table
271 2; Figure 2). We controlled in our analyses for the positive effect of body weight on RMR and
272 NST and for potential effects of sex and age on body weight and metabolic traits (see Table 2).

273 **Backcrossing experiment**

274 The long-term backcrossing of voles from C and W lineages showed that the body
275 weight of adult females was significantly influenced by interaction between the mitochondrial
276 and nuclear genomes (P = 0.007; Table 3). Insertion of the W mitochondrial genome in the C
277 nuclear background led to a significant reduction in body weight of C^W hybrid females (post-
278 hoc test: P = 0.014), whereas no significant reduction in body weight was observed in W^C
279 hybrid females (P = 0.18; Fig. 3). In this model, voles' age and age squared were entered as
280 covariates to account for quadratic changes in body weight with age (P = 0.014; Table 3).
281 Variation in BAT weight was significantly explained by the nuclear genome (P = 0.027; Table
282 3), and not by mitochondrial genome (P = 0.93; Table 3) or the interaction between the nuclear
283 and mitochondrial genomes (P = 0.36; this interaction was dropped from the final model in
284 Table 3). Females with a W nuclear genome had a heavier BAT than females with a C nuclear
285 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 to 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; 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 and others 2005) and that are distributed in two different regions of Europe, with the W and C lineage being found respectively in Western and Central 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-

311 nuclear interactions on variation in body weight and RMR. Overall, our findings turn new light
312 on the influence of the mitochondrial and nuclear genomes on NST and in shaping adaptation
313 to the environment in mammals.

314 The main seat of heat production during NST is within the mitochondria contained in
315 the BAT (Cannon and Nedergaard 2004; Lowell and Spiegelman 2000; Oelkrug and others
316 2015), and most of the proteins enclosed in the mitochondria and responsible for mitochondrial
317 respiration and NST are encoded in the nuclear genome (Calvo and others 2016). Hence,
318 predictably, most of the genetic variants so far associated with variation in NST have been
319 found in the nuclear genome (e.g. Guerra and others 1998; Lelliott and others 2006; Nishimura
320 and others 2017; Whittle and others 2012; Zheng and others 2017, but see Fontanillas and
321 others 2005). The finding of the present study highlighting a significant influence only due to
322 the mitochondrial genome on between-lineage difference in NST is surprizing. This result is
323 even more striking since variation in BAT size was found to be related to the nuclear genome.
324 Our results on NST are nonetheless concordant with findings in first generation interspecific
325 hybrid carnivorous mice (*Onychomys sp*.) (Shipley and others 2016) and first generation
326 crosses between wild and random-bred laboratory house mice (*Mus domesticus*) (Richardson
327 and others 1994) showing strong maternal effects, potentially caused by mitochondrial effects,
328 on animal RMR and NST, respectively (see also Boratyński and other 2016 for sex-specific
329 effects on RMR in introgressed voles). At least two different scenarios could account for effects
330 of the mitochondrial genome on NST.

331 First, mito-nuclear interaction may distort the inheritance of nuclear genes across
332 generations, favouring the co-inheritance of mito-nuclear genes with strong positive epistatic
333 interactions or preventing the co-inheritance of epistatic mito-nuclear genes with
334 incompatibilities which is a general drawback of backcrossing experiments (Ballard and
335 Melvin 2010; Trounce and others 1994). In this study, we analysed hybrid animals that

underwent at least 9 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 cannot fully exclude that between group differences in NST were explained by nuclear genes co-segregating with the mitochondrial genome (Trounce and others 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 signalling from mitochondria to the nucleus (Butow and Avadhani 2004; Nam and others 2017). Accordingly, it has been recently demonstrated that a reduction in mitochondrial respiratory capacity in laboratory mouse BAT activates retrograde signalling pathways that downregulate thermogenic nuclear gene expression, and in turn BAT function and NST capacity (Nam and others 2017). This scenario points out mitochondria as master regulators of the phenotype (Galluzzi and others 2012; Horan and others 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,

360 protein content and mitochondrial function are needed to provide a full picture of the proximate
361 mechanisms leading to the difference in NST between groups.

362 Mitochondrial energy transduction depends on respiratory supercomplexes made of
363 proteins encoded both in the nuclear and in the mitochondrial genomes (Blier and others 2001;
364 Rand and others 2004), and epistatic interactions between those two genomes at the protein
365 level could affect suites of phenotypic traits that rely on energy (Ballard and Melvin 2010;
366 Ballard and Pichaud 2014). A mismatch between these two genomes has been suggested as an
367 importance speciation force (Gershoni and others 2009) that can account for the formation of
368 reproductive barriers at contact zones between closely related but phylogenetically distinct
369 lineages if this mismatch leads to a breakdown in mito-nuclear interactions (Barreto and Burton
370 2013; Burton and others 2006). It predicts the occurrence of significant mito-nuclear interaction
371 on phenotypic traits, and in particular impaired phenotypic values in hybrid (introgressed)
372 individuals. Studies on secondary contact zones between W and C voles showed that those two
373 evolutionary lineages are naturally hybridizing but that there is a strong selection against male
374 hybrids in the wild (Beysard and Heckel 2014; Beysard and others 2015; Sutter and others
375 2013). Furthermore, since W voles are losing ground to C voles in the contact zone, it has also
376 been suggested that hybridisation may be more detrimental to the W lineage (Beysard and
377 Heckel 2014; Beysard and others 2015). In this study, we were able to hybridize C and W voles
378 over more than 10 generations, thus providing no evidence of strong mito-nuclear
379 incompatibilities (Burton and Barreto 2012) between those two evolutionary lineages of
380 common voles. Our results support nonetheless the existence of mito-nuclear effects on the
381 phenotype of female voles, with hybrid voles being lighter and having a higher RMR. Those
382 effects were stronger in C^W than W^C hybrid voles and thus, if anything, hybridisation was more
383 detrimental to the C lineage. There are two important caveats to our results. First, due to time
384 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 and others 2004), which predicts the occurrence of stronger negative mito-nuclear interactions in males than females (Gemmell and others 2004; Innocenti and others 2011). Second, the co-evolutionary process between mitochondrial and nuclear genes is likely to be sensitive to environmental factors (Arnqvist and others 2010; Baris and others 2016; Hoekstra and others 2013), 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 and others 2010; Baris and others 2016; Hoekstra and others 2013). 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 (Ballard and Melvin 2010; Ballard and Pichaud 2014; Blier and others 2001; Dowling and others 2008; Rand and others 2004).

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577

578 **Table 1.** Description of the sample sizes used in the statistical analyses of body weight, resting
579 metabolic rate (RMR), nonshivering thermogenesis (NST) and the size of the brown adipose
580 tissue (BAT) of common voles in the common garden and backcrossing experimental design.
581 Voles in the common garden experiment were from Central (nuclear^{mitochondrial} genomes: C^C)
582 and Western (W^W) evolutionary lineages. Hybrids showing a mismatch between the
583 mitochondrial and nuclear genomes (W^C, C^W) were generated using a back-crossing
584 experiment (see the Methods section for more information).

Trait	Design	Generation	Group				Total
			C^C	W^C	C^W	W^W	
Body weight	Common garden	P	83			59	142
		F1-F2	118			114	232
	Backcrossing	F9-F13	59	97	74	77	307
RMR & 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

585

586

587 **Table 2.** Results of linear mixed models describing the influence of the evolutionary lineage
588 (Central versus Western) on phenotypic traits in adult common voles housed for up to two
589 generation in a common garden environment. Voles' sex, age and log-transformed body weight
590 were included as fixed effects when relevant. Mother identity, father identity and generation
591 were included as random effects. The partition of variance [95% CI] and the sample size are
592 given for each model.

Fixed effect	Estimate	s.e	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
Resting metabolic rate (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 thermongenesis (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

593

594

595 **Table 3**. Results of linear mixed models describing the influence of the mitochondrial
596 (mtDNA) and nuclear genomes (nDNA) and their interaction (mtDNAxnDNA) on phenotypic
597 traits in adult female voles. Data are from a long-term backcrossing experiment (> 8
598 generations) between voles from Western and Central evoluationary lineages. Voles' age and
599 body weight were included as fixed covariates when relevant. Non-significant interactions
600 were dropped from the final statistical model. Mother identity, father identity and generation
601 were included as random effects when relevant. The partition of variance [95% CI] and the
602 sample size are given for each model.

Fixed effect	Estimate	s.e	DF_{num}	DF_{den}	*F*-value	*P*-value
Body weight (g)						
$V_{residuals}$ = 15.412 (*N* = 307 voles), V_{mother} = 2.183 (*N* = 159 mothers), V_{father} = 0.271 (*N* = 94 father), $V_{generation}$ = 15.412 (*N* = 5)						
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 x 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	-2.210E-04	8.962E-05	1	299.1	6.08	0.014

Fixed effect	Estimate	s.e	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 x 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
Brown adipose tissue (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
Resting metabolic rate (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 x 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 capacity (NSTC) (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

603

Fig. 1. Map of Western 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).

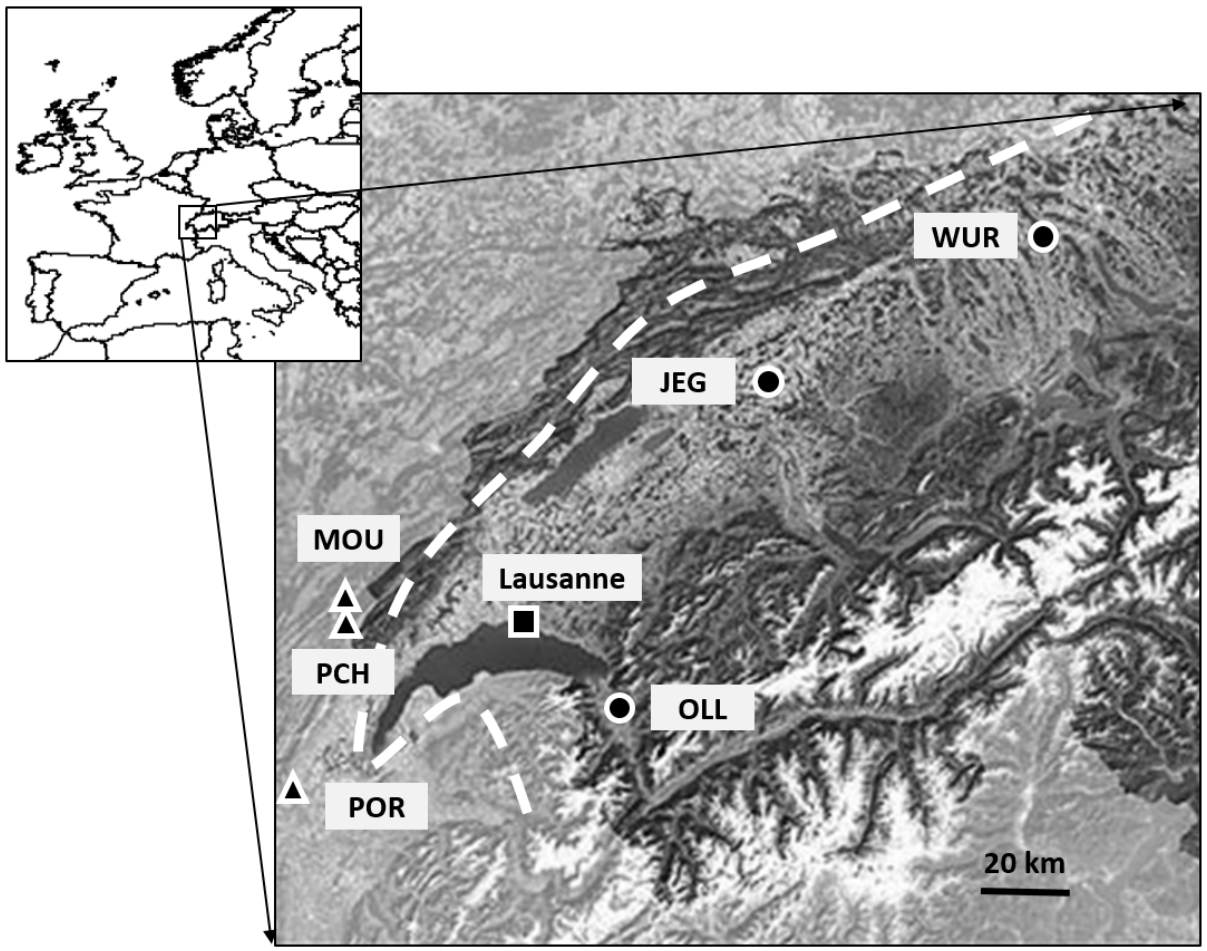

Fig. 2. Body weight, resting metabolic rate (RMR) and nonshivering thermogenesis (NST) as a function of evolutionary lineage (Western: W, Central C) in (**A**) wild caught (P generation) adult common voles and in (**B**) their offspring housed under standardised conditions for two generations (F1, F2). Number of P voles analysed for body weight, RMR and NST are, respectively, 142, 39 and 39. Number of F1 and F2 voles analysed 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.

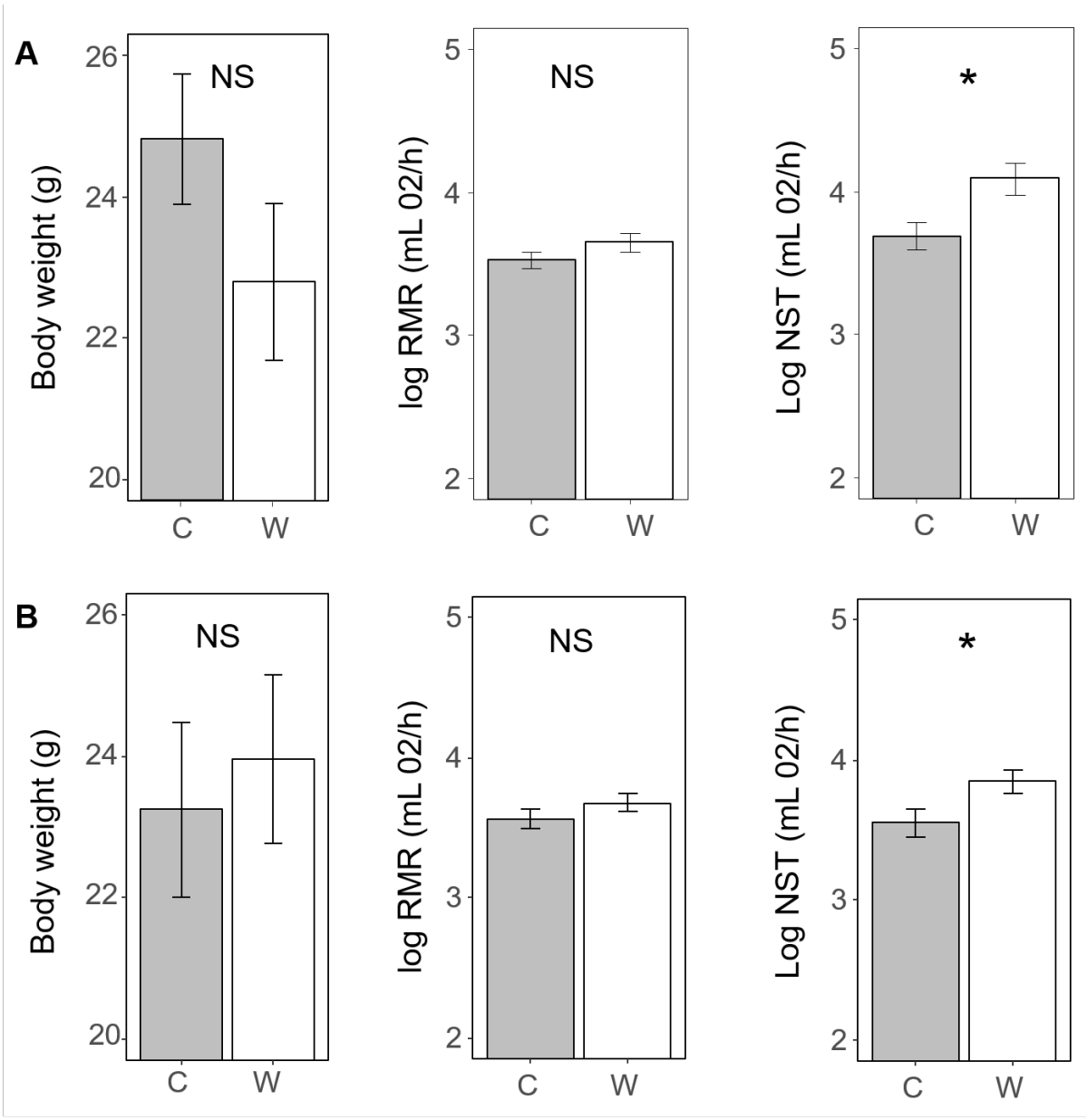

Fig. 3. Total body weight, weight of brown adipose tissue (BAT), resting metabolic rate (RMR) and nonshivering thermogenesis (NST) as a function of nuclear and mitochondrial genomes background ($nuclear^{mitochondrial}$) of adult female common voles from a long-term (>F9) backcrossing experiment. Number of voles analysed 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.