

Article

Experimental evolution of personality traits: open-field exploration in bank voles from a multidirectional selection experiment

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Abstract

Evolution of complex physiological adaptations could be driven by natural selection acting on behavioral traits. Consequently, animal personality traits and their correlation with physiological traits have become an engaging research area. Here, we applied a unique experimental evolution model—lines of bank voles selected for (A) high exercise-induced aerobic metabolism, (H) ability to cope with low-quality herbivorous diet, and (P) intensity of predatory behavior, that is, traits shaping evolutionary path and diversity of mammals—and asked how the selection affected the voles' personality traits, assessed in an open field test. The A- and P-line voles were more active, whereas the H-line voles were less active, compared those from unselected control lines (C). H-line voles moved slower but on more meandering trajectories, which indicated a more thorough exploration, whereas the A- and P-line voles moved faster and on straighter trajectories. A-line voles showed also an increased escape propensity, whereas P-line voles tended to be bolder. The remarkable correlated responses to the selection indicate a common genetic underlying mechanism of behavioral and physiological traits, and support the paradigm of evolutionary physiology built around the concept of correlated evolution of behavior and physiology.

Key words: evolution, behavior, physiology, selection, rodents, *Myodes glareolus*

It has been proposed that evolution of complex morphological or physiological adaptations, such as those required to cope with a specific diet, endure high locomotor activity or invade aquatic habitats, is intimately correlated with evolution of behavioral traits, whose possible scope is limited by the physiological performance (Duckworth 2009; Swallow et al. 2009; Careau and Garland 2012; Wolf and Weissing 2012; Garland et al. 2016). For example, the selection favoring an intensified exploratory activity or active hunting can drive evolution of an increased aerobic exercise capacity, and the selection for herbivorous diet preference can lead to evolution of a complex alimentary system supporting symbiotic digestion. Consequently, the inter-individual variation in behavior and its correlation with non-behavioral traits has become an engaging research

area both in behavioral sciences and evolutionary physiology (Dingemanse and Réale 2005; Réale et al. 2010; Careau and Garland 2012; Foster 2013; Ferrari et al. 2013; Toscano et al. 2016; Yuen et al. 2017). Individuals within a population consistently differ in behavioral response to the same stimuli, often across a wide range of environmental contexts. Such among-individual differentiation in behavior is known as “personality” (Careau and Garland 2012; Carter et al. 2013; Han and Dingemanse 2015), and has been identified in a wide range of animals (Gosling 2001; Wolf et al. 2007; Schuett et al. 2010; Jandt et al. 2013; Wolf and Weissing 2012; Carere and Maestripieri 2013). Inter-correlated sets of personality traits, representing a “behavioral syndrome” (Sih and Bell 2008; Carter et al. 2013; Han and Dingemanse 2015; Chock et al. 2017),

can be further correlated with physiological traits (Koolhaas et al. 2007; Thörnqvist et al. 2015; Houslay et al. 2017; Zidar et al. 2017). Such correlation may reflect common genetic mechanisms, for example, resulting from pleiotropic gene effects, which provide a basis for correlated evolution of the behavioral and physiological traits (van Oers et al. 2005; Bouwhuis et al. 2013; van Oers and Sinn 2013; Dochtermann et al. 2015; Kern et al. 2016; Edwards et al. 2017).

Here we applied an experimental evolution approach and asked how selection for important traits shaping evolutionary path and diversity of mammals, namely the aerobic exercise performance and herbivorous *versus* predatory capability, affected personality traits of a non-laboratory rodent, the bank vole.

A presence of genetic correlations can be revealed by a quantitative genetic analysis of the traits of interest (e.g., Boake 1994; van Oers et al. 2004a, 2004b, Petelle et al. 2015). However, the presence of genetic correlation implies that, when both traits are heritable, a selection acting on one trait leads also to changes in the other trait, that is, a correlated response is observed. Therefore, artificial selection provides a powerful, inferential tool for studying the potential for correlated evolution of behavioral and physiological traits (Boake 1994; van Oers et al., 2005; Swallow et al. 2009). For example, selection for high nest building behavior in house mice resulted in increased body-mass, body temperature, social-aggression and litter size, and decreased food consumption and wheel running activity (Lynch 1994; Bult and Lynch 2000). Importantly, a reciprocal selection for either large body-mass or high aggression resulted in an increased nest building behavior (Lynch 1994; Sluyter et al. 1995). Such a symmetry in responses to selection provides flexibility in inferences based on selection experiments: a change in a behavioral trait in response to selection for a physiological trait implies that selection for the behavioral trait should lead to evolution of the physiological trait, and *vice versa*.

Animal personalities are broadly differentiated on a proactive-reactive coping style continuum (Koolhaas et al. 2007; Réale et al. 2007; Jones and Godin 2009; Coppens et al. 2010; Koski 2014; Roche et al. 2016; Toscano et al. 2016; de Boer et al. 2017; Laubu et al. 2017; Zidar et al. 2017). “Proactive” individuals cope with stressful situation by “flight or fight” response (Jones and Godin 2009; Ferrari et al. 2013; Zidar et al. 2017): they have higher level of activity and are bold to explore unfamiliar space, and act aggressively towards other individuals or predators. They rely on routines based on previous experience and hence cope better in a stable environment, but need longer time to adjust to changes. On the other hand “reactive” individuals are fearful and react to stressors (such as presence of a predator) by “freezing” (Koolhaas et al. 1999; Carere and Maestripieri 2013). They are less active and slower, but more thorough in exploration. They are more behaviorally flexible, and hence adapt better in an unstable environment compared with the proactive ones (Coppens et al. 2010; Carere and Maestripieri 2013; Baugh et al. 2017; Zidar et al. 2017).

The evolutionary origin and the maintenance of the animal personality remains an enigma (Réale et al. 2010; Biro and Stamps 2010; Kight et al. 2013; Snell-Rood 2013; Roche et al. 2016; Edwards et al. 2017). From an adaptive and “optimal phenotype” perspective an individual’s behavior should be plastic enough to render selective advantage (Wolf et al. 2008; Careau et al. 2010), and hence the individuals should show no distinct personalities. However, the scope of the plasticity can be constrained by morphological and physiological limitations (Duckworth 2010), so that personality, morphology and physiology form an integrated suite of

traits that developed to adapt to a particular environment and resource availability (Snell-Rood 2013). Particularly, the development and expression of personality and the level of behavioral plasticity are shaped by nutritional history and specialization (Toscano et al. 2016; Han and Dingemanse 2017). A relation between personality and metabolic rate is also commonly postulated, but the predicted direction of the relationship is not obvious and the pattern varies among species and across environmental circumstances (Biro and Stamps 2010; Careau et al. 2010; Careau and Garland 2012; Šichová et al. 2014; Toscano et al. 2016).

To test the hypothesis that personality traits coevolve with diverse adaptive strategies, we used a unique model system, comprising lines of the bank vole selected for 3 performance traits: the maximum rate of swim-induced aerobic exercise metabolism (A, “aerobic”), ability to maintain body mass on a low-quality herbivorous diet (H, “herbivorous”) and predatory behavior measured as a time needed for killing a cricket (P, “predatory”), and unselected control lines (C) (Sadowska et al. 2008, 2015). Importantly, values of all the selected performance traits depend in the first instance on behavioral characters (Sadowska et al. 2008). The time needed for catching a prey depends first on whether the individual is at all interested in the potential prey—and only in the second instance on its physical ability to efficiently capture the prey. The ability to maintain body mass balance depends first on the “decision” whether to eat the low quality food (or rather wait or search for a better food)—and only then on the physiological capability of efficiently digesting the food. Similarly, because voles do not have to work vigorously during the swimming test (they can “hang” in water without moving), the maximum swim-induced rate of metabolism depends first on the behavioral characteristics such as stress-coping or motivation to exercise, not only on the physiological “aerobic capacity” *per se*. Indeed, behavioral component of the trait had evolved in the A-selected lines, and the swim-induced metabolism of A-line voles approached the true aerobic capacity, whereas voles from the unselected C lines exercised well below their physiological capability (Jaromin et al. 2016). Thus, the selection experiment provides a suitable model for investigating the correlated evolution of behavior and physiological adaptations.

We have already used the model to test hypotheses concerning evolution of high BMR and endothermic thermoregulation, learning capability, motivation to exercise, molecular basis of an increased metabolic rate and predatory propensity, and gut microbiome evolution (Chrzęścik et al. 2014; Sadowska et al. 2015; Konczal et al. 2015, 2016; Jaromin et al. 2016; Kohl et al. 2016). In this study, we asked how the selection affected the voles’ personality traits, assessed as activity and exploration in an open field. Exploratory behavior, which helps animals assess their environment, has considerable fitness consequences as it plays important role in finding resources and mates, and in predatory avoidance (Drent et al. 2003; Dall et al. 2004; Kight et al. 2013; Dall and Griffith 2014; Toscano et al. 2016; Delnat et al. 2017). The open field test is a standard tool for measuring emotionality as well as overall locomotor activity and exploratory propensity (Bronikowski et al. 2001; Carter et al. 2013). It is also held as analogous to ecological situation of exposure to a novel environment, in which individuals differ in tendency of risk taking and tactics of coping with the stressful situation (Dall and Griffith 2014; Toscano et al. 2016) and therefore widely used for assessing animal personality (Šichová et al. 2014; Perals et al. 2017; Yuen et al. 2017). For a semi-fossorial bank vole, the open field provides both a spatial novelty and a stressful environment. Because a more proactive coping style appears to be associated with high locomotor activity (Kern et al. 2016; Baugh et al. 2017),

high metabolic rate (Rezende et al. 2006; Šichová et al. 2014; Toscano et al. 2016), and predatory skills (McGhee et al. 2013; Chang et al. 2016), we expected that voles from the “aerobic” (A) and “predatory” (P) lines, which are characterized by higher home-cage locomotor activity (Koteja et al. 2009), evolved a more proactive personality, manifested as a higher activity and more intensive exploration in the open field, compared with that of voles from the unselected C lines. On the other hand we expected that the less active voles from the “herbivorous” (H) lines evolved a more reactive personality.

Material and Methods

Animals and the selection experiment

This work was performed on a small, omnivorous rodent, the bank voles [*Myodes* (= *Clethrionomys*) *glareolus* (Schreber 1780)] from generation 15 of a multivariate artificial selection experiment. The rationale, history and protocols of the ongoing experiment and details of the animal maintenance and welfare were presented in our earlier work (Sadowska et al. 2008, 2015; Chrzęścik et al. 2014), and are also described in detail in [Supplementary Methods](#). Briefly, selection was applied based on the following criteria: High aerobic metabolism (A)—the maximum 1-min rate of oxygen consumption (VO₂swim), achieved during 18-min swimming at 38°C; Herbivorous capability (H)—body mass change in a 4-day trial, during which voles were fed a low-quality, herbivorous diet (made of dried grass and flour); Predatory behavior (P)—mean ranked time to catch an alive cricket in four 10-min trials (ranks 1–5: cricket caught in 0.5, 1, 3, 6, or 10 min, respectively; rank 6: cricket not caught). The measurements of swim-induced aerobic metabolism and the predatory behavior tests were performed on adults (about 75–95 days old), and the test with low-quality diet on young, still growing animals (starting at day 32). All the trait values used as selection criteria were mass-adjusted (residuals from ANCOVA including also other covariates and cofactors). Four replicate lines for each selection direction and an unselected control (C) were maintained (to allow valid tests of the effects of selection), with 15–20 reproducing families in each of the 16 lines (which allowed to avoid excess inbreeding). The selection was applied mostly within families, but if more than 16 families were available, families in which all individuals scored below the line mean were excluded.

The selection was effective ([Supplementary Figure S1](#)). In generation 15, VO₂swim was 55% higher in A than in C lines (mean ± SD: A: 5.49 ± 0.66 ml O₂/min; C: 3.54 ± 0.43 ml O₂/min). Body mass loss during the 4-day test with low-quality diet was 2.2 g smaller in H than in C lines (H: 1.32 ± 1.21 g; C: –3.79 ± 1.50 g). In P lines 97% whereas in C lines only 13% individuals attacked a cricket (mean ranked time to catch a cricket, P: 1.80 ± 1.11; C: 5.63 ± 1.08).

The animals were maintained in standard plastic mouse cages with sawdust bedding, at a constant temperature (20 ± 1°C) and photoperiod (16h: 8h light: dark; light phase starting at 2:00 am). Water and food (a standard rodent chow: 24% protein, 3% fat, 4% fiber; Labofeed H, Kcynia, Poland) were provided *ad libitum*.

All procedures associated with the breeding scheme, the selection protocol as well as experimental procedures were approved by the Local Ethical Committee for Experiments on Animals, Kraków, Poland (No. 67/2012).

Open field test

The open field was a large circular arena (113 cm diameter, 40 cm high wall) similar to the arena as described in Jónás et al. (2010).

The arena was made of light grey hard PVC, with non-slippery and non-reflective inner surface. The tests were performed in November and December 2012, between 08:00 and 16:00 h. Two replicate trials were performed on each of 191 individuals (80–90 days old): 6 males and 6 females per each replicate line from each of the 3 selection directions and unselected control, except one of P lines in which only 5 males were available. The voles did not undergo any selection trials to avoid a possible influence of such experience on the behavior in open field.

A tested vole was taken from its home cage, placed in a plastic, non-transparent container (same as used during all husbandry manipulations) for 1 min, and weighed (with the container). Then the animal was gently placed on the floor in the arena center, with the container inverted over the animal, and left for another 15 sec. The container was then removed, and the movement of the animal was video recorded in a surveillance recorder (BCS-0404LE-AN) for 5 min with the aid of a surveillance camera (Samsung 3000 PH) fixed 166 cm perpendicular above the center of the arena floor. After the test the animal was collected from the arena and released to its home cage. The arena floor and the inner wall were cleaned thoroughly before and after each trial with moist cloth and then with 90% ethanol to remove any residues of odorants and marks left by the animal. The next test animal was placed only after the arena was thoroughly dry. The test was repeated on the next day following the first trial.

Video analysis

The video records (resolution 704 × 576 pixels, 25 frames/s) were analyzed using tracking software EthoVision XT 9.0 (Noldus Information Technology, Netherlands). An “arena-image” (the region in the video image in which a moving subject-animal is tracked) was set up with calibration scale, enabling to convert pixel coordinates to real-space coordinates, with each pixel approximately equal to 0.26 cm (EthoVision XT Reference manual 2012; Noldus et al. 2001). The arena-image comprised the entire floor of the arena and bottom part of the wall (up to 15 cm high). The arena-image was then divided into virtual zones: the “central zone” (circular area with a diameter of 56 cm), the “middle zone” (a 21 cm ring outside the central zone), the “edge zone” (all the arena-image outside middle zone), and the “wall zone” (an outer part of the edge zone, covering only the wall of the physical arena).

Tracking started automatically 1 s after detection of “center of the body” point of the animal and stopped automatically after 300 s. EthoVision determined the body central point every 0.04 s and calculated distance moved between 2 consecutive frames (time points). To avoid overestimation due to noise in tracked points and changes of body shape, the movement duration and distance were counted after filtering out small movements of body center point, with threshold start velocity 0.08 cm/0.04 s and end velocity 0.07 cm/0.04 s. The start velocity is the velocity above which the subject was considered to start moving, whereas the end velocity is the velocity below which displacements of the subject’s body points is no longer attributed to locomotion but to system noise, body wobble or pivoting on the spot (Noldus Information Technology 2012; Noldus et al. 2001).

We measured the following parameters: the total distance moved by the vole (cm), duration of mobility (s), the mean and maximum velocity (cm/s), meandering coefficient (rad/cm), latency to reach the edge (s), frequencies of visits to the edge zone, wall-seeking (standing or jumping by the wall), and returns from the edge to the central zone. Duration of mobility is the total time (summation of all 0.04 s

time intervals) when the animal was detected as moving based on the filter mentioned above. The mean velocity is the total distance moved by an individual divided by the duration of mobility. The maximum velocity is computed by the tracking software as the maximum frame-to-frame (i.e., across 0.04 s) change of the body center position observed in the entire trial (and multiplied by 25 to obtain cm/s units). The meandering coefficient is the mean frame-to-frame change in direction of movement (unsigned radians) of a subject relative to the distance moved by that subject. Frequency of visits to a particular zone was counted as the sum of instances the body central point was detected immediately following the entry to that zone.

We also calculated proportions of the total time spent moving (duration of mobility divided by total trial time, i.e., 300 s) and of the time spent in the edge and the central zones (duration of time spent in the respective zone divided by total trial time), and proportion of distance covered in the edge and in the central zone (relative to total distance). The proportion of time spent moving provides the same information as the duration of mobility, but we decided that presenting the results in this form will be more effective. The proportions of distance and time in the central zone were computed only for episodes following returns to the central zone (i.e., excluding the time immediately following releasing the animal in the arena).

Statistical analyses

Some of the quantitative variables described above concerned behavior in the edge zone, or behavior of those individuals that at least once visited the edge zone (or at least once left the central zone), and hence could not be quantified for several individuals. For these traits we attempted to analyses 2-part models, in which each individual was characterized by 2 response variables: a binary (1 if the individual visited the zone, 0 if not) and a quantitative, which was set to missing for individuals that did not visit the zone. However, the models failed to converge. Therefore, we performed the analysis separately for the 2 parts (Dammhahn 2012). First, we applied logistic regression to the binary variable to study the effect of selection and other factors on the probability of entering the zone. Next, we performed a typical analysis for quantitative values of the traits, but only for individuals that entered the focal zone.

The quantitative traits were analyzed with generalized linear mixed model for repeated measures design, implemented in GLIMMIX procedure in SAS 9.4 (SAS Institute, Cary, NC, USA), with assumed normal distribution of residuals and restricted maximum (REML) method of estimation. The model included “Selection direction” (4 line Types: T=A, H, P and C), “Sex” (S) and “Trial number” (R=1 or 2) as the main fixed categorical factors, interactions between the fixed factors, “Body mass” (M, averaged for trial 1 and trial 2) as fixed covariate, replicate Line nested within selection direction (L(T)) and its interactions with Sex and Trial number as *G-side* random effects, and the residuals of trial day 1 and day 2 for each individual (ID) as *R-side* random term for repeated measures (details: Supplementary Results). In the initial model we allowed heterogeneous slopes (i.e., interactions between body mass and the categorical predictors) as well as heterogeneous residual variances across the selection and sex groups, and across the 2 repeated trials (“group=T*S type=un” option in the “random” statement of GLIMMIX procedure). In subsequent analyses we stepwise reduced the model, by eliminating non-significant interactions with body mass, and eliminating a non-supported variance heterogeneity (based on AIC criterion comparisons, for models

with the same set of fixed factors but different variance structure of the random effect).

Inspection of residuals in preliminary analyses indicated that, with the exception of mobility duration and the maximum velocity, all other variables had to be transformed to achieve normality. Therefore the analyses were performed on square-root-transformed total distance and average velocity, and \log_{10} -transformed meandering coefficient, frequency of wall-standing (as the frequency traits were zero for some individuals, the raw frequencies were increased by 1 before computing their logs). Edge to Central zone frequency was modeled with negative-binomial distribution with log link function.

The angular-transformation (arc-sin square-root) was applied to the proportions of distance in the edge and central zone, and time spent in the central zone. However, the proportion of the time spent in the edge zone was heavily left skewed, with most of the observations above 50%, but some below 50%, and hence the angular transformation could not normalize the distribution. Therefore, we reversed this variable to proportion of time spent outside the edge zone, and then square root transformed it to achieve normality.

The preliminary analyses supported the assumption of the homogeneity of slopes for most of the variables, that is, all interactions with body mass were not significant and removed from the final models. Thus, the final model had the following structure (with Y representing a dependent variable, and b_0 the overall intercept; subscripts of group indicators are omitted for simplicity):

$$\text{Fixed effects model : } Y = b_0 + T + S + R + T \times S + T \times R + S \times R + T \times S \times R + M$$

$$G\text{-side random effects : } L(T) + S \times L(T) + R \times L(T) + S \times R \times L(T)$$

$$R\text{-side random effect : repeated measures term with subject} = ID(S \times L(T)).$$

The exception was the proportion of time spent in the central zone, for which the slopes differed between sexes, and therefore the final model included also fixed $M \times S$ and random $M \times S \times L(T)$ interactions. The variance structure of the residual term differed between particular variables (Supplementary Results).

Significance of the fixed factors was tested with F test, and Satterthwaite approximation was used for denominator degrees of freedom. Multiple comparisons between the 4 selection directions (classes of T factor) were performed with the Tukey–Kramer correction.

The binary variable “reached the edge zone” was analyzed with a mixed effect logistic regression implemented in SAS GLIMMIX procedure, with binary distribution, logit link function, and residual pseudo-likelihood method of estimation (preferred methods, such as adaptive quadrature or Laplace, could not be applied to the repeated-measures design, in which *R-side* matrix must be modeled). The model included the same main fixed and random effects as that used for the quantitative traits (described above), but did not include interaction effects (models with interactions did not converge). The resulting adjusted least squares means for particular groups were back transformed (“ilink” option in GLIMMIX) to represent probability of reaching the edge zone by an individual from a given group.

To assess “consistency repeatability” of the above traits we fitted simplified versions of the models, with Trial number as the only fixed factor and individual (ID) as the only random effect, and

compound symmetry structure for residual covariance matrix. Repeatability was then calculated as the coefficient of intraclass correlation, $ICC = (ID \text{ variance}) / (ID \text{ variance} + \text{residual variance})$ (Nakagawa and Schielzeth 2010). We also computed the “adjusted repeatability” (Nakagawa and Schielzeth 2010) from models that included fixed effects of Selection, Sex, Trial number and Body mass, and random effect of Replicate Lines (see Supplementary Table S5 for details). For the traits with binary or negative binomial distributions, the repeatability was computed according to Nakagawa and Schielzeth (2010) and Nakagawa et al. (2017). The method of calculation is presented in Supplementary Table S5, together with all the quantities used in the computations. Following recommendation of Nakagawa and Schielzeth (2010), we present the repeatabilities on latent scale (which is the basis for the inferential statistics).

Latency to reach the edge zone (s) was analyzed as a “failure time model” (a variant of survival analysis) for right-censored observations with assumed Weibull distribution, implemented as a non-linear mixed model (NLMIXED procedure, see example 70.5, SAS 9.4 Documentation). For individuals that reached the edge, the actual time of achieving this zone was the non-censored latency time (s). For those that did not reach the zone, the right-censored time was 300 s, that is, equal to the trial length. A binary dummy variable distinguished the censored from non-censored observations. The analysis was performed separately for trial 1 and 2. The model included fixed effects of Selection and Sex groups, coded with dummy variables, and random effect of Line (with normal distribution), added to both of the 2 parameters of the Weibull distribution, namely the scale and shape of the “survival” curve. Significance of effect of selection on the scale and shape parameters was tested with likelihood ratio test, against reduced models in which the respective effects were removed.

Results of descriptive statistics (means \pm SD) and results of the generalized mixed linear and non-linear models (adjusted Least Square Means \pm SE and confidence limits for selection directions, test statistics, and significance levels) are presented in the Supplementary Tables. A complete outputs from the SAS procedures, including diagnostic graphs with residuals distribution, are presented in Supplementary Results. In the main text we provide the main results in graphical form, as the adjusted least square means with 95% confidence limits (LSM [CL]), back-transformed to original scale (Figure 1).

Results

During the 5-min open field test the voles travelled on average a distance of 32 ± 25 m (mean \pm SD from pooled results from the 2 trials; see Supplementary Tables S1, S2 for complete descriptive statistics). In most animals the distance was between 1 cm and 130 m, but one individual covered 210 m in trial 1 and 142 m trial 2. However, on the square-root-transformed scale these extreme values were not outliers (studentized residuals < 3) and hence were retained in the analysis. The meandering coefficient typically ranged from 2 to about 1070 rad/cm, but 2 values were extreme outliers (2300–43,000 rad/cm; studentized residuals > 4). The meandering coefficient averaged 261 ± 2298 rad/cm for all observations, and 116 ± 176 rad/cm after excluding the 2 outliers. As the outliers severely distorted the distribution of residuals, here we present results of analyses from the reduced dataset. However, the results were qualitatively the same as those obtained on the complete dataset (results of both versions of the analysis are presented in the

Supplementary Results). The voles were mobile for $53 \pm 25\%$ (0.7–96%) of the trial duration, with the average velocity of 17.6 ± 7.8 cm/s (1–51 cm/s), and reached the maximum velocity of 80 ± 30 cm/s (1.3–173 cm/s). About 96% of the voles left the central zone, 93% visited the edge zone, and 84% showed at least once wall-seeking behavior (mean frequency 21 ± 31 , maximum 286). The voles spent in the edge zone on average $70 \pm 27\%$ of the total test time and covered there $68 \pm 24\%$ of the total distance. About 66% of the voles made at least one return from the edge to central zone (mean frequency: 3.7 ± 4.8 , maximum 32). After excluding the initial time and distance covered before the first exit from central zone, the voles spent in the central zone $3.7 \pm 4.6\%$ of the trial time (maximum 26%) and covered there $8 \pm 9\%$ of total distance (maximum 74%).

Behavior of the voles differed markedly between the 2 repeated trials (Figure 1, Supplementary Tables S3 and 4). In the second trial, the voles were mobile for only 44% instead of 62% of the trial duration ($F_{1, 179} = 116$, $P < 0.001$), moved at a lower average speed ($F_{1, 139} = 6.02$, $P = 0.015$) and covered about 1.2 m shorter total distance ($F_{1, 175} = 62$, $P < 0.001$). However, in the second trial a higher proportion of the distance was located in the central zone ($F_{1, 107} = 12.7$, $P = 0.001$), the trajectory of the movement was more meandering ($F_{1, 180} = 127$, $P < 0.001$), and the voles achieved higher instantaneous maximum speeds ($F_{1, 18.8} = 6.6$, $P < 0.018$; other variables did not differ significantly between the trials). After accounting for the systematic differences between the 2 trials, the consistency repeatability, estimated as the coefficient of intraclass correlation (ICC), ranged from 30% to 60% (for the quantitative variables) and 13–19% (for the binary variables; Table S5). The adjusted repeatability (estimated as ICC from models including also fixed effects of Selection, Sex and Body mass) ranged from 21% to 57% (for the quantitative variables) and from 10% to 19% (for the binary variables; Table S5).

Most of the variables were not affected by body mass ($P > 0.1$). However, the log-transformed meandering coefficient tended to decrease with mass (slope \pm SE = -0.015 ± 0.009 , $t_{174} = -1.73$, $P = 0.08$) and the proportion of distance covered in the central zone after returning to the central zone from outside (arcsine-transformed) increased with mass (slope: 0.004 ± 0.001 ; $t_{70} = 2.37$, $P = 0.020$). For the proportion of time spent in the central zone, the Sex \times Mass interaction was significant: the arcsine-transformed proportion increased with mass in males (slope: 0.008 ± 0.003 ; $t_{140} = 3.02$, $P = 0.003$) but not for females (slope: -0.002 ± 0.004 ; $t_{124} = -0.54$, $P = 0.59$). The proportion was lower in males than in females at low body mass (at 18 g: $t_{90} = 2.43$, $P = 0.017$), but not at mean or higher mass (at 24 g: $t_{14.9} = 0.63$, $P = 0.54$). For all other variables the slopes were homogeneous, and no significant difference between sexes was observed. No interactions between the effects of selection, sex or trial number were significant.

The selection did not affect significantly the proportion of individuals that reached the edge zone ($F_{3, 185} = 1.50$, $P = 0.22$) or the proportion of those that returned at least once to the central zone ($F_{3, 13} = 1.55$, $P = 0.25$), but several other aspects of the behavior were affected (Figure 1, Supplementary Tables S3–5). The nonlinear mixed model analysis showed that in the first trial day (but not in the second) voles from the A lines reached the edge quicker and at a less dispersed time (i.e., had a lower “shape” and higher “scale” parameters of the Weibull curve) than those from all other lines, for which the parameters were similar (overall likelihood ratio test for the effect of selection with $df = 3$, trial 1, shape: $\chi^2 = 12.0$, $P = 0.007$; scale: $\chi^2 = 10.2$, $P = 0.017$; trial 2, shape: $\chi^2 = 4.5$,

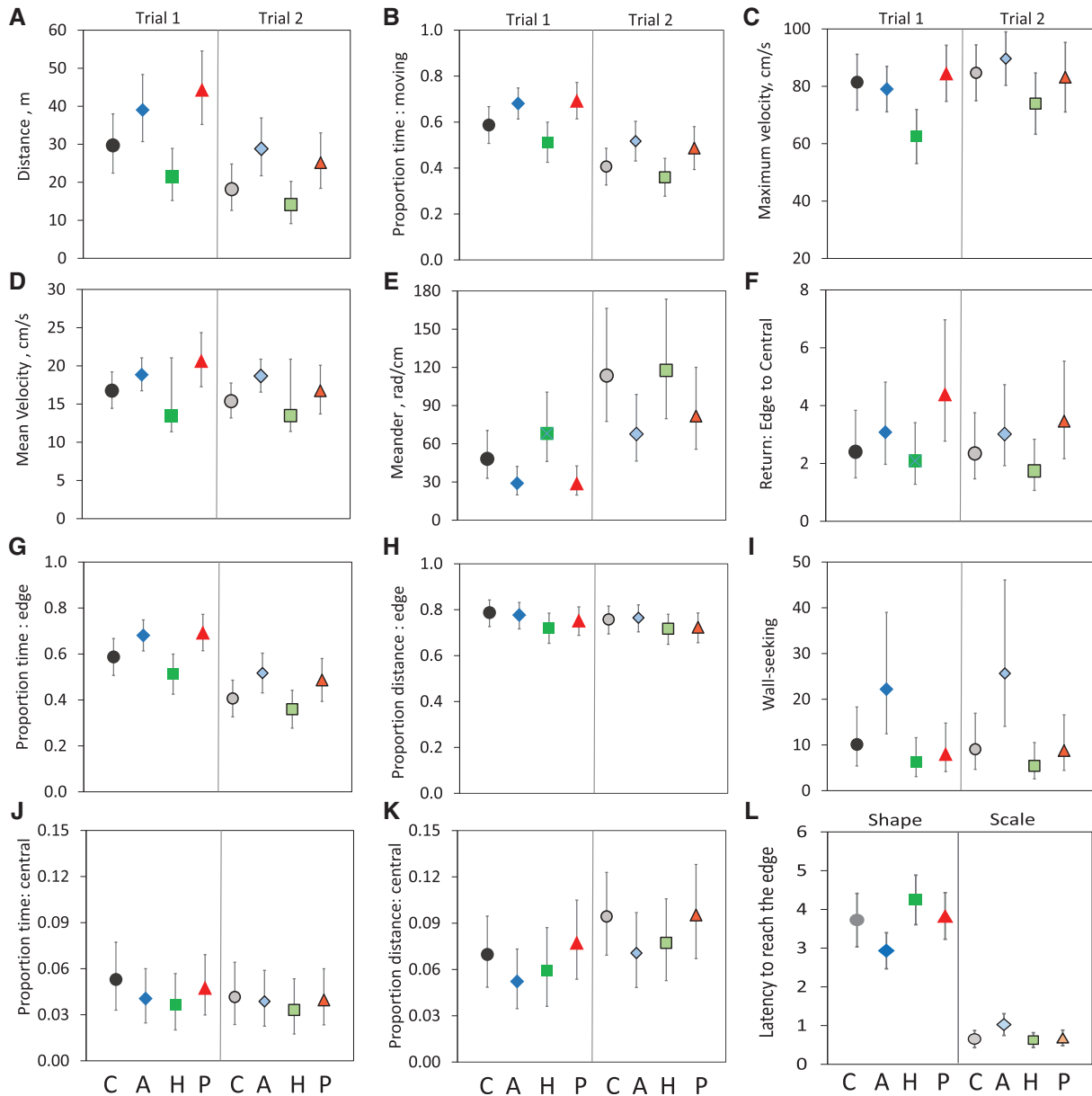


Figure 1. (A–K) Adjusted least-square means from Mixed ANCOVA models ($\pm 95\%$ confidence intervals) of behavioral traits measured in in Open Field test in bank voles from the selection experiment (C: unselected control, A: “aerobic”, H: “herbivorous”, P: “predatory”). Variables that were transformed for the analysis has been back transformed to produce results in original unit. The traits are: (A) Total distance (m), (B) Proportion of time spent moving, (C) Maximum velocity (cm/s), (D) Average velocity (cm/s), (E) Meandering coefficient (rad/cm), (F) Frequency of returns from the edge to central zone (only for individuals that reached the edge zone), (G) Proportion of time spent in the edge zone, (H) Proportion of distance covered in the edge zone, (I) Frequency of wall-seeking behavior (thigmotaxis), (J) Proportion of time spent in the central zone (only following returns), (K) Proportion of distance covered in the central zone (only following returns). (L) coefficients of the Weibull model for the latency to reach the edge zone at the onset of the open field test (“shape” describes the mean latency; “scale” describes variance of the latency).

$P = 0.21$; scale: $\chi^2 = 2.3$, $P = 0.51$; [Supplementary Table S6, Figure S2](#)). For all other traits the effect of selection did not differ between trials 1 and 2, that is, no significant interaction was detected.

Both the duration of mobility and the total distance were highest in the A and P lines, intermediate in unselected C lines, and lowest in the H lines (mobility: $F_{3,17} = 4.78$, $P = 0.014$; distance: $F_{3,13} = 6.37$, $P = 0.007$), although in the pairwise Tukey–Kramer *post hoc* tests the differences were significant only between the extreme A or P and H lines (mobility, A-H: $t_{16} = 3.33$, $P = 0.019$; P-H: $t_{22} = 2.81$, $P = 0.054$; duration A-H: $t_{13} = 3.73$, $P = 0.011$;

P-H: $t_{16} = 3.46$, $P = 0.019$; other comparisons: $P > 0.16$). Voles from the A lines showed also more frequently the wall-seeking behavior than those from H lines, and the values were intermediate in C and P lines ($F_{3,13} = 5.33$, $P = 0.013$; A-H: $t_{12} = 3.79$, $P = 0.011$; other comparisons: $P > 0.06$). The maximum running speed was nearly equal in the A, P and C lines, but markedly lower in H lines ($F_{3,15} = 3.37$, $P = 0.048$; A-H: $t_{14} = 2.98$, $P = 0.042$; other comparisons: $P > 0.10$). Conversely, the meandering coefficient was the highest in H lines, intermediate in C lines, and the lowest in A and P lines ($F_{3,13} = 4.27$, $P = 0.026$; H-A: $t_{13} = 3.01$, $P = 0.043$; other

comparisons: $P > 0.11$). The frequency of returns from the edge to the central zone tended to be highest in P and lowest in H lines, but the effect of selection on this trait was not significant ($F_{3, 13} = 2.22$, $P = 0.13$). The proportions of time spent or distance covered in the edge or the central zones were not affected by the selection ($P > 0.33$).

Discussion

Behavior of the voles in the open field differed markedly between 2 trials performed on 2 consecutive days. In the second trial the voles were generally less active (covered smaller distances), but spend more time in the arena center and moved on more meandering trajectories (Figure 1). Nevertheless, differences among individuals in the 2 trials were largely preserved, as documented by moderately high repeatability of the quantitative traits (coefficients of intraclass correlation of about 30 to 60%), and a lower, but still substantial repeatability of the binary traits (10–20%; Table S5). Thus, the voles do show variation in the personality traits, at least in the context of open field exploration.

The open-field activity and exploratory behavior traits in voles were generally not affected by body mass, and did not differ between sexes. This is contrary to the differences between sexes in exploratory behavior reported in several species of vertebrates (Shchuetz et al. 2010), including mammals. Males and females have different hormonal mechanisms underlying behavioral reactivity (Dammhahn 2012; Luine et al. 2017). Males are often more prone to dispersal whereas females tend to be more resident, and often have larger home ranges, as is also the case in bank voles (Koskela et al. 1997; Sipari et al. 2016). Therefore, males are typically expected to show boldness and more exploratory activity. Despite of differences in the home range, however, bank vole males and females face similar environmental challenges whereas foraging or avoiding predators, which may explain why they react in a similar way to the open field challenge (Haupt et al. 2010; Sipari et al. 2016). The lack of significant difference between sexes in our study is also consistent with the results of open field test in laboratory mice (Jónás et al. 2010; Careau et al. 2012) and personality tests for boldness and exploration in house mice (Auclair et al. 2013). Also in great tits, males and females lack difference in exploration-related traits, even though they differed in other behavioral traits such as learning (Titulaer et al. 2012) and social interaction (van den Meer and van Oers 2015).

The selection we applied affected significantly several aspects of the bank vole behavior in the open field, especially the overall activity measured as the total distance, duration of mobility, mean and maximum running speed, and the straightness of the movement (Figure 1). Zone-specific activities such as time spent, distance or inter-zone crossing, did not vary significantly among animals from the selection lines, which could indicate a lack of genetic covariance between these traits and the traits directly selected for. However, the lack of significant correlated response in the proportions of time spent or distance covered in particular zones could be due to the fact that the proportions were either generally high (in the case of edge zone) or low (in the case of returning to the central zone), and hence the power of detecting significant effects was inevitably low. Thus, further observations, performed in a more complex environment, can still reveal effects of the selection on the behaviors related to specific zones (e.g., open *vs.* covered space).

Voies from the “predatory” and “aerobic” lines moved faster and covered larger distance on relatively straighter trajectories,

compared with those from unselected control lines and the lines selected for “herbivorous” capability (although the difference was statistically significant for the comparisons with respect to the “herbivorous” lines). The trend of high locomotor activity in “predatory” and “aerobic” lines indicates a presence of genetic correlation between locomotor activity and both the predatory tendencies and the aerobic capacity. This complies with the findings from the experiment with laboratory mice selected for high voluntary locomotor activity (wheel running), which showed increased predatory aggression (Gammie et al. 2003) and aerobic exercise capacity (Rezende et al. 2006). Similarly as our voles selected for the activity-related traits, the high-runner mice moved on more straight trajectories in the open field test (made smaller number of turns), although they did not cover larger distance during the test (Bronikowski et al. 2001). Fast exploration in a straighter path indicates a proactive tendency where the animals assume an open field as a risk-free space or accept the risk taking. This observation indicates evolution of a bolder personality in the “aerobic” and “predatory” lines of voles, in agreement with an intuitive expectation. The conclusion is strengthened in the case of “predatory” voles by the observation of an increased tendency (although not statistically significant) of returning to the central zone of the open field, which is usually interpreted as a sign of boldness (Rangassamy et al. 2015; Yuen et al. 2017). We are not aware of other studies on correlated responses to selection for predatory aggression in rodents, but house mice and rats selected for high social aggression also show “proactive” coping style (Koolhaas et al. 1999). Note, however, that although some experiments showed a correlation between the social and predatory aggression (e.g., Sandnabba 1996), the traits represent very different aspects of behavior (e.g., Weinschenker and Siegel 2002), and therefore the similarity in correlated responses to selection for the predatory and social aggression should be treated with caution. Interestingly, animals from the “aerobic” lines moved faster than any others from the starting point to the edge of the open field, and showed an increased frequency of wall-seeking behavior. Such a behavior (thigmotaxis) is usually interpreted as anxiety response, and is stereotypical in mice and rats (Prut and Belzung 2003). Therefore, the high frequency of thigmotaxis in “aerobic” voles could indicate an increased escape seeking and fearfulness. However, the more athletic animals from the “aerobic” lines are predisposed to engage in voluntary exercise (Jaromin et al. 2016; Rudolf et al. 2017), which can be manifested as increased thigmotaxis. Thus, interpretation of the observation remains unclear.

Contrary to the “aerobic” and “predatory” voles, those from the “herbivorous” lines moved less and followed a more meandering path than voles from unselected control lines. Turning and stopping could be a sign of hesitation, which implies that the animals from the “herbivorous” lines are reactive and turn around more often to check for impending danger. However, the increased meandering could indicate also a more thorough exploration, rather than increased fearfulness. Thus, also in this case the results could be interpreted in alternative ways, and resolving the doubts will require performing more specific behavioral tests (Dall and Griffith 2014; Yuen et al. 2017). Remarkably, despite evident importance of evolution of the herbivorous life strategy for the history and diversity of vertebrates, our experiment appears to be only one in which selection for such a trait was performed. However, results of comparative studies demonstrate ecological relevance of the effects we observed. Compared with carnivorous mammals, the herbivorous ones tend to have lower daily movement and foraging distance (Garland 1983). Herbivorous animals are subjected to foraging decision based on the

trade-off between getting quality food-site and predatory risk (Lima and Dill 1990; Banks et al. 1999; McArthur et al. 2014). Consequently, herbivorous animals are typically characterized by a lower locomotor activity, cautious exploration of their foraging sites, freezing response to an imminent danger, and reserving their energy for sudden fleeing when freezing fails to avoid the danger—and at least some aspects of this pattern have been demonstrated in our selection experiment. Interestingly, voles from the “herbivorous” lines have an altered composition of gut bacteria (Kohl et al. 2016), which opens a fascinating possibility for further research on microbiome-behavior nexus (e.g., Heijtz et al. 2011).

Irrespective of the doubts in interpretation of particular observations, our results revealed clearly a presence of genetic correlations between the selected performance traits (subject to direct selection) and personality traits expressed in the open field exploration (in which correlated response to the selection was observed). The evidence for the genetic correlation allows also for inferences concerning the expected effects of selection in a reciprocal direction. Therefore, our results suggest that the selection for traits associated with proactive personality, such as high exploratory activity in a novel environment, would lead to increased aerobic metabolism capacity and predatory propensity. Thus, more generally, the results support the idea of correlated evolution of animal personality traits and physiological adaptations. The results obtained under highly artificial environment do not provide a firm ground for speculations concerning specific consequences of the correlations for the voles functioning under natural conditions. However, the selection experiment is continued, and the selected lines provide not only a unique model for further studies on the neurophysiological and molecular mechanisms underlying the variation in behavior and its correlation with physiological performance traits (e.g., Jaromin et al. 2016; Konczal et al. 2015, 2016), but offers also a possibility of performing field experiments, which can reveal whether and how the direct and correlated responses to the selection affect competitive performance and fitness components of the voles under natural conditions.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at <https://academic.oup.com/cz>.

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