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# On personality, energy metabolism and mtDNA introgression in bank voles

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Consistent interindividual differences in behaviour, or animal personality, are emerging as an important determinant of a wide range of life history traits and fitness. Individual behaviour, however, may be constrained by between-individual variability in energy metabolism and may become unstable owing to intrinsic and extrinsic stressors. Here we tested the relationship between personality and physiology using wild-caught bank voles, *Myodes glareolus*, that varied according to mtDNA type (original or introgressed from *Myodes rutilus*). Personality traits and their within-individual consistency were assessed using an open field test and basal metabolic rate (BMR) was measured in an open-flow respirometer. A significant relationship was found between individuals' consistent (repeatable) personality trait (principal component analysis score reflecting individual differences in proactivity) and their consistent (repeatable) residual BMR (body mass corrected); however, this association depended on mtDNA type and sex. Particularly, the males with original mtDNA showed a positive relationship between proactive behaviour and BMR, which supports the increased-intake model, stating that BMR is positively related to the capacity to engage in costly behaviours. However, this relationship was disrupted in introgressed males, and showed a negative trend in females, suggesting the alternative compensation model. According to our findings, it is likely that consistent differences in behavioural patterns and mtDNA types promote variation between individuals in energy metabolism.


Individual variability in behaviour is omnipresent and currently intensively studied (Careau, Bininda-Emonds, Thomas, Réale, & Humphries, 2009; Carvalho et al., 2013; Lantová, Šíchová, Sedláček, & Lanta, 2010). Repeatable interindividual differences in behaviour (or suites of correlated behaviours) have been referred to as animal personality (according to the broad-sense personality concept, see Réale, Dingemanse, Kazem, & Wright, 2010). Personality represents an important biological trait because of its intraindividual consistency (Bell, Hankison, & Laskowski, 2009), heritable basis (Ariyomo, Carter, & Watt, 2013; Drent, Van Oers, & Van Noordwijk, 2003) and linkage with fitness (Adriaenssens & Johnsson, 2011; Smith & Blumstein, 2008).

Personality appears to be strongly expressed, especially under novel, risky or challenging situations (Carvalho et al., 2013; Wilson, Clark, Coleman, & Dearstyne, 1994). Depending on the nature of the reaction, conspecifics can be categorized along a continuum, ranging from reactive to proactive personality types, differing in suites of correlated behaviours, or personality traits (Koolhaas et al., 1999). In comparison with reactive individuals, proactive ones are behaviourally characterized by a higher level of general activity (Koolhaas et al., 1999), elevated exploratory (Verbeek, Drent, & Wiepkema, 1994) and risk-taking tendencies (Dammhahn & Almeling, 2012; Frost, Winrow-Giffen, Ashley, & Sneddon, 2007), higher aggression (Øverli et al., 2004; Wilson, de Boer, Arnott, & Grimmer, 2011) and lower behavioural plasticity (Coppens, De Boer, & Koolhaas, 2010). At the physiological level, active phenotypes show lower hypothalamus–pituitary–interrenal activity (Silva et al., 2010), higher sympathetic and lower parasympathetic reactivity (Verbeek, Iwamoto, & Murakawi, 2008) and elevated testosterone activity (Koolhaas et al., 1999). This behavioural and physiological covariation has resulted in a hypothesis that personality types mirror variation in metabolic rate (Atwell et al., 2012; Biro & Stamps, 2010; Careau & Garland, 2012; Careau, Thomas, Humphries, & Réale, 2008; Réale, Garant, et al., 2010). Interindividual variation in energy metabolism may represent an important biological signal, as repeatability, heritability and fitness of metabolic rates have been documented (Blackmer et al., 2005;

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Boratyński, Koskela, Mappes, & Oksanen, 2010; Boratyński & Koteja, 2010; Boratyński, Koskela, Mappes & Schroderus, 2013; Ketola, Boratyński, & Kotiaho, 2014; Sadowska et al., 2005; Szafrańska, Zub, & Konarzewski, 2007). Therefore the rate of energy metabolism can be a fitness-related factor, limiting the expression level of personality types.

Several mechanistic models of a potential relationship between personality and energy metabolism can be postulated (reviewed in: Careau & Garland, 2012; Konarzewski & Książek, 2013). The physiologically based increased-intake model (Nilsson, 2002) predicts a positive association between energetically demanding behaviours and metabolic rate, leading to elevated energy output in proactive individuals (Biro & Stamps, 2010; Careau et al., 2008). Conversely, according to the compensation model (Blackmer et al., 2005; Olson, 1992) increased energy requirements of proactive animals must be compensated for by the expenses of another component of the fixed energetic budget, causing a negative association between proactive behaviours and energetics. Recent studies give empirical evidence for both of these models (reviewed in: Biro & Stamps, 2010; Careau et al., 2009; Careau & Garland, 2012). Nevertheless, it is critical to note that many of these studies were conducted on laboratory animals and yielded contradictory or unconvincing support. These contradictory results might have emerged because the metabolic–personality relationship is affected by variable factors, such as resource availability (Killen, Marras, Metcalfe, McKenzie, & Domenici, 2013) and genetic polymorphisms in energetic machinery. As energy is mainly generated by mitochondria, which are characterized by their own genome and enzymatic pathways, the rate of mitochondrial processing might be crucial for displaying energetically demanding personality traits. The mitochondrial processes of oxidative phosphorylation depend on both mitochondrial and nuclear-encoded enzymes; therefore, the evolution of metabolic pathways is constrained by the coevolution of the mitochondrial and nuclear genomes (Rand, Haney, & Fry, 2004). Disruption of coevolved gene complexes by interspecific mitochondria transfers (Arnold, 2006; Melo-Ferreira et al., 2011) can potentially affect adaptation and the evolution of fitness-dependent and energetically limited traits (Ballard & Melvin, 2010; Doiron, Bernatchez, & Blier, 2002; Ropiquet & Hassanin, 2006).

In this study we tested whether personality is associated with a physiological trait, basal energy metabolism, and whether the link between behaviour and physiology is dependent upon the type of the mitochondrial genome the animals carry. Wild-caught bank voles, *Myodes* (or *Clethrionomys*) *glareolus*, including individuals affected by introgressive hybridization of mtDNA from the red vole, *Myodes rutilus* (Tegelström, 1987), were repeatedly exposed to an open field test and their basal metabolic rate (BMR) was subsequently measured using open-flow respirometry. We predicted that the voles proactively responding to an unknown environment would also be characterized by higher BMR, according to the increased-intake hypothesis. This effect would be reduced by mtDNA introgression that putatively disrupted coevolved mitonuclear physiological pathways.

## METHODS

### *Study Animals*

This study was conducted on the most common, and the best-studied, small mammalian species in Finland. Bank voles carry two kinds of mitochondrial genomes: one that is original, native to this species, and one that was acquired by introgression from its relative, *M. rutilus*. Adult bank voles were captured in six Finnish populations (Tammela: 60°48′N,23°58′E, Virolahti: 60°35′N,27°34′E, Kannus: 63°50′N,23°55′E, Sotkamo: 64°07′N,28°23′E, Kolari: 67°19′N,23°46′E and Savukoski: 67°17′N,28°09′E) in July–August of 2008 (Boratyński et al., 2011). The number of trapped animals was decided based on previous research, specifically on trait variability and repeatability, and represents the minimum number necessary for comprehensive investigation of physiological and behavioural traits in voles (Boratyński & Koteja, 2009, 2010; Lantová et al., 2010; Lantová, Zub, Koskela, Šichová, & Borowski, 2011). Previous knowledge based on decades of monitoring for commercial purposes (by METLA, Finnish forestry institution) and help from the Finnish ethical committee, ensured a study design with minimum impact on the local bank vole populations. Animals were trapped using 300 live-traps (Ugglan Special multiple-capture, Grahnab, Hillerstorp, Sweden, 24 × 6 cm and 9 cm high, average body mass of voles was 20 g) placed in lines (≥9), distributed >2 km from one another to decrease the chances of capturing relatives, thus decreasing impact on the local colony. Trapping was designed to reduce the capture of other species and the time spent by voles in the traps. Traps were placed in shaded locations and covered with aluminium protective covers. Each trap contained sunflower seeds and pieces of raw potato, to provide food and water between trap checks in the evenings and mornings (spanning around 12 h). Trap installation and subsequent checking were designed in our previous experimental study and optimized to reduce animals' stress (Boratyński et al., 2010). Trapping did not cause any physical damage to the voles and there was no evidence of aggression among individuals that were trapped at the same time (this happened in four cases; most of the voles were trapped individually). After capture, voles were placed in individual cages (43 × 26 cm and 15 cm high) with bedding of wood shavings and hay, provided with moist food (potato), dry food (Labfor 36; Lactamin AB, Stockholm, Sweden) and water ad libitum, and transported immediately to the animal facility (either local, in one of the forestry field stations, or the animal facility at the University of Jyväskylä). To reduce stress during long transport by air-conditioned truck (the longest transport route was 9 h, from Kolari to Jyväskylä, Finland), animals were kept inside their individual cages with a surplus of hay and moist and dry food, and 1.5 h of recovery breaks were included (transport had no apparent impact on the animals' health). Captured voles were finally housed in the animal facility at the University of Jyväskylä. Each vole was housed in a separate, individual cage (43 × 26 cm and 15 cm high) with wood shavings and hay as bedding, and cardboard tubes for environmental enrichment, in a 16:8 h light:dark photoperiod and at 20 ± 2 °C with standard food (Labfor 36, Lactamin AB, Stockholm, Sweden) and water ad libitum. Overall, 220 (99 females and 121 males: 17/17, 17/19, 22/22, 17/24, 17/26, 9/13; females/males from Tammela, Virolahti, Kannus, Sotkamo, Kolari, and Savukoski, respectively) individuals were captured. Although trapping was conducted during a season of low breeding activity for bank voles (connected to natural between- and within-year population density cycles) 21 captured females were pregnant and were thus provided with additional bedding and allowed to give birth and to nurse successfully. Small tissue samples (1–2 $mm^2$) were taken from the ear of all animals for genetic investigations under anaesthesia (with isoflurane, IsoFlo vet, Orion Pharma, Turku Finland, administrated by the Experimental Animal Unit of the University of Jyväskylä). With the controlled inhalation system, isoflurane was given at a maximum 0.5–1.0% volume in breathing air to each animal by certified experimental animal authorities in Finland (a member of the Experimental Animal Unit). Recovery after the procedure took 15–20 s, after which period the animals were inspected and returned to their individual cages. During trapping, 13 individuals of other *Myodes* species (*rutilus* and *rufocanus*) were also captured

and transported to the laboratory, which was done under permit (see below). All captured voles were used to set up a breeding colony and estimate lifetime repeatability of morphophysiological parameters, and they were kept for their entire lives in the virus-proof animal facility at the University of Jyväskylä, according to the specifications in the permits. Each vole was included in three metabolic and two behavioural trials. Water, food and cage conditions were checked daily and bedding was changed at least monthly by animal facility workers. During cage inspections and bedding changes, the health of individuals was inspected (suspected unhealthy individuals were observed for several minutes, for multiple times); however, no evidence of poor health was found in the colony. Voles were carefully handled (with the use of soft tissue gloves) by trained animal care workers. All study 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).

### *Behavioural Tests*

Personality types of the bank voles were estimated (Fig. 1) by using open field tests, which have become standard behavioural experiments in personality research of animals (e.g. Lantová et al., 2010; Martin & Réale, 2008). Behavioural trials were conducted between 0900 and 1500 hours, and took up to 5 min per individual. Each animal was carefully transferred inside a home tube shelter from its cage and placed into a corner of an experimental arena. The arena was 90 cm wide, 90 cm long and 60 cm high, and made from white, hard PVC; it was cleaned between trials using 90% ethanol. The arena was illuminated by a lamp with a 75 W bulb placed at a height of 1.5 m and by the lights in the experimental room (70 lx in the arena). After each trial, animals were weighed to the nearest 0.1 g on digital scales and their head width was measured (a proxy of structural size) to the nearest 0.1 mm with a digital calliper. All trials were recorded for 5 min (starting when the animal entered the arena) with a digital camera. Video records were analysed using Modular Tracking system 1.07 (custom designed and purchased from M. Kučera, Institute of Physiology, Academy of Sciences, Czech Republic). Since entering and exploring open unprotected space is considered to reflect an individual's activity and boldness, and remaining in a corner or showing slow locomotion along walls reflects insecurity and/or stress (Archer, 1973; Stam, Croiset, Akkermans, & Wiegant, 1997), we divided the space of the arena into three zones: (1) corners (15 × 15 cm), (2) edge (15 cm wide zone along the walls, the corners excluded) and (3) the central space. In each of these zones we analysed the length of the trajectory of the individual's locomotion (Path Corner, Path Edge, Path Space) and time spent in the zone (Total Duration Corner, Total Duration Edge, Total Duration Space). The length of the overall trajectory (Path Total) was also measured. To evaluate the stability and repeatability of the behaviours, the tests were conducted twice, once in May and once in July 2010, always at least 8 days before or after metabolic trials.

### *Basal Metabolic Rate*

Basal metabolic rate (BMR) was estimated with measurements of oxygen consumption (ml/h) on an eight-channel, open-flow respirometric system (Sable Systems, Henderson, NV, U.S.A.) with the Fc-1B O2 (Sable Systems) analyser (Fig. 1). Respirometric trials ran during daytime for 7 h 30 min, which is the optimal time necessary to estimate metabolism at rest while reducing stress to voles. Seven animals were measured per day. Voles were weighed and their head width was measured prior to being placed into the Plexiglas chambers (180 ml) and trials were conducted within their thermoneutral zone: 30.0 ± 0.5 °C (Petrusewicz, 1983). Air flows for each chamber were measured with Flow-Bar (Sable Systems, Henderson, NV, U.S.A.) and air used in the respirometry system was dried with silica gel. Trials were run for 7 h 30 min and samples of dried (with Drierite desiccant) air were taken sequentially from seven measurement chambers and one reference chamber every 15 min. Oxygen levels measured during the trials were used for calculation of oxygen consumption of bank voles at rest, in a postabsorptive and nonreproducing state (Boratyński et al., 2011). The first metabolic trial was conducted 2 months after capturing animals, in October–November 2008. At least 8 days after the first behavioural trial another metabolic trial was run, in May 2010.

### *Genetic Investigations*

The animals were also subjected to genetic investigations (Fig. 1; Boratyński et al., 2011) that detected distinct mitochondrial lineages and signalled introgression of mtDNA from a close relative, *M. rutilus*. Previous sequencing, using tissue samples clipped from ears (GenBank accession numbers: JF929975–JF930131, JX477265–JX477369, JF930082–JF930131), showed that Savukoski and Kolari populations comprise completely, and the Sotkamo population partly, introgressed individuals (Boratyński et al., 2011; Boratyński et al., 2014). Here we included clade affiliation of all individuals, with either introgressed or original mitochondrial DNA, in all statistical analyses.

### *Statistical Analyses*

#### *Variations, correlations and repeatability*

Body condition was defined here as a standardized residual value of body mass regressed against head width (Schulte-Hostedde, Zinner, Millar, & Hickling, 2005). Body mass-corrected BMR was defined here as a standardized residual value of BMR regressed against body mass. To normalize the data, as the traits

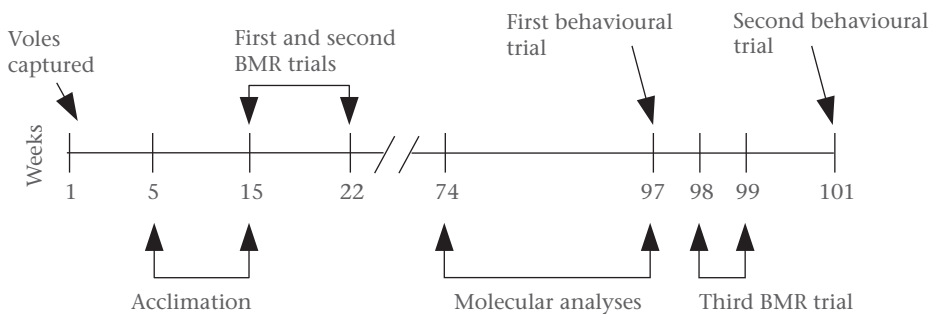


**Figure 1.** Timeline of the study. Week 1 began 2 August 2008.

were skewed (Kolmogorov–Smirnov test: $P < 0.05$), body mass, head width and BMR were log transformed prior to analyses. Pearson partial correlation coefficients between morphological, physiological and behavioural variables (based on the most balanced data set, $N = 116$) included population of origin, individual ID, mtDNA type (with two levels for original and introgressed mitochondria), sex and measurement trial (two repeated measurements) as cofactors. Repeatability of traits was estimated as the intraclass correlation coefficient ($\tau$) on two consecutive measurements collected between 16 and 57 (with mean of 27.1) days of morphological and behavioural trait measurements (Falconer & Mackay, 1996; Hayes & Jenkins, 1997) using the ICC package in R (Wolak, Fairbairn, & Paulsen, 2012) from absolute or mass-corrected (linear residual values) trait values. Long-term repeatability (more than 1 year) was also estimated on morphophysiological traits to test persistence of the level of metabolic rate over the life span of bank voles.

*Principal component analysis*

Using principal component analysis (PCA), a standard multivariate method used in studies of animal personality (e.g. Lantová et al., 2011; Martin & Réale, 2008; Timonin et al., 2011), composite behavioural variables reflecting individual reactions to an open field test were obtained. We decided to apply PCA analyses (e.g. instead of alternative, but more complicated, multivariate mixed-modelling methodology) to follow the well-established protocol in this kind of research and to make this study more comparable with other similar experiments. Moreover, PCA allowed us to reduce a large number of potentially correlated variables to a smaller number of linearly uncorrelated variables (principal components) while retaining the maximal amount of variation (Timm, 2002). This statistical method provides a useful tool, since separate analysis of open field behaviours may lead to misleading results (Gosling, 2001), as they may mirror expression of different personality traits (Archer, 1973; Stam et al., 1997). PCA was conducted on behavioural traits of 116 bank voles. The correlation matrix of measured behavioural traits, composed of the length of the trajectory of individual locomotion in each zone of the experimental arena and the time spent in these zones (Path Corner and Total Duration Corners, Path Edge and Total Duration Edge, Path Space and Total Duration Space), and the overall length of the trajectory reflecting locomotion rate of an individual throughout the test, were reduced to the limited number of principal components selected according to the Kaiser–Guttman criterion (eigenvalues $> 1$; Kaiser, 1991) and scree plot position. PCA scores were used in further analyses evaluating both repeatability and the metabolism–personality association. PCA was conducted in CANOCO 4.5 (Microcomputer Power, Ithaca, NY, U.S.A.).

*Linear mixed models and model selection procedures*

To evaluate and quantify the possible sources of variation of behaviour we constructed analyses based on the linear mixed model and $AIC_c$ model selection procedure in R (libraries: MuMIn and nlme; R project; The R Foundation for Statistical Computing, Vienna, Austria, http://www.r-project.org). The models were fitted with the restricted maximum likelihood (REML) method and $P$ values were estimated from a $t$ test based on REML estimates of the variance. We tested whether variables significantly explained the variation in PC1 and PC2 derived from the PCA analysis on behavioural traits (on the most balanced data set, $N = 116$). The most complicated model for selection procedure included the following fixed factors: population of animal origin (P, six levels), mtDNA type (M, two levels), sex (S, two levels), trial for repeated measurements (T, novel environment trials, two levels), continuous predictors (covariates): body condition (C), residual BMR (rB), body mass (BM), two-way and three-way interactions between factors with one continuous predictor (covariate) and a random factor describing individuals' identity [ID, full model included: P+M+S+T+C+rB+BM+(P*C)+(M*C)+(S*C)+(T*C)+(P*rB)+(M*rB)+(S*rB)+(T*rB)+(P*BM)+(M*BM)+(S*BM)+(T*BM)+ID]. Furthermore, the model selected by the $AIC_c$ (delta $AIC_c > 2$) method was tested in sex-specific analyses. If no single best model could be decided by the delta $AIC_c > 2$ criterion, all alternative models were considered. As the initial analysis on the complete data set failed to select a unique best model (the most comprehensive models are presented in Table 3) but always depicted interaction with sex as an important predictor, we repeated the procedure in two data sets partitioned according to sex, after excluding all interactions in which the effect of sex was involved.

## RESULTS

*Repeatability and Correlations*

All repeatability estimates of different types of behaviours, except for 'Path Corner', were significant both for absolute values (ANOVA) and for values that accounted for variation in body mass (ANCOVA; Table 1). PCA scores were also repeatable; PC1 had a repeatability of 1 which reflects that variation in PC1 occurred mainly at the between-individual level. Also, morphophysiological characters were significantly and highly repeatable (Table 1). Repeatability of BMR and head width were much lower for long-term estimates (0.37 and 0.29; for body mass-accounted values) than for short-term estimates obtained previously for the same experimental colony (0.62 and 0.74; from Boratyński et al., 2011, presented also in Table 1 for comparison). Pearson partial correlation analyses (which accounted for population, individual ID, mtDNA type, sex and repeated trial) showed a weak association between behavioural and morphophysiological characteristics (e.g. correlation between BMR and Total Duration Space: $r = -0.16$, $P < 0.05$; Appendix Table A1). All correlations were low ($|r| \leq 0.16$) and none of them remained significant after accounting for multiple tests (Bonferroni correction for all analyses: critical $\alpha$ of 0.00064; correction per character: critical $\alpha$ of 0.0042). Correlations between behavioural characters ($|r| \geq 0.28$) and between morphophysiological traits ($|r| \geq 0.39$) remained significant after correcting for multiple tests (Table A1).

*PCA of Behavioural Variables*

Behaviour in the open field test was best described by the first principal component PC1, which explained in total 84.9% of the total variability (Table 2). This component had positive loadings with boldness to explore central parts of the arena (Total Duration Space, Path Space) and negative loadings with the tendency to occupy its edge (Total Duration Edge; Table 2). The rest of the variability in behavioural traits was explained by the second PCA axis (PC2) which mainly described the time spent in the arena corner.

*Variation Analyses*

Interindividual variation in behaviours loaded on the first principal component (PC1) was best explained (according to $AIC_c$: $w_i = 0.27$) by the model including condition, mtDNA type, residual BMR (body mass corrected), sex, open field test session, and interactions between residual BMR with both sex and mtDNA type as factors (Table 3). As the best five models similarly explained variation in the data (within delta $AIC_c = 2$; probably caused by correlations between some of the predictors: Table A1) and all of them

**Table 1**
Repeatability estimates of behavioural and physiological characters for two repeated measurements

| Trait | Absolute values | | | Body mass corrected (residuals) | | |
|---|---|---|---|---|---|---|
| | *N* | τ(95%CI) | $P_{ANOVA}$ | *N* | τ(95%CI) | $P_{ANCOVA}$ |
| Raw behavioural traits (short time interval ≈27 days) | | | | | | |
| Path Corner | 119 | 0.07(−0.11–0.25) | 0.224 | 56 | 0.09(−0.26–0.35) | 0.476 |
| Total Duration Corner | 119 | 0.16(−0.03–0.33) | 0.046 | 56 | 0.35(0.05–0.55) | 0.008 |
| Path Edge | 119 | 0.56(0.43–0.67) | <0.0001 | 56 | 0.69(0.52–0.79) | <0.0001 |
| Total Duration Edge | 119 | 0.32(0.15–0.47) | <0.001 | 56 | 0.35(0.06–0.56) | 0.011 |
| Path Space | 119 | 0.52(0.38–0.64) | <0.0001 | 56 | 0.82(0.71–0.88) | <0.0001 |
| Total Duration Space | 119 | 0.29(0.11–0.44) | 0.001 | 56 | 0.51(0.27–0.67) | <0.001 |
| Path Total | 119 | 0.56(0.42–0.67) | <0.0001 | 56 | 0.77(0.64–0.85) | <0.0001 |
| Behavioural trials (short time interval ≈27 days) | | | | | | |
| Log body mass | 56 | 0.95(0.92–0.97) | <0.0001 | | | |
| No. of faeces* | 79 | 0.25(0.02–0.44) | 0.018 | 33 | 0.30(−0.13–0.55) | 0.114 |
| PC1* | 108 | 1.00(1.00–1.00) | <0.0001 | 56 | 0.99(0.98–0.99) | <0.0001 |
| PC2 | 108 | 0.43(0.26–0.57) | <0.0001 | 56 | 0.32(0.02–0.53) | 0.013 |
| Morphophysiological trials (long time interval >1 year) | | | | | | |
| Log body mass | 56 | 0.71(0.55–0.82) | <0.0001 | | | |
| Log head width* | 56 | 0.44(0.20–0.62) | <0.001 | 56 | 0.26(−0.01–0.48) | 0.014 |
| Log BMR* | 56 | 0.52(0.31–0.69) | <0.0001 | 56 | 0.37(0.14–0.59) | <0.0001 |
| Morphophysiological trials (short time interval ≈35 days)† | | | | | | |
| Log body mass | 26 | 0.80(0.61–0.90) | <0.0001 | | | |
| Log head width* | 25 | 0.85(0.70–0.93) | <0.0001 | 25 | 0.77(0.55–0.89) | <0.0001 |
| Log BMR* | 26 | 0.80(0.60–0.90) | <0.0001 | 26 | 0.50(0.15–0.74) | <0.001 |

BMR: basal metabolic rate.
* Significant effect of covariate: body mass.
† Recalculated from Boratyński et al., 2011.

included the three-way interaction between sex, mitochondria type and residual BMR, we proceeded with the analyses separately between the sexes.

The analyses performed separately for the sexes revealed that body condition significantly affected only females' behaviour. Moreover, for females' mitochondrial type, BMR and body mass had no significant effects on principal components (Table 4). In males, the interaction between mitochondrial type and metabolic rate was significant indicating that males with the original type of mitochondria had a positive relationship between PC1 score and BMR, whereas in introgressed males the relationship between PC1 score and BMR was not evident (Table 4, Fig. 2). PC2 did not associate significantly with any of the variables included in the study (Appendix Table A2).

## DISCUSSION

Studies on the association between energetics and personality represent a relatively new research paradigm, although linkage between individual metabolic and behavioural phenotypes and fitness has already been highlighted (but see e.g. Castañeda, Figueroa, Bacigalupe, & Nespolo, 2010). As is typical for many complex traits, behaviour and physiology are prone to constraints and trade-offs set by intrinsic and extrinsic environmental factors, which compromise the tests of their relationship with fitness. Here we found that individual metabolic rate, personality and the relationship between them can be sex and context dependent, and may be altered by genetic processes, such as introgression.

**Table 2**
Summary of principal component analysis (PCA) of behavioural traits of 116 bank voles subjected to the open field test

| Behavioural trait | PC1 | PC2 |
|---|---|---|
| Path Corner | −0.13 | −0.44 |
| Total Duration Corner | −0.16 | **−0.99** |
| Path Edge | −0.07 | 0.05 |
| Total Duration Edge | **−0.96** | 0.27 |
| Path Space | 0.19 | 0.09 |
| Total Duration Space | **0.98** | 0.20 |
| Path Total | 0.09 | 0.05 |
| Total variance | 0.85 | 0.15 |

Behavioural traits include overall locomotion rate, path total and length of trajectories and time spent in each part of the experimental arena. Values in bold contribute significantly to a particular component (i.e. PCA loadings values >0.6; Stevens, 2002).

Since biologically meaningful variability is conditioned by consistency of individual patterns (Bell et al., 2009), the first important step was to demonstrate statistical repeatability of the traits on which we focused. In our study, behaviours of interindividual reactions to an unknown environment (open field test) were strongly repeatable over a considerable part of the bank voles' lives (4 weeks; only 24% survive 4–5 months in a natural situation: Boratyński & Koteja, 2009). Similarly, Korpela, Sundell, and Ylonen (2011) documented substantial repeatability in further behaviours such as mobility, risk taking and exploratory behaviour, aggressiveness towards pups and dominance, indicating the presence of personality-based differences in bank vole behaviour. Regarding physiological traits, both BMR and body mass were highly consistent, which agrees with previous studies on bank voles (Boratyński & Koteja, 2010; Boratyński et al., 2011; Labocha, Sadowska, Baliga, Semer, & Koteja, 2004; repeatability of metabolism reviewed in: Nespolo & Franco, 2007; White, Schimpf, & Cassey, 2013).

In line with our prediction, we found partial evidence for a metabolism–personality syndrome, as there was a positive association between PC1 score and BMR in nonintrogressed bank vole males (Fig. 2a). According to published works on rodent open field behaviour, PC1 could be interpreted as a personality trait reflecting individual differences in proactivity (Archer, 1973; Stam et al., 1997). The positive linkage between proactivity and BMR provides support for the increased-intake model (Nilsson, 2002), rooted in the assumption that proactive personality types (characterized by e.g. elevated levels of general activity, novelty seeking, resistance to stress, aggressiveness and earlier maturation) have a higher energetic output. Similar patterns have been documented in a variety of animal species (reviewed in Biro & Stamps, 2010; Careau & Garland, 2012; but see Timonin et al., 2011). The positive association between proactivity and BMR was not observed for

**Table 3**
Model components and parameters from the $AIC_c$ model selection procedure for explaining variation in PC1 (proactive behaviour)

| Data sets and model components | Model parameters | | | | |
|---|---|---|---|---|---|
| All data | *df* | Log likelihood | $AIC_c$ | Delta | Weight |
| C+M+rB+S+T+(M*rB)+(M*S)+(rB*S)+(M*rB*S) | 12 | −775.1 | 1586.3 | 0.00 | 0.27 |
| BM+M+rB+S+T+(M*rB)+(M*S)+(rB*S)+(M*rB*S) | 12 | −772.2 | 1586.4 | 0.12 | 0.26 |
| C+M+P+rB+S+T+(M*rB)+(M*S)+(rB*S)+(M*rB*S) | 13 | −770.1 | 1587.1 | 0.76 | 0.18 |
| BM+M+P+rB+S+T+(M*rB)+(M*S)+(rB*S)+(M*rB*S) | 13 | −767.2 | 1587.2 | 0.88 | 0.17 |
| C+BM+M+rB+S+T+(M*rB)+(M*S)+(rB*S)+(M*rB*S) | 13 | −773.8 | 1588.0 | 1.70 | 0.12 |
| Females only [starting model: C+M+rB+T+(M*rB)]† | | | | | |
| C+T | 5 | −387.6 | 784.9 | 0.00 | 0.44 |
| C+M+T | 6 | −381.8 | 786.4 | 1.43 | 0.22 |
| C+rB+T | 6 | −390.7 | 787.3 | 2.31 | 0.14 |
| T | 4 | −387.8 | 787.4 | 2.44 | 0.13 |
| C+M+rB+T | 7 | −384.8 | 788.7 | 3.79 | 0.07 |
| Males only [starting model: C+M+rB+T+(M*rB)]† | | | | | |
| M+rB+T+(M*rB) | 7 | −389.1 | 799.1 | 0.00 | 0.66 |
| C+M+rB+T+(M*rB) | 8 | −390.5 | 800.5 | 1.39 | 0.33 |
| C+rB+T | 6 | −400.2 | 809.5 | 10.43 | 0.0 |
| rB+T | 5 | −399.7 | 809.8 | 10.73 | 0.0 |
| C+M+rB+T | 7 | −394.4 | 810.7 | 11.64 | 0.0 |

Starting model for the complete data set included population of animal origin (P: six levels), mtDNA type (M: two levels), sex (S: two levels), open field trial (T: repeated, two levels) as fixed factors, body condition (C), residual basal metabolic rate, BMR (rB), body mass (BM) as continuous predictors, two-way and three-way factorial interactions between M and S with C, rB or BM and a random factor describing individual identity.
† BM, C and P simultaneously kept in sex-specific starting models did not affect final results.

females in our study. Rather, the behavioural–energetics association showed a negative trend, suggesting the compensation model may be more plausible for females (Fig. 2a). This type of sex discrepancy has been suggested by some previous studies (Bouwhuis, Quinn, Sheldon, & Verhulst, 2014; Lantová et al., 2011; Rezende, Gomes, Chappell, & Garland, 2009) and may mirror both sex differences caused by social organization (territorial females versus males with overlapping home ranges, Bujalska, 1990) and a greater investment on the part of females in reproduction, together

**Table 4**
Mixed-model analyses on the factors affecting PC1 (proactive behaviour)

| All data | *Value* | *SE* | *df* | *t* | $P_{REML}$ |
|---|---|---|---|---|---|
| Intercept | 212.35 | 102.75 | 112 | 2.07 | 0.041 |
| C | −0.08 | 0.04 | 46 | −2.06 | 0.045 |
| M | 89.98 | 192.78 | 112 | 0.47 | 0.640 |
| rB | −0.30 | 0.07 | 46 | −4.43 | 0.0001 |
| S | −154.54 | 69.81 | 112 | −2.21 | 0.029 |
| T | −10.80 | 0.02 | 46 | −525.37 | <0.0001 |
| M*rB | 0.35 | 0.16 | 46 | 2.12 | 0.039 |
| M*S | −6.04 | 116.66 | 112 | −0.05 | 0.960 |
| rB*S | 0.29 | 0.05 | 46 | 5.35 | <0.0001 |
| M*rB*S | −0.30 | 0.10 | 46 | −3.09 | 0.003 |
| Female† | | | | | |
| Intercept | 78.11 | 38.95 | 57 | 2.01 | 0.050 |
| C | −0.11 | 0.05 | 25 | −2.19 | 0.038 |
| T | −10.80 | 0.02 | 25 | −438.14 | <0.0001 |
| Male‡ | | | | | |
| Intercept | −96.80 | 52.96 | 56 | −1.83 | 0.073 |
| M | 77.92 | 73.64 | 56 | 1.06 | 0.295 |
| rB | 0.30 | 0.05 | 22 | 5.56 | <0.0001 |
| T | −10.78 | 0.03 | 22 | −391.61 | <0.0001 |
| M*rB | −0.28 | 0.07 | 22 | −4.13 | <0.001 |
| Nonintrogressed males (with original, bank vole, mtDNA) | | | | | |
| Intercept | −96.80 | 57.95 | 27 | −1.67 | 0.106 |
| rB | 0.30 | 0.08 | 10 | 3.88 | 0.003 |
| T | −10.78 | 0.06 | 10 | −191.94 | <0.0001 |
| Introgressed males (with mtDNA from red vole) | | | | | |
| Intercept | −18.87 | 46.22 | 29 | −0.41 | 0.686 |
| rB | 0.02 | 0.02 | 11 | 0.85 | 0.414 |
| T | −10.79 | 0.02 | 11 | −597.48 | <0.0001 |

Analyses were performed for all the individuals, females and males separately. Factors added were selected using $AIC_c$ values (see Table 3).
† Effect of BM was nonsignificant ($P > 0.05$) when included in the model.
‡ Effect of C was nonsignificant ($P > 0.05$) when included in the model.

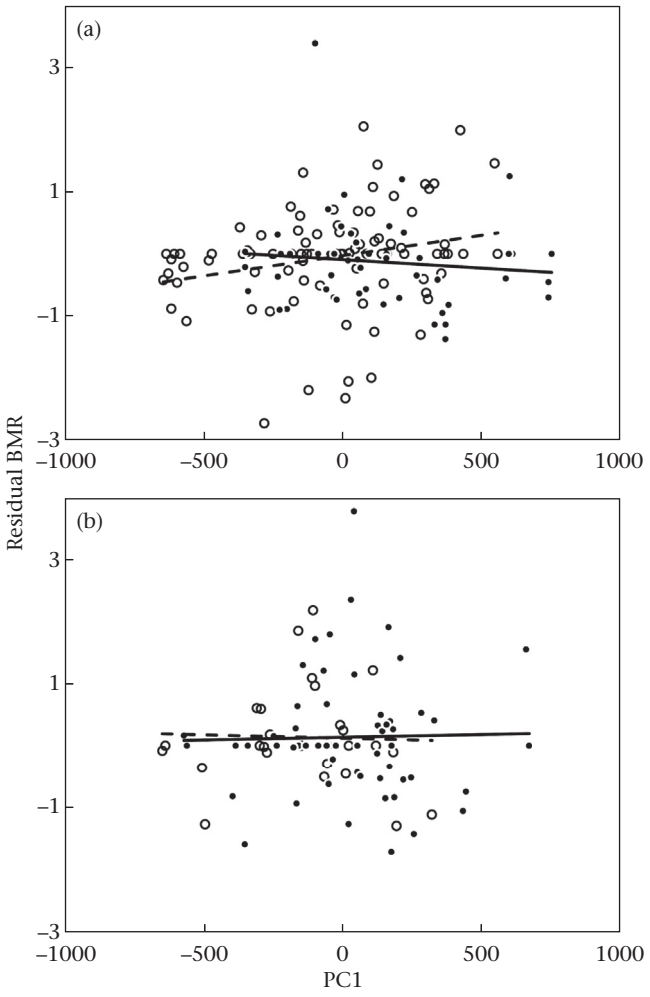


**Figure 2.** The relationship between proactive behaviour (PC1) and body mass-corrected basal metabolic rate (BMR) conducted separately for (a) females (open dots and dashed line) and males (solid dots and solid line) that had the original mitochondrial type and (b) females (open dots and dashed line) and males (solid dots and solid line) with the introgressed mitochondrial type.

with the fact that bank voles usually rely on scattered food resources (Jensen, 1982).

In general, our study supports the suggested positive linkage between energy metabolism and individual personality, at least in males. However, this relationship was affected not only by the sex of the individuals but also by the type of the mtDNA they carried. The relationship between proactive behaviour and BMR was evident only in males with their original mtDNA, whereas it was absent in the male voles that carried introgressed mtDNA from *M. rutilus* (Fig. 2b). The nonsignificant negative trend observed in females also disappeared in introgressed voles (Fig. 2b). The disruption of important coevolved mitochondrial and nuclear gene complexes might be responsible for these effects (Rand et al., 2004; Runck, Matocq, & Cook, 2009). This possibility, together with the findings of a previous study on additive genetic correlation between resting metabolic rate and exploratory behaviour, suggests that the covariation between personality and metabolism might carry an important genetic component in rodents (Careau et al., 2011). Lack of correlations between metabolism and behaviour in introgressed voles is expected if the mtDNA introgression causes metabolic and/or behavioural malfunctions that disrupt normal physiology. Alternatively, if the metabolic rate constrains personality, it can be hypothesized that through introgression of mtDNA, individuals could acquire novel physiological machinery that may allow them to overcome these limitations. In this study we could not explicitly test between these two alternative hypotheses, which would require future experimental manipulation of phenotypes. However, a marked increase in phenotypic variability was not observed in the introgressed voles (Rand et al., 2004), which would suggest the disruptive character of introgression. Thus, and in concordance with our previous results (Boratyński et al., 2011, 2014), we cautiously hypothesize that the introgressed mtDNA type might carry specific, probably adaptive, features.

### *Conclusions*

Our study provides new evidence for recent hypotheses suggesting that consistent behavioural patterns, or personality traits, promote variation between individuals in energy metabolism, and vice versa. Here we demonstrated the individual consistency of these traits across biologically meaningful periods of the lives of the bank voles tested, and showed evidence of a mutual linkage between personality and BMR. However, the physiology–behaviour association differed significantly between the sexes and was affected significantly by the mtDNA type. In particular, introgression disrupted this association. The observed sex differences might reflect behavioural differences between the sexes, and less physiological dependency of female behaviours. Mitochondrial dependency suggests either that introgressed mtDNA from related species disrupted the bank voles' normal phenotypes, or that the captured mtDNA encodes beneficial features that allow individuals to overcome physiological limitations for expressing costly behavioural traits. These alternatives need to be verified experimentally.

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## Appendix

**Table A1**
Pearson partial correlations between behavioural and morphophysiological characters

| | Path Corner | Total Duration Corner | Path Edge | Total Duration Edge | Path Space | Total Duration Space | Path total | No. of faeces | Log body mass | Log head width | Log BMR | rBMR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Total Duration Corner | **0.54**$^{***}$ | | | | | | | | | | | |
| Path Edge | **0.34**$^{***}$ | −0.06 | | | | | | | | | | |
| Total Duration Edge | −0.01 | −0.17$^{*}$ | 0.08 | | | | | | | | | |
| Path Space | 0.21$^{*}$ | −0.14 | **0.92**$^{***}$ | −0.13 | | | | | | | | |
| Total Duration Space | **−0.28**$^{**}$ | **−0.35**$^{***}$ | −0.05 | **−0.86**$^{***}$ | 0.20$^{*}$ | | | | | | | |
| Path Total | **0.30**$^{**}$ | −0.09 | **0.97**$^{***}$ | −0.05 | **0.99**$^{***}$ | 0.10 | | | | | | |
| No. of faeces | −0.11 | −0.08 | 0.01 | −0.11 | 0.02 | 0.14 | 0.01 | | | | | |
| Log body mass | 0.03 | 0.02 | −0.03 | 0.11 | −0.05 | −0.12 | −0.04 | 0.19$^{*}$ | | | | |
| Log head width | −0.05 | 0.02 | −0.16$^{*}$ | 0.12 | −0.13 | −0.12 | −0.15 | 0.09 | **0.40**$^{***}$ | | | |
| Log BMR | 0.10 | 0.07 | 0.03 | 0.12 | 0.03 | −0.16$^{*}$ | 0.03 | 0.01 | **0.57**$^{***}$ | **0.39**$^{***}$ | | |
| rBMR | −0.02 | 0.01 | 0.16$^{*}$ | 0.06 | 0.15$^{*}$ | −0.06 | 0.16$^{*}$ | −0.09 | 0.11 | −0.03 | **0.72**$^{***}$ | |
| Condition | 0.06 | 0.01 | 0.04 | 0.07 | 0.01 | −0.07 | 0.02 | 0.16 | **0.92**$^{***}$ | −0.01 | **0.45**$^{***}$ | 0.13 |

Correlations accounted for variation in the population of the origin, individual ID, type of the mtDNA, sex and the number of repeated trial. In bold are values corrected for multiple tests, both within characters (for 12 tests) and for all analyses (for 78 tests). BMR: basal metabolic rate; rBMR: linear residual values of log BMR corrected for log body mass; condition: linear residual values of log body mass corrected for log head width.
$^{*}P < 0.05$; $^{**}P < 0.0006$; $^{***}P < 0.00001$.

**Table A2**
Model components and parameters from $AIC_c$ model selection procedure for explaining variation in PC2

| Data sets and model components | Models parameters | | | | |
|---|---|---|---|---|---|
| All data* | *df* | Log likelihood | $AIC_c$ | Delta | Weight |
| Population | 4 | −243.4 | 488.2 | 0.00 | 0.32 |
| MtDNA type | 4 | −242.4 | 488.6 | 0.39 | 0.26 |
| NULL | 3 | −243.2 | 489.6 | 1.48 | 0.15 |
| Population+Trial | 5 | −244.2 | 489.9 | 1.74 | 0.13 |
| Population+rBMR+Population*rBMR | 6 | −245.6 | 489.9 | 1.74 | 0.13 |
| Females only [starting model: P+M+rB+T+(P*rB)]* | | | | | |
| NULL | 3 | −114.4 | 232.3 | 0.00 | 0.33 |
| Population | 4 | −115.4 | 233.1 | 0.81 | 0.22 |
| MtDNA type | 4 | −114.2 | 233.2 | 0.86 | 0.21 |
| rBMR | 4 | −115.5 | 234.2 | 1.93 | 0.13 |
| Trial | 4 | −115.0 | 234.5 | 2.17 | 0.11 |
| Males only [starting model: P+M+rB+T+(P*rB)]* | | | | | |
| NULL | 3 | −126.1 | 256.5 | 0.00 | 0.28 |
| Population | 4 | −126.5 | 256.7 | 0.15 | 0.26 |
| MtDNA type | 4 | −126.7 | 257.3 | 0.77 | 0.19 |
| rBMR | 4 | −126.8 | 257.7 | 1.27 | 0.15 |
| Trial | 4 | −126.6 | 258.4 | 1.87 | 0.11 |

MtDNA type: type of the mtDNA hosted by individuals, referring to original bank vole or introgressed from red vole mtDNA; rBMR: residual basal metabolic rate corrected for variation in body mass, both log transformed: trial: behavioural measurement trial.

* No effects were significant in the analyses including either all or separate variables from all five models.