Evol Biol (2016) 43:37–47
DOI 10.1007/s11692-015-9347-2

RESEARCH ARTICLE

The Sex Specific Genetic Variation of Energetics in Bank Voles, Consequences of Introgression?

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Received: 11 May 2015 / Accepted: 14 September 2015 / Published online: 23 September 2015
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Abstract Interaction between mitochondrial and nuclear genomes is expected to affect energetic phenotypes of traits linked to mitochondrial physiology, further influencing the fitness. A rodent, the bank vole (*Myodes glareolus*), has a population structure completely or partially introgressed with mitochondria from its relative, the red vole (*M. rutilus*). Females that carried either bank vole mitochondria or mitochondria from the introgressed species were repeatedly mated with males of both mtDNA types. We found that in males, but not in females, morpho-physiological phenotypes are affected by sire type, causing decreases in body mass (BM) and basal metabolic rate (BMR; including BM corrected, rBMR) in individuals sired by fathers carrying introgressed mitochondria. Higher effect sizes for the proportion of additive genetic variation (and 5.6, 1.9 and 3.6 times higher narrow sense heritability for BM, BMR and rBMR, respectively), and lower for proportion of environmental variation were detected in progeny of non-introgressed males. Our data indicate that co-adapted and possibly co-introgressed nuclear genes related to energetic physiology have an important role in adaptation to the northern conditions in bank voles, and that sex linked nuclear genes are a potential source for variation in basal metabolic rate.

Keywords Basal metabolic rate · Fitness · Geographic variation · Heritability · Hybridization · mtDNA introgression · *Myodes* (*Clethrionomys*)

Introduction

The mitonuclear compatibility hypothesis (affinity between nuclear and mitochondrial inherited proteins) is based on the correspondence between mitochondrial and nuclear genomes to produce functional and effective oxidative phosphorylation processes (OXPHOS) (Arnqvist et al. 2010; Hadjivasiliou et al. 2012; Hill and Johnson 2013; Wolff et al. 2014). It has been shown that traits that are linked to mitochondrial physiology can signal cellular respiration quality, and that the progeny phenotype and fitness will depend on the type of the mitochondria and nuclear genomes carried by the mating parents (Hill and Johnson 2013). Therefore, compatibility between genomes is expected to affect progeny energetic physiology, and indirectly influence their fitness (Arnold et al. 2012; Arnqvist et al. 2010; Boratyński and Koteja 2010; Wolff et al. 2014; Zub et al. 2014; Williams et al. 2015). Although components of energetic physiology are encoded on mtDNA, the majority of the genetic background of the quantitative physiological traits are encoded on nuclear genomes. Therefore, an adaptive genetic signal, e.g. reflected by the variation in the DNA sequences (e.g.: Boratyński et al. 2014), might be independent from variation in mitochondria, especially if the discrepancies between mitochondrial and nuclear genomes do not cause physiological malfunctions.

Electronic supplementary material The online version of this article (doi:10.1007/s11692-015-9347-2) contains supplementary material, which is available to authorized users.

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A small rodent, the bank vole (*Myodes glareolus*), is known to have population genetic structure that is completely or partially, naturally introgressed with mitochondria from its relative, the red vole (*M. rutilus*), primarily in northern populations (Tegelström 1987; Kotlík et al. 2006, 2015; Filipi et al. 2015). The introgression of mitochondria may have influenced fitness in this system, as indicated by the traces of selection in a mitochondrial marker (cytochrome b) and environmental constraints of mitotype distribution, suggesting that the introgressed mitotype inhabits colder and drier habitats then the original one (Boratyński et al. 2014). There are some indications that mitonuclear interactions affect the phenotype of introgressed bank voles. Detected effects of mitochondria on physiological (body mass and basal metabolic rate) (Boratyński et al. 2011) and behavioural (personality) (Šíchová et al. 2014) traits suggest incompatibility between the introgressed mitochondrial and original nuclear genomes. It was shown that the level of basal metabolic rate was decreased in introgressed females, and that the proactive behaviour (linked to higher reproductive output, Mariette et al. 2015) was only positively correlated with basal metabolism in non-introgressed males, implying that the effect of mtDNA introgression bears physiological and developmental costs. However it was not inferred if the observed effects are due to direct nuclear/mitochondrial effects or indirect, mitochondrial-nuclear interactions. Moreover, an experiment that would control for variable confounding effects (like population of origin and common early environment) to test effects on the energetic phenotype has not been conducted.

In the breeding experiment presented here the females that carried either bank vole or introgressed (from its phylogenetically closely-related species) mitochondria were repeatedly mated with both mitochondrial types of males (introgressed and non-introgressed; Fig. S1 in Supplementary Material). The effect of parental types on the progeny's morphological (body mass) and energetic (basal metabolic rate) phenotypes was investigated to test direct mitochondrial/nuclear and mito-nuclear interaction effects. Proportions of additive genetic and common environment components of variance and covariance were estimated between crosses, to test for a signal of decreased genetic variation in introgressed (and northern) populations exposed to putatively stronger selection. Massive genome wide introgression is expected to elevate additive genetic components, whereas introgression of an adaptive fragment might deplete it, due to the purging effect of selection, especially in more energetically demanding, and constrained, northern conditions (Gaitán-Espitia et al. 2013). We found that sons of fathers (sires) that carried introgressed mitochondria (northern origin) have significantly lower values of basal metabolism and body mass, and that the additive genetic effects of traits tended to be lower, while the environmental effect tended to be higher, for all traits in introgressed (northern origin) males.

Materials and Methods

Animals, Breeding Colony and Crosses

Experiments were conducted on populations of bank voles (*M. glareolus*) that carry two kinds of mitochondrial genomes, one native to this species and one that was acquired by introgression from its relative (*M. rutilus*) (Tegelström 1987; Boratyński et al. 2014; Kohli et al. 2015; Filipi et al. 2015). Animals for this experiment, F0 generation, were live trapped in boreal forest habitats typical for this species, from four populations in Finland (Tammela: 60°48′N:23°58′E, Virolahti: 60°35′N:27°34′E, Kolari: 67°19′N:23°46′E and Savukoski: 67°17′N:28°09′E). Northern populations (Kolari and Savukoski) experienced colder climates that affect the length of the snow cover [January mean temperature (°C) for: Tammela: −7.1, Virolahti: −7.3, Kolari: −14.5 and Savukoski: −13.3]. Animals from the F0 generation were live trapped and transported to the laboratory at the University of Jyväskylä where all procedures were accomplished. All individuals from generation F0 were scored according to one mitochondrial and six nuclear, unlinked, DNA fragments (Boratyński et al. 2011, 2014). This investigation revealed that two of the populations (North West and North East populations from Kolari and Savukoski, respectively) exhibited only introgressed mitochondria, from phylogenetically closely related (and native to Finland) red voles (*Myodes rutilus*), while nuclear DNA fragments (haplotypes) were shared among all populations from Finland. The results indicate that original mtDNA type is missing (or in very low frequencies), in those two populations. Matings (avoiding matings between relatives) within populations were conducted for one generation on 30 dams and 45 sires (generation F0), that successfully bred at least once, giving birth to 202 progeny (generation F1). A cross-breeding experimental design was based on the genetic information (mtDNA affiliation) of the F0 generation, assuming mammalian maternal inheritance of mtDNA. Generation F1 was used to conduct the cross breeding experiment based on 33 dams and 43 sires. Each dam and each sire were mated multiple times within and between its parental populations and according to matching and miss-matching mtDNA types, producing 123 (118 with complete phenotypic data) progeny (Fig. S1). Breeding protocols developed in our laboratory were applied as follows: voles were paired for 2 weeks, after that time males were separated from females, while females were separated from offspring 2 weeks after parturition (Boratyński et al. 2013). After the

weaning period, the sexes were kept in separate cages until the breeding procedure was applied to produce the next generation, or until animals were placed in individual cages two weeks prior to metabolic measurements. All procedures adhered to ethical guidelines for animal research in Finland (Finnish National Animal Experiment Board, permission numbers: ESLH-2008-04660/Ym-23 and ESLH-2009-09663/Ym-23).

Phenotype, Basal Metabolism and Body Mass

To describe potential consequences of parental nuclear and mitochondrial types on animals, two traits related to morphology and physiology were measured in all individuals of the pedigree: body mass (BM) and basal metabolic rate (BMR) (Naya et al. 2013; White and Kearney 2013; Sadowska et al. 2015). An eight-channel open-flow respirometric system (Sable Systems, Henderson, NV), developed in our previous research (Boratyński et al. 2011), based on the Fc-1B O_2 (Sable Systems) analyser was used to estimate oxygen consumption on post-absorptive animals, at rest and at thermo-neutral temperature. Seven animals were weighed prior to metabolic measurements (±0.1 g) and placed in the respirometry system for 7.5 h. Air passing thought eight chambers (seven with animals and one reference), was sampled sequentially every 15 min and passed through an oxygen analyser for recordings. The metabolic rate (consumption of oxygen) was calculated using O2 measures with the equation: $VO2 = \{Vi\ (FdO2)/[1 - FeO2\ (1 - RQ)]\}$ (VO2—oxygen consumption rate, Vi—flow rate before measurement chamber, FdO2 - difference between O2 fractional concentrations before and after the measurement chamber, FeO2—fractional concentration of O2 after the measurement chamber), assuming respiratory quotient (RQ = CO2 eliminated/O2 consumed) of 0.75 (Koteja 1996).

Statistical Effects of Parental Types

To test for parental type (determined by mtDNA type) effects on progeny phenotype (dependent variables: BM, BMR and residuals from linear regression of BMR on BM referred to as rBMR), we applied a mixed model strategy on the F2 generation crosses between populations and mtDNA types. Statistical significance was tested in models including (independent) fixed effects of: sex of progeny, mtDNA type of mothers (dam; 2 levels) and fathers (sire; 2 levels) and interaction between them, as well as random effects of: maternal (4 levels) and paternal (4 levels) populations of origin, and maternal and paternal mtDNA types nested within their population of origin. Interactions between random effects of maternal and paternal populations were tested in preliminary analyses, and as their effects were negligible ($\chi^2_{REML} < 4.35$, df = 15, $p > 0.99$), they were excluded from final tests. Analyses were performed in R with "lme4", for generalized linear mixed model, and "LMERConvenienceFunctions", for log likelihood ratio test, packages. The tests included 52 females and 66 males, progeny of 33 dams and 43 sires.

Additive Genetic and Common Environment (Co)variations

The total number of individuals in the pedigree (generations F0–F2) in the quantitative genetic analysis was 415. To estimate the proportion of additive genetic and common environment (and heritability and maternal) effects on individuals morpho-physiological phenotype (BM, BMR and rBMR), we applied a mixed model strategy and univariate models on the three generation pedigree dataset (Wilson et al. 2010). Random effects included additive genetic variance (V_a), population affiliation (to account for possible population specific variation; V_p) and common environment effects (to account for environmental and genetic maternal effects; V_c). Models included sex as a fixed factor for analysis in the full dataset. Sets of linear models of increasing complexity were fitted hierarchically to determine the appropriate random effects structure for traits. The significance of the additive genetic and common environmental effects was assessed with a DIC (deviance information criterion) comparing the full model with restricted models (Wilson et al. 2010):

$$y = sex + population + e$$
$$y = sex + population + animal + e$$
$$y = sex + population + animal + dam + e$$

where "y" is the vector of phenotypic (BM, BMR or rBMR) observations; "sex" is the vector of fixed effect; "animal", "population", "dam" and "e" are the vectors of direct additive genetic, population specific, common environmental (both genetic and environmental effects), and residual effects (environmental and non-additive effects), respectively. If ΔDIC (difference in deviance information criterion, DIC, between hierarchical models) is smaller than 5, then the difference between models is interpreted as negligible, whereas for ΔDIC larger than 10 the model with smaller DIC values is considered much better (Barnett et al. 2010). Narrow-sense heritability (h^2), maternal effects (m^2) and coefficients of additive (CV_a) and common environmental (CV_c) effects were calculated as follows:

$$h^2 = V_a/(V_a + V_p + V_c + V_e)$$
$$m^2 = V_c/(V_a + V_p + V_c + V_e)$$
$$CV_a = 100 \times \sqrt{V_a}/X$$
$$CV_c = 100 \times \sqrt{V_c}/X.$$

Analyses were run using the Bayesian method with the MCMCglmm package in R (Hadfield 2010). The posterior distributions of model parameters were assessed with Markov Chain Monte Carlo (MCMC) simulations with a chain length of 50 million iterations, sampled every 5000th iteration, with the first 500,000 iterations being neglected (burnin). Genetic correlations were estimated with bivariate models in similar, hierarchical, way and additive genetic (r_a), common (r_c) and general environmental (r_e) correlations were calculated as follows:

$$r_a = COV_a/\sqrt{(V_{aBMR} \times V_{aBM})}$$
$$r_c = COV_c/\sqrt{(V_{cBMR} \times V_{cBM})}$$
$$r_e = COV_e/\sqrt{(V_{eBMR} \times V_{eBM})},$$

where COV_a, COV_c and COV_e are additive genetic, common and general environmental covariations between BMR and body mass (BM), respectively. All statistical analyses we conducted in R 3.1.1 (http://cran.r-project.org/) on the Bio-Linux 8 platform (Field et al. 2006).

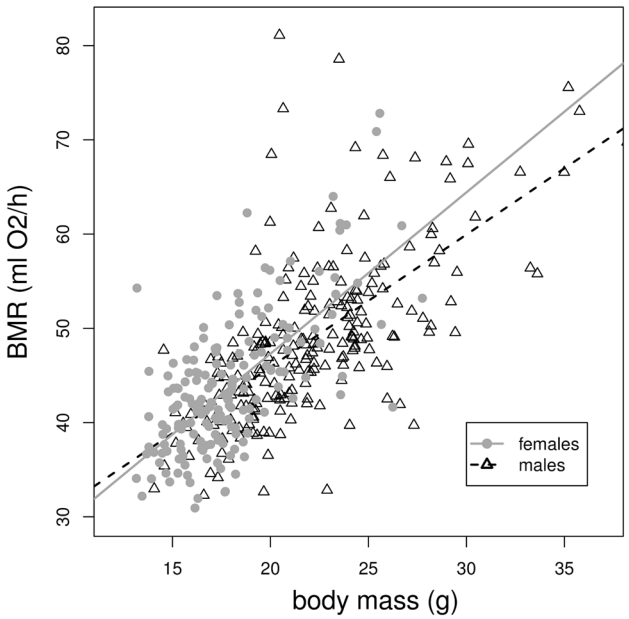

Fig. 1 Phenotypic correlation between basal metabolic rate (BMR) and body mass in bank vole colony

Results

Phenotypic Variation

The body mass of animals from all three generations varied from minimum values of 13.2 to maximum values of 38.2, with mean of 20.2 and median of 19.5 grams. Males were always bigger than females ($p < 0.0001$, for all data sets). The basal metabolic rate varied from minimum values of 26.3 to maximum values of 81.1, with mean of 46.5 and median of 45.3 ml O2/h. Males reflected a higher level of the absolute oxygen consumption rate than females ($p < 0.005$), but not when the rate accounted for body mass variation ($p > 0.16$). All traits were previously described to be highly repeatable in this species (Labocha et al. 2004; Boratyński and Koteja 2009), and in this particular colony (Šíchová et al. 2014). The correlation was high and significant between BM and BMR ($r = 0.69$, $t = 19.3$, $df = 402$, $p < 0.0001$; Fig. 1) but none of the traits significantly correlated with age of individuals during measurements ($p > 0.3$, in all comparisons). The body mass and BMR correlation partitioned to sexes and generations were also significant (females: $r = 0.47$, 0.69, 0.74; males: $r = 0.49$, 0.68, 0.79; $p < 0.005$ for F0, F1 and F2, respectively). After accounting for variation in BM by calculating linear regression residuals, residual basal metabolic rate (rBMR) was highly correlated with BMR ($r = 0.73$, $t = 21.3$, $df = 399$, $p < 0.0001$). BMR and rBMR correlations partitioned to sexes and generations were also high and significant (females: $r = 0.79$, 0.83, 0.92; males: $r = 0.74$, 0.76, 0.81; $p < 0.0001$ for F0, F1 and F2, respectively).

Effects of Parental Types on Progeny Phenotype

In the cross-breeding experiment (Fig. S1), the basal metabolic rate (BMR), but not body mass (BM), was affected by paternal type by offspring sex interaction [BMR: Estimate (±SE) = −0.11 (±0.08), $t = -1.49$, $p = 0.014$; BM: Estimate (±SE) = −0.03 (±0.07), $t = -0.49$, $p = 0.205$], indicating the genetic type of sire has a divergent effect on sons versus daughters phenotype (Fig. 2). The same effect was found for body mass corrected BMR [rBMR: Estimate (±SE) = −3.49 (±2.69), $t = -1.30$, $p = 0.046$]. Moreover, progeny sex explained variation in BM, indicating sexual size dimorphism [bigger males: Estimate (±SE) = 0.24 (±0.05), $t = 4.39$, $p < 0.0001$)], therefore further tests were conducted separately for sons and daughters. In sex specific analyses, neither dam nor sire significantly explained variation in BM, BMR or rBMR in daughters. However, the genetic type of sire, but not dam, significantly explained variation in sons phenotypes, for all investigated traits (Table 1). Sons, sired by fathers characterized by mitochondria introgressed from other species, were significantly smaller and had lower basal metabolic rates. After accounting for variation in body mass, basal metabolic rate (rBMR) was

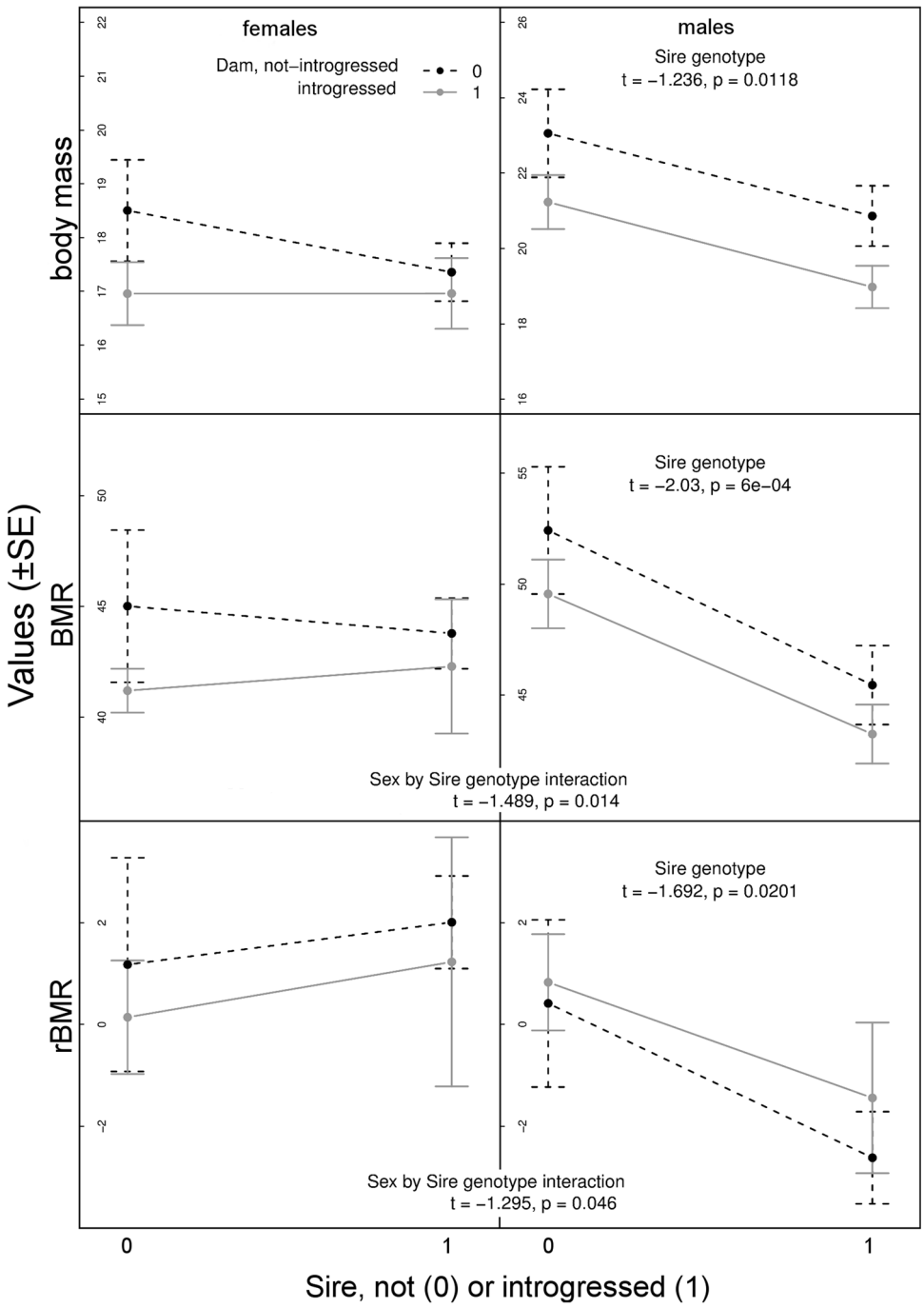

Fig. 2 The effect of parental types on progeny body mass, BMR and residual BMR (rBMR) in bank voles, derived from cross breeding experiment between parents characterized by two mtDNA types: introgressed from red vole and native to bank vole, not-introgressed. Maternal types (dam) are indicated with: *black*, *dashed line* (not-introgressed) and *grey*, *line* (introgressed mtDNA types). Paternal types (sire) mated with dams are indicated on the x-axis with "0"(not-introgressed) and "1" (introgressed)

still lower in male progeny sired by introgressed fathers (Table 1 and S1; Fig. 2).

Additive Genetic and Common Environmental (Co)variations

For all individuals, all traits have a significant (ΔDIC $>$ 19) component of additive genetic variation, with narrow-sense heritability (further referred as heritability) of $h^2 = 0.28$, 0.29 and 0.14 for BM, BMR and rBMR respectively. Also, common environmental effects were highly significant for BM and BMR (ΔDIC $>$ 23), and moderately for rBMR (ΔDIC $=$ 9). In "cross types" (referring to crosses within mtDNA introgressed and non-introgressed populations), additive genetic effects were highly significant for BM and BMR (ΔDIC $\geq$ 29), and moderately significant for rBMR (ΔDIC $=$ 7 and 11;

Table S2). The common environmental effects were significant for both BMR and rBMR ($\Delta DIC \geq 13$) and moderate for BM ($\Delta DIC = 7$) for introgressed data, but in non-introgressed populations only for BM and BMR ($\Delta DIC \geq 11$; for rBMR: $\Delta DIC = -2$). Because the fixed effect of sex was also significant in models for BM and BMR (Table S2), further analyses were performed on males and females separately in both cross types (Tables 2, 3). For females, the only high and significant additive genetic effect was detected for BM for introgressed populations, whereas the common environmental effect significantly affected metabolism (Table 2; Fig. 3). In non-introgressed females, additive and common environmental effects were low and insignificant ($\Delta DIC \leq 6$; Table 2; Fig. 3). For males, significant additive genetic effects were detected for all traits in both introgressed and non-introgressed populations ($\Delta DIC \geq 11$), with higher effect sizes of additive effects and heritability for individuals originating from non-introgressed populations (Table 3; Fig. 4). When populations were partitioned (however including both sexes), additive genetic effects differed marginally between northern and southern populations for residual values of BMR but not for body mass (Fig. S2). None of the common environmental effects were significant in males ($\Delta DIC \leq 4$; Table 3; Fig. 4).

Overall, a genetic correlation between BMR and BM was high, positive and significant when including [$r_a = 0.65$ (0.28–0.83); $\Delta DIC = 40$] and excluding [$r_a = 0.71$ (0.53–0.84); $\Delta DIC = 111$] common environmental effects. Furthermore, common [$r_c = 0.70$ (0.44–0.85); $\Delta DIC = 40$] and general [$r_e = 0.57$ (0.43–0.65); $\Delta DIC = 40$] environmental correlations were high in this study. Within cross types, the genetic correlation was high, positive and significant in crosses between introgressed populations [$r_a = 0.65$ (0.33–0.84); $\Delta DIC = 41$], but not significant after including common environmental effects [$r_a = 0.40$ (−0.15–0.76); $r_c = 0.60$ (0.17–0.86); $r_e = 0.63$ (0.46–0.74); $\Delta DIC = 18$]. In crosses between non-introgressed populations, the genetic correlation was significant in models without [$r_a = 0.61$ (0.22–0.81); $\Delta DIC = 49$] and with maternal effects [$r_a = 0.64$ (0.10–0.82); $r_c = 0.62$ (0.01–0.86); $r_e = 0.43$ (0.16–0.65); $\Delta DIC = 19$]. When further partitioned according to sex, genetic correlations were significant for all datasets ($\Delta DIC \geq 16$), except for non-introgressed females ($\Delta DIC = 4$), and none of the common environmental correlations were significant (HPD for all estimates overlapped with 0; Table S3).

Table 1 Results of mixed model testing the effects of parental (dam: "MtGD" and sire: "MtGS") types on log transformed body mass (BM) and basal metabolic rate (BMR), and residual values of BMR (calculated for linear regression model including BM)

Data	Dep.	Effect	Est.	SE	t	*p*
Daughters						
	BM	(Intercept)	2.93	0.09	31.50	
		MtGD	−0.08	0.09	−0.83	0.334
		MtGS	−0.06	0.07	−0.90	0.407
		MtGD^{x}MtGS	0.06	0.07	0.84	0.451
	BMR	(Intercept)	3.80	0.13	28.76	
		MtGD	−0.08	0.14	−0.59	0.375
		MtGS	0.01	0.06	0.14	0.806
		MtGD^{x}MtGS	−0.01	0.09	−0.07	0.972
	rBMR	(Intercept)	1.67	3.49	0.48	
		MtGD	−1.53	3.74	−0.41	0.495
		MtGS	1.33	2.03	0.66	0.380
		MtGDMtGS	−0.24	2.94	−0.08	0.947
Sons						
	BM	(Intercept)	3.15	0.12	26.20	
		MtGD	−0.11	0.12	−0.87	0.160
		MtGS	−0.06	0.05	−1.24	0.012
		MtGD^{x}MtGS	−0.05	0.07	−0.68	0.512
	BMR	(Intercept)	3.97	0.12	33.63	
		MtGD	−0.08	0.12	−0.64	0.366
		MtGS	−0.11	0.05	−2.03	0.001
		MtGD^{x}MtGS	−0.03	0.07	−0.39	0.676
	rBMR	(Intercept)	0.41	1.45	0.28	
		MtGD	0.41	1.84	0.23	0.250
		MtGS	−3.03	1.79	−1.69	0.020
		MtGD^{x}MtGS	0.76	2.44	0.31	0.749

Analyses were conducted separately for daughters and sons (data) and for dependent (dep.) variables. For results for random effects of parental population affiliation see: Table S1

Discussion

Quantitative genetic investigation of the bank vole colony showed that both body mass and basal metabolic rate had significant levels of additive genetic variation and heritability, and thus can respond to natural selection. The traits share a significant proportion of additive genetic variation, as reflected by high levels of genetic correlation, that can constrain their evolution (Sadowska et al. 2009, 2015). Intriguingly, our cross-breeding experiment between populations of different types of mitochondria showed that the effect of parental genetic type on progeny depends on both parental and progeny sex, and that it has a stronger effect on physiological than morphological characteristics.

In the cross-breeding experiment between sires and dams of two types of mitochondria (one populating

Table 2 Females components of variance (±SE) from quantitative genetic analyses of body mass (BM) and basal metabolic rate (BMR), and residual values of BMR

	Components of variance				ΔDIC	Coefficients of variance			
Traits	A(HPD)	P(HPD)	C(HPD)	E(HPD)		h^2(HPD)	c^2(HPD)	CV_A(HPD)	CV_C(HPD)
Introgressed									
BM	2.49(0.76–5.22)	2.31(0.17–17.17)	–	2.48(0.97–4.32)	35	0.35(0.04–0.64)	–	9.09(5.44–13.42)	–
	0.83(0.16–3.81)	1.27(0.10–12.10)	1.19(0.20–3.47)	2.60(0.83–4.00)	4	0.08(0.01–0.48)	0.14(0.02–0.42)	7.40(3.09–11.64)	6.99(3.30–11.11)
BMR	23.11(4.28–51.46)	27.71(1.25–166.56)	–	29.56(14.77–54.85)	22	0.20(0.02–0.58)	–	10.97(5.82–16.93)	–
	4.71(1.50–37.61)	0.05(0.84–126.50)	19.36(4.76–52.26)	25.71(7.16–41.45)	23	0.08(0.01–0.43)	0.27(0.05–0.54)	7.97(3.17–14.10)	10.00(6.03–17.29)
rBMR	0.14(0.03–0.53)	0.45(0.01–2.38)	–	0.65(0.40–1.03)	6	0.09(0.01–0.40)	–	19.93(10.30–38.46)	–
	0.08(0.02–0.39)	0.01(0.01–1.88)	0.32(0.09–0.90)	0.44(0.20–0.70)	27	0.04(0.01–0.31)	0.33(0.05–0.57)	15.16(7.88–33.00)	31.34(17.14–50.65)
Not-introgressed									
BM	1.50(0.35–6.64)	5.06(0.29–21.84)	–	8.16(4.44–12.07)	5	0.09(0.01–0.43)	–	6.58(3.61–14.05)	–
	1.04(0.19–5.92)	0.04(0.23–23.18)	1.56(0.26–5.49)	6.61(3.52–10.90)	6	0.07(0.01–0.37)	0.08(0.01–0.32)	5.50(2.79–13.19)	7.46(3.18–12.83)
BMR	9.15(1.49–33.27)	0.36(1.42–116.15)	–	41.36(24.03–63.76)	5	0.11(0.01–0.42)	–	6.93(3.44–13.43)	–
	5.36(1.39–30.60)	7.37(1.15–109.29)	6.47(1.06–28.55)	35.05(20.56–58.64)	4	0.07(0.01–0.36)	0.06(0.01–0.32)	6.71(3.18–12.94)	5.79(3.00–12.46)
rBMR	0.12(0.03–0.44)	0.01(0.01–1.57)	–	0.78(0.47–1.12)	2	0.07(0.01–0.34)	–	17.27(9.04–31.93)	–
	0.09(0.02–0.38)	0.11(0.02–1.24)	0.08(0.02–0.40)	0.72(0.47–1.12)	0	0.05(0.01–0.28)	0.06(0.01–0.27)	16.01(7.62–29.52)	13.45(7.06–29.84)

Components of variance: A—additive genetic, P—population affiliation, C—common environment, E—residual environment. Coefficients: h^2—heritability, proportion of additive genetic variance to phenotypic variance, c^2—proportion of common environment variance to phenotypic variance, coefficients of additive genetic (CV_A) and common environment (CV_C) variations in % [(SD/mean) × 100]. HPD—95 % higherst posterior density, ΔDIC—difference in deviance information criterion (DIC) between hierarchical model, with ΔDIC: < 5—differences between models negligible, 5–10—significant, marginal differences between models, ΔDIC > 10—model including additional effect is much stronger and explains more variation in data

Table 3 Males components of variance (±SE) from quantitative genetic analyses of body mass (BM) and basal metabolic rate (BMR), and residual values of BMR

Traits	Components of variance				ΔDIC	Proportions of variance		Coefficients of variance	
	A(HPD)	P(HPD)	C(HPD)	E(HPD)		h^2(HPD)	c^2(HPD)	CV_A(HPD)	CV_C(HPD)
Introgressed									
BM	4.57(0.91–14.82)	6.17(0.51–72.15)	–	15.15(8.02–21.88)	16	0.11(0.01–0.46)	–	10.24(5.47–18.33)	–
	2.13(0.42–10.95)	−0.02(0.33–56.79)	2.98(0.62–11.26)	14.24(8.23–20.37)	4	0.06(0.01–0.35)	0.08(0.01–0.33)	8.08(3.78–15.63)	9.18(4.27–15.81)
BMR	32.16(7.30–68.05)	0.52(2.01–190.40)	–	47.91(27.50–80.53)	24	0.30(0.04–0.56)	–	12.40(6.43–17.44)	–
	9.44(2.52–46.99)	0.48(1.51–153.15)	15.05(2.89–50.81)	49.79(29.51–79.49)	2	0.07(0.01–0.39)	0.15(0.01–0.37)	8.52(3.96–14.52)	8.89(4.03–14.90)
rBMR	0.34(0.04–0.83)	0.41(0.03–1.88)	–	1.01(0.58–1.47)	11	0.12(0.03–0.46)	–	20.07(9.88–32.97)	–
	0.14(0.04–0.73)	0.15(0.02–1.65)	0.10(0.03–0.51)	1.03(0.62–1.46)	−1	0.07(0.01–0.38)	0.07(0.01–0.25)	16.84(8.01–30.42)	11.72(6.84–25.20)
Not-introgressed									
BM	9.71(4.31–15.28)	1.06(0.24–18.90)	–	2.54(1.00–6.61)	74	0.62(0.21–0.83)	–	13.45(9.09–16.97)	–
	6.29(0.66–11.60)	1.47(0.20–14.93)	2.09(0.33–9.45)	3.28(1.10–7.33)	−6	0.29(0.04–0.66)	0.12(0.02–0.47)	11.23(4.86–15.29)	7.46(2.98–13.58)
BMR	42.95(16.39–73.99)	9.48(1.39–89.69)	–	19.98(10.13–45.85)	51	0.56(0.18–0.77)	–	14.04(8.60–17.81)	–
	42.08(11.01–68.78)	0.57(1.10–78.79)	6.02(1.41–28.88)	19.72(10.00–46.93)	−1	0.46(0.12–0.70)	0.06(0.01–0.29)	13.17(7.09–17.05)	4.98(2.85–11.27)
rBMR	0.51(0.07–0.98)	0.01(0.03–1.61)	–	0.58(0.32–1.12)	21	0.43(0.05–0.62)	–	35.91(17.14–51.57)	–
	0.45(0.05–1.00)	0.01(0.02–1.36)	0.06(0.02–0.29)	0.61(0.30–1.12)	−2	0.34(0.04–0.58)	0.05(0.01–0.17)	33.51(15.33–51.78)	13.35(8.00–27.23)

northern and one southern areas in Finland), we did not find strong support for the mito-nuclear incompatibility hypothesis (Wolff et al. 2014). Furthermore, our results do not support the direct mitochondrial involvement in the expression of the BMR (or body mass), energetic physiology trait measured here, as proposed in our previous work (Boratyński et al. 2011). Our results rather imply the importance of the sex linked nuclear genes, as indicated by the significant effect of sire type by progeny sex interaction on the variation in basal metabolic rate between the crosses. The detailed analyses revealed that sons sired by fathers carrying introgressed mitochondria (from north populations) had lower values of body mass and basal metabolic rate. The lack of effects of maternal and paternal cross types on the daughter's phenotype, and the strong effect of paternal, but not maternal types on son's phenotype suggest strong male biased expression of nuclear inherited genes. The strongest and most consistent phenotypic effect of our crossing procedure was found on BMR, and not on body mass. This suggests that the change in the body mass could have been affected by the correlative response to changes in BMR via strong and significant genetic correlation.

As highlighted by Lane (2011) variable apoptotic thresholds might optimize the match of mitochondrial and nuclear genes after mtDNA introgression, causing changes in phenotype that affect fitness. Therefore, if crucial parts of the nuclear genome could have been adjusted through co-adaptation or co-introgression with mitochondrial genomes, carrying advantages over the original mitochondrial-nuclear machinery, it could have caused the change in phenotype and potentially increased fitness (Puurtinen et al. 2009). In our study system, the northern population of bank voles, *M. glareolus*, carry mtDNA and probably also some of the nuclear genomic regions, introgressed from red voles, *M. rutilus,* adapted to northern conditions. Together with our previous findings of signs of selection on a mitochondrial marker (Boratyński et al. 2014), the (sex biased) phenotypic effects of the breeding experiment between mitochondrial types presented here suggests that indeed introgression could have affected adaptation, but mtDNA is probably not a direct target of selection, as it did not affect body mass or BMR. However, we cannot exclude that mtDNA links to fitness through another set of phenotypic traits. Here, the results indicate that the nuclear-inherited and male expressed genes carry a signal of adaptation.

The lower level of basal metabolic rate in male progeny sired by fathers originating from northern populations suggests the importance of this character as an adaptation to the harsh northern conditions (Fig. 2). It implies that, according to the "compensation" model of energetic

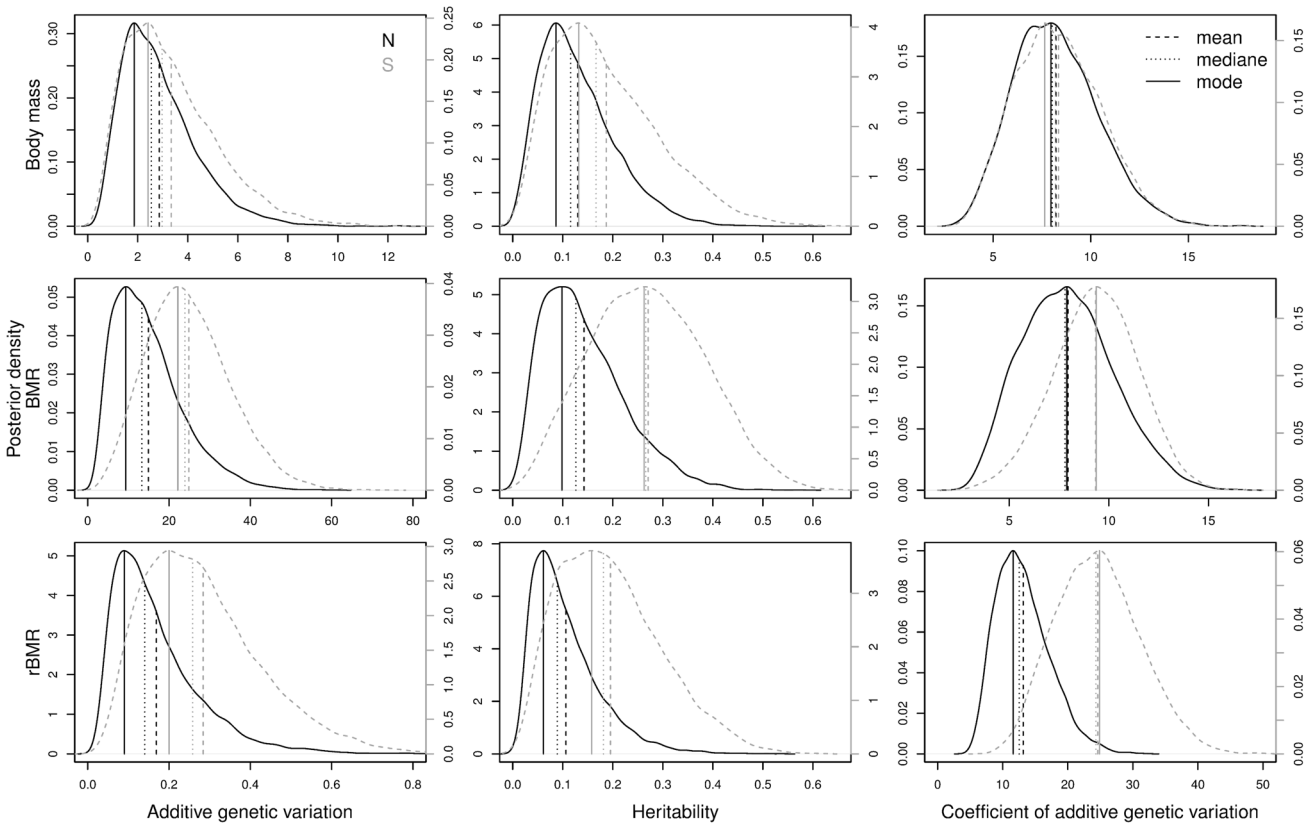

Fig. 3 Posterior density distributions for body mass, BMR and residual BMR (rBMR) for introgressed (N, *black lines*) and non-introgressed (S, *gray dashed lines*) bank vole females

physiology, males with lower maintenance costs could allocate more energy to growth (and survival) and reproduction (Ketola and Kotiaho 2009; Burton et al. 2011; Nespolo et al. 2014). This mechanism might be especially beneficial in non-productive environments where population densities, and (mate) competition, remain low (Einum 2014), as in the northern part of Finland, where a low level of available resources promotes individuals of economic physiology. This mechanism was previously proposed for Finnish populations of bank voles, where overall selection on BMR was suggested to promote lower values of maintenance metabolism (Boratyński et al. 2013). The detection of mitonuclear co-adapted or/and co-introgressed genes, and their physiological functions, would verify if an adaptive process is responsible for the massive observed mitochondrial introgression and the signal of adaptation in bank voles.

The observed male biased effects on phenotype suggest that nuclear genes co-introgressed together with mitochondria from the northern circumboreal red vole, *M. rutilus*, species (Tegelström 1987), or that the co-adaptation to acquired mtDNA happened after introgression. Signals for such potentially co-introgressed nuclear genes were not detected in previous molecular studies (with limited genome coverage) (Boratyński et al. 2011, 2014). Instead, the current study revere low effect sizes of additive genetic variations of BMR and rBMR in males from introgressed populations, which suggests both that there is a higher proportion of fixed alleles and stronger selection acting in those, compared to the non-introgressed, populations. This tentative finding, together with the strong latitudinal segregation of mtDNA types (intogressed populations occurring in the northern areas), suggests that diverse selection pressures and introgression between populations might have played an important adaptive role in this system. Finding that the male linked genes have a strong effect on expression of BMR is suggestive of co-introgression between mitochondrial and nuclear genomes, highlighting the importance of the interplay between introgression and selection.

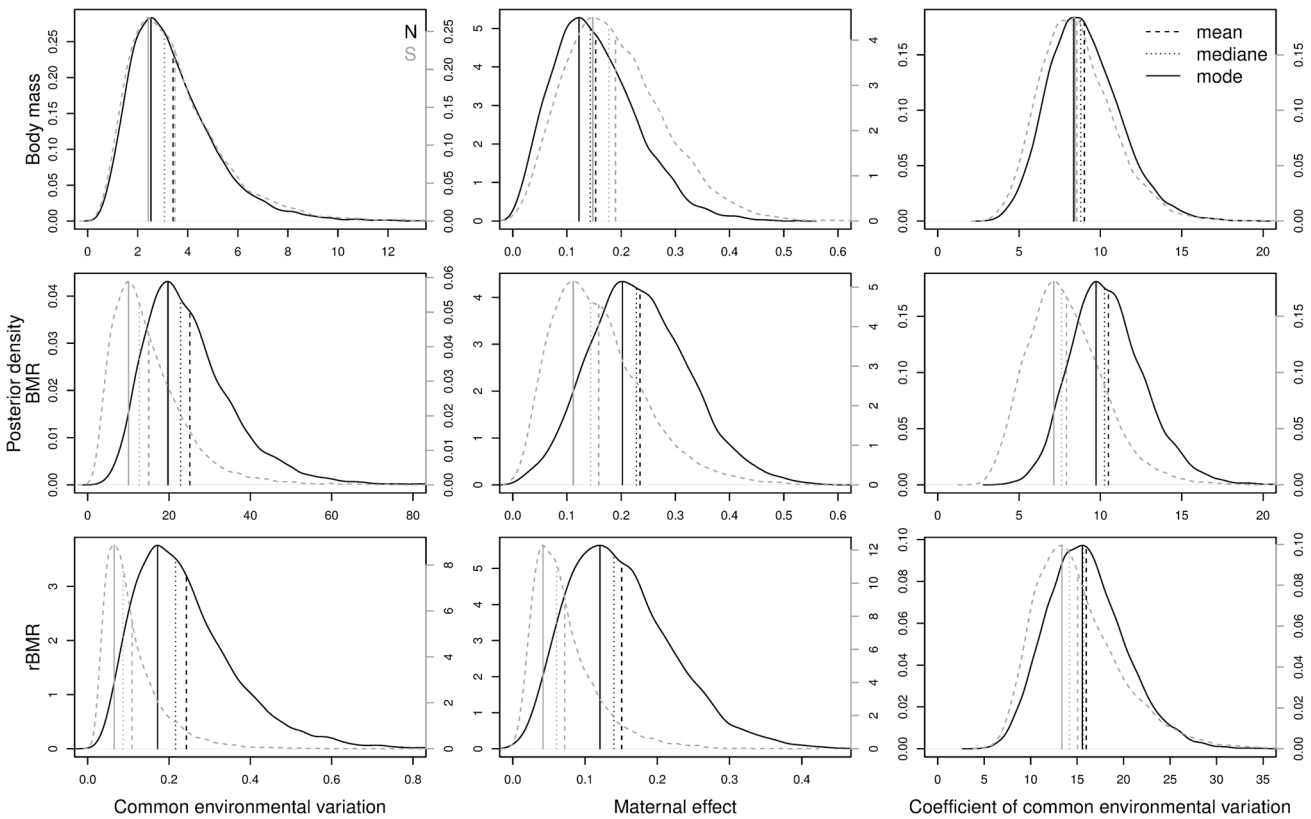

Fig. 4 Posterior density distributions for body mass, BMR and residual BMR (rBMR) for introgressed (N, *black lines*) and non-introgressed (S, *gray dashed lines*) bank vole males

Acknowledgments We acknowledge Paulina A. Szafranska, Mikael Mökkönen and one anonymous reviewer for comments and corrections of the manuscript. This work was supported by Finnish Academy of Science (Grants Numbers: 257340 to EK, 278751 to TK and 132190 to TM). ZB is post-doctoral grantee of the Foundation for Science and Technology, Portugal (SFRH/BPD/84822/2012).

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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