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**Underlying causes and stability of
intraspecific variation in behaviour of
microtine rodents**

Ph.D. Thesis

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Annotation

Two crucial issues related to personality in non-human animals have been identified: firstly, the underlying ‘causes’ of personality and, secondly, the stability of behavioural repertoires (essentially the defining feature of personality) have both been the subject of long-standing debate. This thesis is focused on investigating stable inter- and intra- individual differences in the behaviour of wild-caught bank voles, *Myodes glareolus* (that varied according to mtDNA type), and in common voles, *Microtus arvalis*. The first study, using wild voles, revealed empirical evidence about the effects of sex and mtDNA type on individual differences in basal metabolic rate and in behaviour in the open field which provides measures of approach and avoidance (here, generically termed ‘proactivity’) and which relate to several theoretical conceptualisations of animal personality. The second study demonstrated the presence of stable individual differences in reactions to exposure to open field test and radial maze in common voles, which were shaped by the social environment and in turn, related to cognitive efficiency. The same species was used in the third study documenting the distinct temporal patterns of behavioural plasticity that manifested over repeated exposures to the open field test. We suggest that this distinct temporal patterning in habituation, whilst it varied over time, was predictable in nature and therefore a reflection of a stable underlying personality. To conclude, this body of thesis work draws together a number of influencing factors, and considers their contribution to animal personality.

Declaration [in Czech]

Prohlašuji, že svoji disertační práci jsem vypracoval samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury. Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své disertační práce, a to v nezkrácené podobě elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dále s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. zveřejněny posudky školitele a oponentů práce i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou Národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

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List of papers and author's contributions

The thesis is based on the following papers:

- I. ŠÍCHOVÁ, Klára, Esa KOSKELA, Tapio MAPPES, Petra LANTOVÁ, and Zbyszek BORATYŃSKI. On personality, energy metabolism and mtDNA introgression in bank voles. *Animal Behaviour*. June 2014, vol. 92, pp. 229 - 237. ISSN 0003 - 3472. DOI: 10.1016/j.anbehav.2014.04.011. IF = 3.405 [Klára Šíchová analysed the data and wrote the manuscript together with ZB; the study was designed by PL and ZB]

- II. LANTOVÁ, Petra, Klára ŠÍCHOVÁ, František SEDLÁČEK, and Vojtěch LANTA. Determining behavioural syndromes in voles – the effects of social environment. *Ethology*. February 2011, vol. 117, no. 2, pp. 124 - 132. ISSN: 0179 - 1613. DOI: 10.1111/j.1439 - 0310.2010.01860.x. IF = 1.947 [Klára Šíchová collected the data and participate in the writing of the manuscript; the study was designed by PL]

- III. ŠÍCHOVÁ, Klára, Gabriela URBÁNKOVÁ, Rachel HORSLEY, Nella MLADĚNKOVÁ, Jan RIEGERT, Petra STARCK-LANTOVÁ, and František SEDLÁČEK. Distinct temporal patterns of behavioural plasticity across repeated trials of the Open Field may reflect 'personality' in the common vole, *Microtus arvalis*. (manuscript) [Klára Šíchová designed the study, collected the data together with GU and NM, analysed the data together with JR and wrote the manuscript]

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CHAPTER I.

General Introduction

1. Overview

Individual differences in the behaviour of non-human animals (commonly labelled as (animal) personality, temperament or behavioural syndromes) have been intensively studied. Owing to the rapid development of the area, considerable terminological and conceptual heterogeneity is still present in the literature. **Introduction** therefore discusses the meaning of the most frequently used terms labelling individual differences in behaviour, that is, 'personality'; further, several proximate causes of its existence, and commonly accepted models of its structure are presented and discussed. Finally, the aims of the thesis are summarised in the last part.

The first empirical part of the thesis covers two studies focused on the proximate factors that contribute to the presence of stable individual differences, since their investigation is crucial for understanding of the presence and maintenance of personality. In the first study (**Chapter II**) we responded to recent calls to give empirical attention to the suggested link between personality and energy. Therefore, in wild-caught bank voles, *Myodes glareolus*, that varied according to their mtDNA type (original or introgressed from *Myodes rutilus*), personality traits and their intra-individual consistency were assessed using an open field test and basal metabolic rate (BMR, 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 also showed a negative trend in females, suggesting the alternative compensation model.

In the **Chapter III**, inter-individual differences in reaction to a novel environment were tested in common voles, *Microtus arvalis*, revealing the first documentation of their existence in this species. Moreover, the effect of several social factors (number and sex of siblings and identity of the family group) was investigated. The animals were repeatedly tested in two behavioural experiments – novel environment (NE) test and radial-arm maze (RAM) test, from which seven behavioural traits were extracted. Individual-specific responses to NE corresponded with the performance in the maze, which revealed behavioural syndromes and possible trade-offs. Anxiety was determined by the size of the family group, whereas escape-related behaviours and maze-exploring tactic differ between particular family groups. Our study revealed the importance of the social factors in the development of personality and the possible association between personality and cognitive efficiency in non-human animals.

Temporal stability of personality expression represents a hot topic in current behavioural studies. It has been suggested that the behavioural reaction norms approach (BRN) can explain and integrate both inter- and intra-individual behavioural stability, therefore it was this approach that was adopted. Our study, covered in **Chapter IV**, provides some of the first empirical evidence about the validity of the relationship between inter- and intra- individual stability and has important implications that may be crucial for a wide spectrum of behavioural studies. In this study, voles repeatedly exposed to the open field test displayed stable intra-individual differences in exploratory activity, reflecting distinct temporal patterns of behavioural plasticity. Individuals that can be (according

to generally accepted construct of animal personality), characterised as highly explorative/proactive in initial test sessions displayed an elevated habituation rate (expressed by rapid waning of the exploratory activity across repeated exposures to the open field) in comparison to neophobic animals with low proactivity in the initial session. Moreover, the shape of temporal activity patterns (individual habituation rate) was affected by the number of siblings. Our study provides an important step in characterizing individual variation in behaviour, and highlights the importance (at least in the species of vole studied) of taking both inter- and intra- individual differences into the account when the open field is used for repeat testing.

Finally, all findings are summarised in **Chapter V**.

2. Introduction

2.1. *What is animal personality?*

Behaviour is the most plastic phenotypic trait that enables organisms to respond rapidly to changes in on-going extrinsic (and intrinsic) conditions; it is therefore highly adaptive from an evolutionary perspective (Hazlett, 1995). The ability to generate the most appropriate reaction to actual conditions (i.e., display unlimited behavioural plasticity) is expected to be highly beneficial, nevertheless, there is extensive evidence demonstrating that intraspecific variation in behaviour shows rather a stable character in a wide array of animal species including both vertebrates (Gosling, 2001) and invertebrates (Mather & Logue, 2013). Moreover, a heritable base of inter-individual differences has been documented (e.g. Drent et al., 2003; Herborn et al., 2010; Stamps & Groothuis, 2010) that interacts with environmental factors (e.g., Carere et al., 2005; Trillmich & Hudson, 2011) which leads to the assumption that intraspecific variation is a final product of natural selection rather than just a raw material for its acting (Réale et al., 2007).

Several theoretical explanations of intra- individual differences in animal behaviour have been postulated; they are based mainly on behavioural ecology concepts and evolutionary theory is a unifying feature (Nettle & Penke, 2010). The first part of them works with the ultimate causes of intraspecific variation including (i) social niche specialisation theory (Bergmuller & Taborsky, 2010); (ii) theories based on the game theory that assume that individual differences can be maintained via negative frequency-dependent selection (acting on e.g. alternate mating or fighting strategies, Wolf & Weissing, 2010) and (iii) sexual selection theories (Schuett et al., 2011).

The second group of the most generally accepted theories can be labelled as “mechanistic” (i.e., they assume the role of proximate factors); they are based on the idea that behavioural plasticity is restricted by morphological and/or physiological limits inherent to the particular organism, represented by constraints related to its ability to capture and process information (Dall et al., 2004) or reflecting variability in metabolic rate, which generates the ‘pace-of-life syndrome’ (Réale et al., 2010; see **Chapter II** for empirical evidence). Further, some theories also expect the role of non-genetic factors (e.g., the hormonal and/or social environment acting across different phases of ontogeny; Wolf, 2000); see the part 2.3. *Why personalities vary between individual members of a species* for more details and **Chapter III and IV** for empirical studies.

The aforementioned theories have arisen as a reaction to the initial boom of studies describing the presence of individual behavioural differences across a variety of non-human animal species (timed around the 90’s of the last century); currently, the main attempts are addressed to obtain empirical support for hypothetical constructs leading to creation of robust theory (a really challenging goal when the richness of the taxa is taken into the account). Rapid growth of the research field is still restricted by terminological uncertainties, since the basic constructs have been generated as a result of the interaction between two distinct fields - human psychology and behavioural ecology (Nettle & Penke, 2010).

Whilst there has been considerable variation in the specific terminology and definitions used (resulting from differences in conventional focus in different research areas, as well as inconsistency regarding particular levels of variation and/or historical usage; McKay & Haskell, 2015), stable individual differences in animal behaviour have been most commonly labelled as personality/animal personality (e.g. Groothuis & Carere, 2005), temperament (e.g. Réale et al., 2000) or as a behavioural syndrome

(Sih et al., 2004). These terms are frequently used interchangeably, despite the fact that they denote somewhat differing phenomena. Their meanings will now be clarified and discussed.

“Personality” refers to a set of individual behavioural responses to a range of stimuli or situations that constitute a behavioural style; the response sets and overall style can be used to derive measurable personality dimensions, or traits that reflect the structure of behavioural variability in a given population (e.g., Gosling, 2001; Uher, 2008). The main assumption of this is that personality-related behavioural expressions must be consistent over time (e.g., showing test-retest reliability) and across related tests (e.g., concurrent validity; Réale, 2007). It is important to note that the conceptualisation of animal personality is distinct from conceptualisations of human personality from both a theoretical and a methodological point of view. Ethologists use the term ‘animal personality’ in similar way to the way psychologists use the term ‘personality dimension’ (in relation to humans), in other words, a specific trait rather than the constellation of traits that are typically viewed as human personality (Uher, 2011). This originated owing to the fact that animal studies are commonly focused on a single behavioural trait - e.g., measuring of variation in “boldness” (e.g., Wilson et al., 1993) which provides reference information that one individual is more or less ‘bold’ than the rest of the individuals (distributed along a continuum of ‘boldness’ in given population), but does not describe the comprehensive, holistic character ‘personality type’ that is a typical goal of human psychology (Hofstee, 1994).

‘Temperament’ is most commonly defined as a basic, underlying trait (Budaev, 1997; Box, 1999) reflecting early predominant dispositions that together with experiences, *leads to, but does not constitute* the “fully evolved” personality (Rothbart, 2007). Nevertheless, this idea also frequently appears in fields that are not directly related to early life and development. Behavioural ecology

defines “temperament” as individual reactions to challenging situations (Réale et al., 2007) or a concept reflecting variation in behaviours associated with affect (Dugatkin, 2009). ‘Temperament’, then, usually reflects individual behavioural responses in a single context assessed on a measurement scale (e.g. “handling temperament” quantified by the latency of escaping from some restraint). Overall, it is possible to extract the definitional differences between “temperament” and “personality” as follows (i) “temperament” commonly measures one-context level of behavioural variation; and (ii) “temperament” may refer to both variation between individuals and populations, since any unit of the interest can be observed and interpreted and (iii) “personality” refers more to the complex structure of behavioural variation that cannot be quantified (McKay & Haskell, 2015).

Before the definition of “behavioural syndrome”, it is necessary to specify some associated concepts, specifically, what is meant by ‘situation’ and ‘context’, when discussed in relation to consistent behavioural individuality. According to one of the most influential papers (Sih et al., 2004), situation reflects a set of environmental conditions at a given point in time. Different ‘situations’ could include different levels presented in given environmental gradient (e.g., different levels of predator risk) or various suites of conditions across time (e.g., breeding vs. non breeding season). By contrast, ‘context’ refers to a functional behavioural category (e.g., mating, parental care, dispersal etc.). A behavioural syndrome is then defined as a suite of correlated behaviours reflecting consistency in behaviour between individuals across multiple situations and contexts (e.g., see Sih et al., 2004). Therefore ‘behavioural syndrome’ is the property of a population or a species; an individual can exhibit a behavioural type within the syndrome (e.g., bold vs. shy behavioural types).

Based on the preceding literature and theory, this thesis operationalised stable individual differences as ‘personality’ for two main reasons. Firstly, to avoid using the term ‘temperament’, since its meaning is strongly connected with the prediction of trait heritability and early expressions, nevertheless, animal behaviour in adulthood can be considerably shaped also by unpredictable experiences across the life span (Gosling, 2001). Secondly, the term ‘personality’ is preferable to ‘behavioural type’ in order to eliminate these current terminological discrepancies and to highlight the potential links with human studies, although of course it is too soon to argue that the meaning of ‘personality’ is interchangeable across human and non-human psychology, since in the majority of animal research, just a part of this complex structure has been studied. It is not necessary to use a distinct term in animal studies until the robust concept of this field is established.

Since this thesis is theoretically based mainly on the concepts originating from behavioural ecology, the term ‘personality’ will be frequently used in its ‘broad sense’ (fitting the concept of behavioural syndromes, Sih et al., 2004) that, contrary to the ‘narrow sense’, allows the study of any ecologically relevant behaviour across one or more ecologically relevant contexts (see Réale et al., 2010). At this point it is highly relevant to clarify *what personality levels are actually measured in animals?*

2.2. *What personality levels are measured in animals?*

Firstly, it is necessary to illustrate the differences between a ‘state’ and a ‘trait’ - the terms used in human psychology. A ‘state’ can be describe as short-term emotional reaction induced by intrinsic and/or external triggers, that, in turn produce physical, behavioural,

cognitive and psychological reactions (Spielberger & Sydeman, 1994). Contrary to ‘states’ generating temporally fluctuating emotional mode, a ‘trait’ reflects the stable, consistent and enduring disposition of an individual (Allport & Odbert, 1936). Nevertheless, the borders of these terms can be tight especially when some ‘states’ displays a chronic occurrence - it can be than labelled as a ‘trait’ - see further text (Forgays et al., 1997).

The theoretical concept of animal personality is rooted in the Trait Theory (also called as “Dispositional”) of human personality (initially suggested by Gordon Allport). According to this approach, personality can be described by particular ‘personality traits’ reflecting an individual’s abiding characteristics shared by all or some of the conspecifics and that can, but not necessarily, vary among these individuals (Réale et al., 2007). In other words, inter-individual differences displayed in a given group (population) that are consistent across time and different contexts (Funder, 2001). At this point, it is important to briefly clarify the exact meaning of the terms ‘consistent’ and ‘stable’ in the context of the trait theory. Individual differences are labelled as ‘consistent’ indicating that behaviour of an individual is predictable across time and/or different contexts even if its level or degree changes (Budaev & Zworykin, 2002) - an individual that is bolder than the conspecifics when confronted with predator is assumed to maintaining its ranking in ‘boldness’ (compared to its con-specifics) position across other contexts (e.g., confrontation with novel environment) even while the absolute value of its boldness may change); by contrast, ‘stability’ refers to a behaviour/characteristic that tended to be stable (i.e., underlying personality characteristics), however, these term are frequently used interchangeably.

Trait personality theory is the basic tool of animal studies; in its use, approaches can be divided to *complex* and *fragmental*. The former one is based on the use of theoretically predefined personality

models widely accepted in human psychology; the most relevant ones will be discussed. The Big Five model (Costa & McCrae, 1992; Digman, 1990; Winter & Barenbaum, 1999) allows the reduction of most of personality traits to five broad dimensions (Extraversion, Agreeableness, Conscientiousness, Neuroticism, and Openness to Experience) regardless of language or culture. Therefore it is highly beneficial for its usability in animal researches. It has been applied to describe intraspecific variation in some non-human animals (e.g., hyenas, domestic cats, orang-utans, and African grey parrots; Gosling, 2001) revealing the likely universal applicability of at least three of its dimensions: Extraversion (reflects the type of individual reaction to novel stimuli); Agreeableness (characterizes tendencies toward aggression and dominance) and Neuroticism (encompasses individual differences in coping with challenging situations).

Similar to the Big Five model, one of its forerunners was postulated by Hans Eysenck (used e.g. in Wistar rats, Garau & Garcia-Sevilla, 1984) including dimensions Psychoticism, Extraversion, and Neuroticism (PEN) and is fully biologically-based. It suggests the involvement of e.g., baseline activation in the ascending reticular activating system (ARAS) in explaining variation in introversion-extraversion in terms of over- and under-reactivity of this system (respectively), which in turn leads to avoidance or the seeking out of stimulation, to maintain optimum levels of stimulation in this system. It has been postulated as criticisms of the Big Five model assumed to inappropriately operate with the personality hierarchical structure. PEN model integrates Agreeableness and Conscientiousness to the new superfactor labelled as Psychoticism (in PEN explained also by impulsivity) and excluded Openness to Experience. A biological base of particular dimensions can be postulated: *psychotism* is related with the elevated testosterone levels; *extraversion* is based on the general arousal in the ARAS that can be measured by e.g., skin conductance, brain waves, or sweating;

neuroticism activation thresholds in the sympathetic nervous system or visceral brain; a part of the brain responsible for the fight-or-flight response in the face of risky situations measurable by heart rate, blood pressure and muscular tension. PEN model was modified by Jeffrey Gray (1970) by rotating of the dimensions Extraversion and Neuroticism leading to additional ones - impulsivity and anxiety; high impulsivity leads to high sensitivity to reward (the behavioural activation system, BAS), while high anxiety is associated with sensitivity to punishment. The model is supported by a body of animal studies and it is used in comparative studies concerning addiction. The relevance of each of these conceptual approaches reinforces the appropriateness of the types of animal testing paradigms implemented here that have direct relevance to the aforementioned characteristics.

Fragmental studies are methodically rooted in traditional ethological approaches; typically fewer personality dimensions are under focus and they are not typically predefined (frequently the bottom - top approach using principal component analysis is used); choosing of the traits is commonly directed by their ecological and/or evolutionary aspects. The most relevant models varying according the species studied and are represented by: shy - bold continuum (fish, Wilson et. al., 1993, 1994), slow - fast explorers (birds; Drent et al., 2003) reactive - proactive coping styles (rodents, Koolhass et al., 1999) principally reflecting the Extraversion - Introversion - a central dimension in human psychology. The concept is based on the assumption that reactions of individuals confronted with the challenging situation (e.g., the presence of a predator or novelty) can be distributed along a continuum from extreme shyness (reactive coping/slow exploration) to extreme boldness (proactive/fast exploration). The position on this continuum is tightly related with fitness since it has a heritable basis and affects success in mating, feeding, and competition, adjusting to environmental changes

and responding to predators (e.g. Dugatkin, 2004). For further details see the part 2.6. *Measuring personality in animals*.

An example of the theory absolutely consistent with the evolutionary - ecological approach is the model suggested by Réale et al. (2007) which assumed that differences in shyness - boldness axis are induced by risky situations (e.g., presence of predator), and suggests that the reaction to novelty is labelled as exploration – avoidance. Three more axes of animal intraspecific variability are also included into the model: general activity (measured in non-risky and non-novel environment); aggressiveness (an individual's agonistic reaction toward conspecifics); and sociability (an individual's reaction to the presence or absence of conspecifics, except aggressive behaviour). The model was suggested for the field of behavioural ecology and evolutionary biology and based on the assumption that each trait should be defined according to the ecological situation in which it was measured. It is important to note, that the model does not presuppose the correlation of whole the traits as it is expected in personality (Costa & McCrae, 1992) and behavioural syndromes (Sih et al., 2004). The validity of this model remains to be confirmed by empirical studies, since it is in its pioneer state.

2.3. Why does animal personality vary between individual members of a species?

The presence of a variety of personality types in animal populations has been a target of several theoretical models (e.g., Dall et al., 2004; Sih et al., 2012; Wolf & Weissing, 2010). Intraspecific behavioural differences are assumed to be a result of interaction between inherited genetic backgrounds and environmental

factors (Broadhurst, 1965; Sluyter et al. 1996; Bouchard & Loehlin 2001; van Oers et al., 2004). Particular personality traits may be heritable to different extents (Drent et al., 2003), however, it can be generalised that heritability is higher in environments with highly predictable conditions (e.g., Dingemanse et al., 2002). An important role in shaping individual behavioural patterns is currently attributed also to the social environment (e.g. Sih & Bell, 2008); however, this field is still under studied (Bergmuller & Taborsky, 2010). The effect of social environment will be discussed in detail, since it is an important part of this thesis, in which the effect of siblings' number and their sex on intraspecific behavioural variability in microtine rodents is tested. Empirical investigation of social environment role is crucial for a wide array of behavioural studies and it can bring more light into the evolutionary processes of personality maintenance.

Since personality is considerably shaped mainly during the early ontogenetic phases (e.g. Rödel et al., 2008a), members of the family group represent the most important part of the social environment “setting” for the behaviour of the organism to develop the most successful way (Carere et al., 2005; Champagne, 2006). One of the most important mechanisms contributing to aforementioned process can be represented by different intensities of the parental investment (maternal and/or paternal care), especially in altricial young (Rödel et al., 2008b). Since parental investment (i.e. the female and/or male expenditure - e.g. time, energy - that benefits the offspring) represents one of the most energetically costly parts of reproduction (Thompson, 1992), considerable intraspecific variation in its levels may be expected (Budaev et al., 1999) and has been investigated in many vertebrates (e.g., Zworykin et al., 1998; Drent & Marchetti, 1999, Calatayud, 2001). It has been documented that the intensity of parental investment increases boldness, neophilia and resistance to stress expressed in adulthood

(e.g. Maestripieri, 1993; Bard, 1996; Wöhr, 2008; Rödel et al., 2010). Nevertheless, it is important to note that the intensity of maternal care may be considerably affected by the characteristics of the young, especially their number and sex (Deviterne et al., 1990; Dimitsantos et al., 2007). Therefore, these are further important components of the social environment that are involved in shaping adult behavioural patterns.

Litter size is thought to be negatively related to both neonatal weight and the growth rate across the life span (e.g. Mendl, 1988; Rödel et al. 2008a; Hudson et al., 2009). Similar trends can be present also at the intra-litter level, where the size can be positively related with the amount of the maternal milk obtained during competitive interactions between siblings, leading in turn to a higher growth rate (e.g., Drummond et al., 2000; Bautista et al., 2005; Rödel et al., 2008a). Variability in neonatal weights can be explained by different positions in the uterus that can lead to irregular blood supply of particular embryos (e.g., Argente et al., 2003; Foxcroft et al., 2006). This is strongly demonstrated in the species with numerous litters including microtine rodents (Foxcroft et al., 2006). To date, an exact mechanism explaining the position of an embryo in the uterus has not been investigated (Fowden et al., 2009).

Inter-individual differences in neonatal weights and growth rate strongly affect the extent of success in competitive interactions amongst siblings beginning directly after birth (Bolles & Woods, 1964; Tanaka, 1998). Especially in rodents, some of maternal nipples may be preferred because of the variability in their shapes, accessibility, quantity of colostrum production, nutritional quality of the milk and the frequency at which the mother forces the young away (Donald, 1937; Ewer, 1959; Jeppesen, 1982; Fraser & Lin, 1984). Neonatal rodents with the higher weights are at an advantage, since weight is also positively related with maturation

of motoric abilities (Bautista et al., 2010) allowing longer suckling of maternal milk under competition pressure from siblings (Bautista et al., 2005). Interestingly, in altricial species, different levels of motor abilities can also extend the duration of staying at the central part of the sibling huddle. This position is beneficial because of the enhanced thermoregulation (Bautista Garcia-Torres et al., 2008; Bautista et al., 2010) which allows higher efficiency in the processing of nutrients derived from maternal milk leading, again, to increasing of the growth rate (Bautista et al., 2003; Bautista, Garcia-Torres, et al., 2008; Rödel et al., 2008a). On the other hand, the absence of siblings in typically multiparous species limited competitive abilities in the adulthood (Nicolas et al., 2011) and also may decrease the probability of adulthood survival (Bautista et al., 2003; Rödel, et al., 2008a; Rödel, 2009).

From the point of view of intraspecific behavioural variability, the effect of aforementioned factors on adult behavioural expression has not been satisfactorily studied. A positive correlation between litter size and emotional stability has been documented in rats (Dimitantos et al. 2007). A similar pattern has been investigated in great tits reacting to a novel environment, since birds from numerous clutches displayed elevated levels of neophilia and boldness (Carere et al., 2005). Nevertheless, the link between behavioural characteristics and number of siblings is still not clear since literature also points to an opposite trend in their relationship (La Barba & White, 1971) or its absence (Grotta & Ader, 1969). Nevertheless, this discrepancy can be explained by heterogeneity of used rodent strains and behavioural tests, or by unnatural manipulation with which the litter size was reduced to required number (Dimitantos et al., 2007).

Prenatal position may affect the behaviour of an individual via both different supplies of nutrients, hormones and immune agents, as well as varying efficiency in the removal of toxic metabolic products

(Coan et al., 2008). Interestingly, embryos can be irregularly affected by the stress hormones (glucocorticoids) produced by the mother during the pregnancy (Kapoor et al., 2006; Götz & Stefanski, 2007; Dardnaudéry & Maccari, 2008). Based on these findings, it can be hypothesised that irregular hormonal supply in multiple litters can be beneficial in challenging conditions, where an elevated variability of personality types are required (Sih & Bell, 2008).

As already mentioned, intraspecific variation in behaviour of multiparous species may be affected prenatally as a result of heterogeneous conditions presented into the uterus. When both males' and females' embryos are presented in the uterus their development can be considerably affected by the irregular hormonal gradient. The sensitive period of the brain development is accompanied with the intensive realising of testosterone from the males' fetuses (Harris & Levine, 1965). Testosterone, however, readily diffuses through the encephalic barrier and can thus also influence development of the adjacent fetuses leading to morphological, physiological and behavioural changes. This phenomenon was labelled as "intrauterine position effect" (hereafter IUP, Ryan & Vandenberg, 2002).

IUP can influence adult behaviour of both females' and males' fetuses. Females developing between two males (2 M female) are exposed to the highest levels of testosterone (vom Saal & Bronson, 1980). In comparison with 0 M females (exposed to zero male' fetuses during prenatal development), 2 M females are generally more active (also in dispersal contexts, Ryan & Vandenberg, 2002), less anxious (Hauser & Gandelman, 1977) and more aggressive (vom Saal, 1989). IUP also affects mating success (since 2 M females are less preferred by the males than 0 M ones; vom Saal, 1989) likewise, reproductive success, since 2 M females give birth to a fewer litters; moreover, their sex ratio is commonly biased in the behalf of the males leading to increased

probability of 2 M females that can be assumed as an interesting mode of non-genetic heredity (Clark et al., 1993; Vandenberg & Huggett, 1994).

From an evolutionary point of view, IUP can be crucial in populations with frequent size fluctuations. Limited food resources and high social stress lead to high levels of the stress hormones in mothers which can cause selective desorption of male foetuses (Krackow, 1992), since males need a higher parental investment which is hardly feasible in unfavourable conditions. IUP has been investigated in both wild and laboratory rodents (Krackow, 1992; Hemmer 1992) and it can be also expected in microtine rodents (Boyce & Ellis, 2005).

2.4. How does personality vary within a particular individual?

Individuals may differ not only in their average behaviours, but also in the extent of intra-individual behavioural consistency (e.g., Boyce & Ellis, 2005; Nussey et al., 2007) indicating the presence of intraspecific variability in behavioural plasticity (Coppens, 2010; Dingemanse et al., 2010, Réale & Dingemanse, 2010). For example, highly aggressive individuals generally display more limited behavioural plasticity when compared with their less aggressive conspecifics (e.g. ural owls, Kontiainen, 2009; laboratory mice, Koolhass et al., 1999); a pattern documented also in humans (Ellis et al., 2006). Taking together, individual level of behavioural plasticity (also called responsiveness) suggested to have an intrinsic base, since it is frequently expressed as a syndrome (i.e., also functionally different behaviours of an individual displayed the similar flexibility extent when compared with the values of conspecifics; Sih & Bell, 2008). Some theoretical explanations of the

presence of individual extents of behavioural plasticity have been suggested (Wolf et al., 2010; Snell-Rood, 2013; Briffa et al., 2015), nevertheless, necessary empirical evidence is still scarce. Dingemans et al. (2010) suggested using the behavioural reaction norm (BRN) approach in study of both ‘activational’ and ‘developmental’ behavioural plasticity (Snell-Rood, 2013). This refers to differential activation of an underlying network (or producing a different behavioural phenotype) across some contextual gradient (e.g., temporal, social or environmental). According to this concept, both intraspecific variability in ‘personality’ type (i.e., *elevation* of the reaction norm) and variety extent of behavioural plasticity (i.e., slope of the reaction norm) can be simultaneously presented in a given population; their relationship, if there is any, can vary across different populations, Dingemans et al. (2010) suggested five possible scenarios (see Fig. 1).

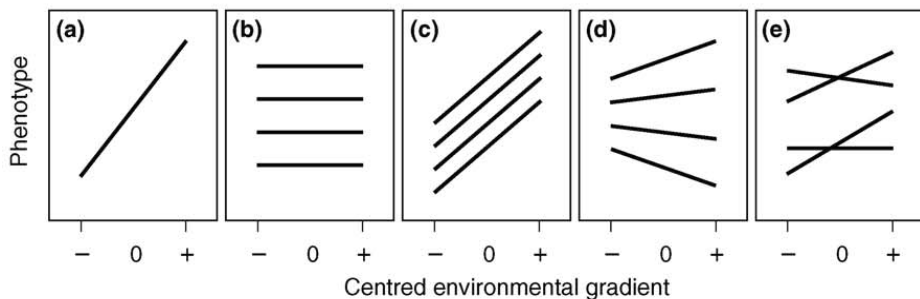


Fig. 1. Five possible scenarios of the relationship between the ‘personality’ type (i.e., the elevation of the reaction norm) and individual behavioural plasticity displayed by a population across a contextual gradient (e.g., temporal, social and/or environmental): (a) there is no variability in ‘personality’ types and individuals display the same behavioural plasticity; (b) different ‘personality’ types are present and behavioural plasticity is absent; (c) different

‘personality’ types are present displaying the same extent of behavioural plasticity; (d) different ‘personality’ types are present and the extent of behavioural plasticity is dependent on them; (e) different ‘personality’ types are present and the extent of behavioural plasticity is not dependent on them (according to Dingemanse et al., 2010).

According to Fig. 1, empirical studies of this are highly required, since validity of the last scenario (e) may change considerably the view of intraspecific behavioural variability. Taken together, investigation of the individual expressions on more heterogeneous scale (e.g., different contexts and/or longitudinal scales) may bring a new insight into the both levels of intraspecific variability. This topic is studied using BRN approach in this thesis (see **Chapter IV**) in common voles, *Microtus arvalis*; the development of their reactions to repeated exposure to the same situation (multiple testing in open field) was investigated. The study was conducted across the entire life span with the aim to empirically contribute to answering of the following question: *Is animal personality stable?*

2.5. *Is animal personality stable?*

One of the basic assumptions of the personality concept is the stability of its expression across the biologically relevant period of the lifetime (Caspi et al., 2005; Réale et al., 2007). Traditionally, it is believed that the major changes in individual behaviour are timed to the sexual maturation, since during that time many important changes in the personality expressions will occur (Müller & Schrader, 2005). Most animal studies are based on this assumption, although without sufficient empirical support; it is surprising since personality development is present also after maturation, and this has been

repeatedly documented in both macaques and humans (Capitanio, 1999; Caspi et al., 2005). Similar trends may be expected also in animals, since the establishment of the individual behaviour is tightly related with the maturation of the prefrontal cortex (Koolhass, 1999) which may not be complete by sexual maturity (Gage, 2002). However, from an evolutionary point of view, it can also be hypothesised that if the “endpoint” of personality development really exist (Stamps & Groothuis, 2010; Groothuis & Trillmich, 2011), since intraspecific differences in behaviour are assumed to be closely linked to intra-individual differences in metabolic rate that may change during the lifetime as a function of the challenging environment (Biro & Stamps, 2008).

Taken together, studying the development of animal personality will be necessarily related to both short-term investigations (to reveal information about the consistency of inter-individual differences in its expression) and long-term observations to determine its changes over a life span (Stamps & Groothuis, 2009), that is intra-individual differences. Long term studies of animal personality are scarce which results mainly from the way animal personality is commonly measured.

2.6. *Measuring personality in animals*

Two basic methods are commonly used in the quantification of personality characteristics in animals - *rating of traits* by a knowledgeable observer and *coding of behaviours* that are relevant for the study topic (Gosling, 2001). *Rating of traits* is based on the assessment by a knowledgeable individual of predefined suites of adjectives reflecting theoretically expected levels of intraspecific

variability (personality dimensions; Weiss et al., 2006). *Trait rating* allowing a more complex description of the personality, nevertheless, its usability is considerably limited when natural populations are studied (that is highly relevant for the studies of ecological and evolutionary aspects of personality). *Coding of behaviours* is based on the gathering the data during the test situation imitating nature, one that is relevant for a given personality trait (e.g., exposure to novelty) or direct coding of the behaviours during the observation of the animals in natural conditions (Freeman et al., 2011). Axes of the variation can be extracted *post hoc* using principal component analysis of variance; this method was used in two papers included into this thesis (see **Chapter II** and **III**). This method is highly relevant for studies of natural populations, since the relationship between particular variation axes (assumed to reflect ‘personality’ traits) can vary in populations as a function of the environmental gradient (e.g., various levels of the predator pressure; Vazire, 2007). *Coding of behaviours* was used also in the final paper (**Chapter IV**), where the lengths of trajectories moved during repeated exposure to open field test were measured assumed to reflect underlying exploration - avoidance trait).

Coding of behaviours is most commonly based on the exposure of an individual to the behavioural test reflecting some biologically relevant natural or contrived situation. A situation predicted to make manifest basic personality dimensions is represented by the confrontation with novel stimuli (e.g., environment, object); individual reactions to the situation display both consistent (e.g. Wilson et al., 1993) and heritable base (Drent et al. 2003) confirmed also in natural, ecologically valid conditions (Dingemans et al., 2010). Most commonly, animals are confronted with a novel environment represented by open field test; procedure originally designed for assessment of the exploratory behaviour and emotional stability in rats (Hall, 1934). The test is initiated by a placing of an

individual to a lighted large area; the intensity of the lighting is positively related to the level of induced anxiety level (Archer, 1973); escaping is prevented by the walls. Both lighting and exposure to open space can negatively affect the tendency to explore the area (a reaction rooted in the rodent psychology, Archer, 1973); level of the anxiety is then reflected by the thigmotaxis, elevated defecation or freezing (Hall, 1934; Archer, 1973). Behavioural display during the open field test is assumed to reflect boldness (Toms et al., 2010), exploration (Conrad et al., 2011; Garamszegi et al., 2012) and spontaneous activity (i.e., displayed under situation not related to novelty and/or risk; Carter et al., 2013). Validity of the former trait has been a subject of debate, since it is not clear if the behaviour in open field indeed mirrors general activity. There is a body of studies giving contrary evidence (Burns, 2008; Finger et al., 2016); nevertheless, the prediction of the presence variability in exploration and boldness is robust. The main limitation of the open field is its usability in longitudinal studies, since the character of the patterns displayed across repeated exposure are not fully clear, and after the first exposure the environment is no longer novel.

According to the current state of knowledge, confrontation with novelty reveals a robust prediction of intraspecific variation in behaviour documented in a wide array of the rodent species (e.g. Simon et al., 2004; Hall et al., 1934; van der Staay et al., 1990). Investigation of the presence of the inter- and intra-individual differences and evaluation of the stability and consistency of these differences is the crucial first step to more complex personality studies in a given species (Réale et al., 2007). Microtine rodents represent an ideal mammalian model for studying animal personality from both ecologically and evolutionary perspectives, since it is a species that shows high reproduction rate, short life span and is exposed to regular population fluctuations.

2.7. *Aims of the thesis*

- demonstration of intraspecific behavioural variation in the common vole (*Microtus arvalis*) and the bank vole (*Myodes glareolus*)
- evaluation of the variation consistent basis indicating its trait character
- testing the relationship of the behavioural traits (assumed to reflecting personality traits) with factors assumed to be linked to them: metabolic rate, factors of social environment
- assessment of relationship between individual consistency and plasticity

4. References

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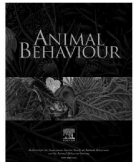
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CHAPTER II.

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

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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.

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Individual variability in behaviour is omnipresent and currently intensively studied (Careau, Bininda-Emonds, Thomas, Réale, & Humphries, 2009; Carvalho et al., 2013; Lantová, Šichová, 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, Dingemans, 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, & Murakami, 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; Szafránska, 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 mitochondrial 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 mitochondrial 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, Síchová, & 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, Grahnb, 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 mm²) were taken from the ear of all animals for genetic investigations under anaesthesia (with isoflurane, IsoFlo vet, Orion Pharma, Turku Finland, administered 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 (Petrušewicz, 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

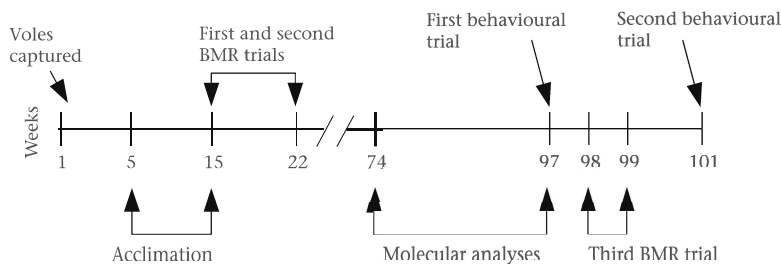


Figure 1. Timeline of the study. Week 1 began 2 August 2008.

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 (τ) 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 Boratynski 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 α of 0.00064; correction per character: critical α 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	$r(95\%CI)$	P_{ANOVA}	N	$r(95\%CI)$	P_{ANOVA}
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, mitochondrial 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, Racigalupo, & 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.

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 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).

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

Data sets and model components	Model parameters				
	df	Log likelihood	AIC _c	Delta	Weight
All data					
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.

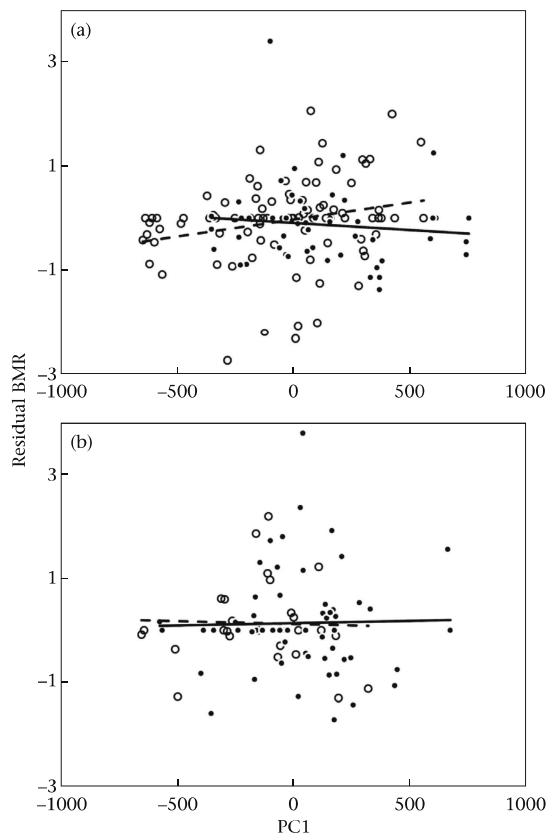


Figure 2. The relationship between proactive behaviour (PC1) and body mass-corrected basal metabolic rate (BMR) conducted separately for (a) females (open dots and dashed line) and males (solid dots and solid line) that had the original mitochondrial type and (b) females (open dots and dashed line) and 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				
	df	Log likelihood	AIC _c	Delta	Weight
All data*					
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.

CHAPTER III.

*Determining behavioural syndromes in voles –
the effects of social environment*

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Determining Behavioural Syndromes in Voles – The Effects of Social Environment

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Abstract

Animal personality and behavioural syndromes have experienced rapid increase in interest in the last few years because of their possible role in the evolution of life histories. However, there is still a scarcity of studies concerning the mechanisms maintaining variation in behaviour as well as addressing their relationships to each other. In this paper, we tested the main assumptions of personality, focusing on the individual variability and repeatability of behaviour, and the identification of behavioural syndromes using the common vole (*Microtus arvalis*) as the species being studied. We also studied the effects of family group characters (group size, sex ratio and affinity to family) on the behaviour in this rodent. The animals were repeatedly tested in two behavioural experiments – novel environment (NE) test and radial-arm maze (RAM) test, from which seven personality traits were extracted. The study revealed that inter-individual variance in vole behaviour was consistent and repeatable. Individual-specific responses to NE corresponded with the performance in the maze, which revealed behavioural syndromes and possible trade-offs. Anxiety was determined by the size of the family group, whereas escape-related behaviours and maze-exploring tactic were more dependent on the affinity to the family. It seems that the development of personality traits we identified here is determined by the effects and structure of the family groups. Further studies are needed to confirm whether the observed variance in vole personalities is maintained by the fitness costs and benefits of the opposite tactics (here fast-random vs. slow-systematic exploration) in more natural circumstances.

Introduction

Consistent behavioural differences in how animals respond to novel or challenging situations have been shown throughout the animal kingdom during the last decade (e.g. Gosling 2001; Bell et al. 2009). These individual tactics are defined as the consistency of an individual's behavioural responses over time and/or across situations (Budaev 1997; Sih et al. 2004a,b; Réale et al. 2007), and they have been labelled by several terms such as temperament,

coping style or behavioural type. In this paper, we are using two of them – 'personality' and the analogous 'behavioural syndrome'. Personality refers to consistent inter-individual variability, and the behavioural syndrome refers more to suites of correlated behaviours resulting from differences in reactions. Both concepts have currently established an accepted position in ecological and behavioural research, because it has been shown that they are often associated with, for instance, survival (Réale & Festa-Bianchet 2003; Dingemanse et al. 2004),

reproductive success, reproductive strategies (Armitage 1986; Réale et al. 2000; Both et al. 2005; Hollander et al. 2008), parental care (Budaev et al. 1999), natal dispersal (Dingemanse et al. 2003) and exploratory behaviour (Verbeek et al. 1994).

One of the goals of current behavioural research is to demonstrate and clarify the existence of consistent behavioural responses, because they can have ecologically and evolutionary important implications. Sih et al. (2004a,b) and Bell (2007) brought up two such considerations – (1) personality can represent limited plasticity if individuals have a ‘tendency’ to behave a certain way generally and do not modify behaviour optimally according to the immediate circumstances; and (2) correlations among behavioural traits can act as evolutionary constraints because genetic correlations between traits can cause a correlated response to the selection on nontarget traits (Lande & Arnold 1983). When we ask for the explanation of the existence of individual differences, we can focus on their origin and maintenance in the population. DeWitt et al. (1998), Dall et al. 2004 and Wolf et al. (2008) provide the ultimate explanations of evolutionary stable strategies (frequency dependent selection favours a rarer phenotype) or positive feedback mechanisms (it pays off to respond consistently as it brings lower costs or higher benefits). Further mechanism may be represented by life history trade-offs, which frequently have been found to result from behavioural syndromes (e.g. Bell 2005; Duckworth 2006; Wolf et al. 2007; Smith & Blumstein 2008). It has been shown, for instance, that more active individuals tend to be bold, aggressive and form routines, but on the other hand they are often worse parents, superficial explorers and non-adaptable to changing environment (for reviews see Sih et al. 2004a,b or Dingemanse & Réale 2005). These trade-offs result in fitness variance of different personality types under different circumstances (e.g. Réale & Festa-Bianchet 2003; Dingemanse & de Gode 2004; Dingemanse et al. 2004) and consequently maintain the variability in populations.

The origin of individual differences may be studied from the developmental (ontogenetic) perspective. Personality develops during ontogeny, and although it is supposed to be more or less stable during adulthood, it may be shaped during early (prenatal as well as postnatal) ontogenetic period. Social environment plays a crucial role in the development of mammals. The effects of (reciprocal) interactions between parents and offspring, or among peers, on phenotype have already been studied (e.g. Mousseau & Fox 1998; Wolf & Brodie 1998; Kölliker 2005;

Emack et al. 2008). Wolf et al. (1998) extended this perspective to interactions among other relatives and even to unrelated individuals. They suggested that the phenotypic resemblance among individuals sharing a (social) environment may increase not only because of their genetic similarity but also because the traits expressed in one individual are often influenced by traits that are expressed in conspecifics (see Wolf et al. 1998 for indirect genetic effects).

In this paper, we present the results of experiments focusing on personality and behavioural syndromes and on the possible mechanisms leading to their existence. Our study species, the common vole (*Microtus arvalis*), is a fossorial, multiparous species with polygynous mating system and maternal care where females can share the nest with sisters and/or daughters and form colonies (de Jonge 1983; Boyce & Boyce 1988). Moreover, as male antagonism decreases during winter and animals overwinter in mixed groups, this creates a system where complicated networks of mutual interactions would be expected to take place. To reveal variance and consistency in vole behavioural responses, we tested the animals repeatedly in two experiments: the novel environment (NE) and the radial-arm maze (RAM) tests. We determined the repeatability of the common vole behaviour and predicted that behavioural traits would co-vary positively across individuals, if consistent individual variation persists across different behavioural traits according to the concept of personality and behavioural syndromes (Sih et al. 2004a,b; Garamszegi et al. 2009). Further, we asked whether social (familial) environment played a role in the development of inter-individual differences.

Methods

Animals

We tested 61 adult voles (31 males and 30 females in both experiments) bred in the laboratory, which were fifth generation offspring of field-caught animals. The voles were reared in 12 groups of related individuals, here called family groups (mean size \pm SD, 7.8 ± 3.9 individuals; mean sex ratio \pm SD, 0.51 ± 0.24), and kept in polycarbonate cages ($70 \times 47.5 \times 20.5$ cm) containing wood shavings and plastic tubes as shelters. Water and commercial food pellets were available *ad libitum*, and their diet was enriched with fresh grass and old bread. The animals were individually marked by fur-clipping, which was renewed every week, and they were weighed prior to the experiment. Rearing was performed at room

temperature (18–22°C) under the L:D 12:12 photoperiod. The animals were cared for in accordance with the principles and guidelines of the ethical standards, and the project was permitted by the Departmental Commission for Animal Protection of the Ministry of Education, Youth and Sports under the reference number 9703/2006-30/300. After the experiments, the voles stayed in the laboratory and were used for further breeding and maintaining of the colony.

Experiments

Novel environment test

Novel environment test is widely used in personality research of mammals. Above all, it reveals traits like activity and exploration, fearfulness or emotionality. Behaviour in unknown environment is commonly regarded as the fundamental index of general individual behaviour (Walsh & Cummins 1976).

Our NE was represented by a glass enclosed area (85 × 63 × 25 cm) with opaque walls and a floor divided into 145 squares. Light intensity was 80 lx. Each animal was recorded for 5 min on a CCD camcorder placed 1.5 m above the enclosed area; the camcorder was connected to a computer placed in an adjacent room. The records were analysed using The Observer Video-Pro 3.0 (Noldus Information Technology, Wageningen, The Netherlands). We measured latency, frequency and total duration of locomotion, immobility, self-grooming, rearing, gnawing and scratching the vivarium walls; furthermore, we counted the number of jumps, defecation activity (number of faeces) and the latency to voluntarily enter the vivarium from a carry-box (transparent jar). The number of passed squares represented travelled distance. The vivarium was cleaned with water and ethanol after each experiment. Each individual was tested three times with a period of 2 wk between the trials. The tests were performed between 9 AM and 4 PM (light phase).

Radial maze

The apparatus used in the second experiment was an eight-arm radial maze (RAM, Olton & Samuelson 1976), made from Plexiglas and placed on a laboratory table. Compared with simple arena maze, maze represents more complex, and for rodents living in systems of underground tunnels, also more natural environment. The maze had a centre platform of 25 cm in diameter and arms 40 cm long and 10 cm wide. Voles were food deprived for 17 h before being

placed into the maze and all the arms were baited with a leaf of clover (*Trifolium repens*). Such period of fasting is not harmful for voles (Pierce et al. 2005) and enhances the motivation to explore the maze, which is an important condition for this kind of experiments (Miller & Dess 1996).

The experiment began by carefully removing the vole individual, in a jar, from the home cage and placing it inside the starting arm of the maze. This arm remained closed for the next 2 min so that the animal could adjust to the new environment. The starting arm remained in the same position during all the experiments. Each animal was allowed to explore the whole maze freely until it had entered all eight arms. Afterwards, the maze was cleaned with water and ethanol.

We defined two tactics of exploring the maze and labelled them according to Teskey et al. (1998), who observed similar phenomenon. An algorithmic pattern was defined as one where the animal entered adjacent arms in a clockwise or anticlockwise direction. Nonalgorithmic animals were those who ran the maze without adhering to any consistent or definable pattern (Teskey et al. 1998). The patterns were designated as the proportion of the number of adjacent (algorithmic pattern) or nonadjacent (non-algorithmic pattern) arms entered. Parameters used for the statistical analyses were as follows: mean number of visited arms per total time (total number of visited arms divided by time needed to visit all eight arms), algorithmic pattern, nonalgorithmic pattern and number of re-entries to already visited arms. We carried out three testing series (14 d separating the series); the tests were performed during the light phase.

Statistics

We used principal component analyses (PCAs) to reduce the number of behavioural variables measured in the NE and RAM experiments. PCAs were conducted separately for both experiments on the correlation matrix with the aim to illustrate the relationship between behavioural variables. The selection of the number of principal components to retain followed the Kaiser–Guttman criterion (Kaiser 1991). To facilitate interpretation, principal components were subjected to a varimax rotation. This orthogonal rotation maximizes the variance of the PCA scores within the principal components across the variables and thus offers the best opportunity of interpretation (Tabachnick & Fidell 1989). The composite behavioural variables with the value of PCA

score higher than 0.4 were used to describe each principal component. The resulting PCA score per individual in each retained principal component was then used as a composite behavioural variable in further analyses. All analyses were performed in a statistical package R (R development core team 2008). PCAs were calculated using library ‘psych’.

To identify the primary differences among individuals along composite behavioural variables, we used linear mixed effect models (LMMs). The models were fitted to address how the composite behavioural variables (response variables) are related to these fixed effects: sex (categorical), sex ratio within family ($M/(M + F)$); continuous, size of family (continuous) and the trial order (continuous). To account for variation among individuals in composite behavioural variables, and for repeated observations of individuals, we fitted the models with individual identity (ID) and affinity to family (ID nested in family) as the random effects. LMMs were built using the function lmer in R library ‘lme4’. p-Values and Bayesian highest probability density (95% HPD) intervals were computed using Markov chain Monte Carlo simulations in library ‘languageR’. We used a likelihood ratio test with one degree of freedom (Pinheiro & Bates 2000) to test the random effects by comparing a LMM with both random effects and a model with either ID or family in the random component. First, to get an idea how strong the effects are, models without fixed effects were compared. Afterwards, the same procedure was used on models with fixed effects included. Only the results for Log-likelihood Ratio Tests (LRT) based on models with fixed effects are presented because the significance of random effects was not affected by the fixed effects. Inclusion of ID as random enabled us to determine the repeatability as the proportion of the phenotypic variance expressed by the individuals (Lessells & Boag 1987; Falconer & Mackay 1996); i.e. inter-individual variation in intercepts. Repeatability may serve as a rough estimate of the heritability of a trait (Falconer & Mackay 1996; Boake 1989; but see also Hayes & Jenkins 1997 or Dohm 2002) and, which is valuable for the study of personality, gives evidence about the consistency of the behaviour (Boake 1989). Variance explained by both ID and family group was calculated as $(V_f + V_i)/(V_f + V_i + V_e)$.

We extracted the best linear unbiased predictors (BLUPs) from the mixed models, which predict the random effect of each individual vole independent of the other terms in the respective models standardized to a mean of zero (Kruuk 2004; Martin & Reale

2008). The BLUPs have an advantage of being less sensitive to extreme values within the data than separate regression estimates (Pinheiro & Bates 2000). They therefore provide more appropriate estimates of the behavioural profile of an individual than the mean of all the measurements of that individual. We used these BLUPs to determine correlations between the composite behaviour variables extracted in the NE and the RAM experiments. We used Pearson’s product moment correlations in a pair-wise manner among all the variables.

Results

Following the PCA analyses, we retained four principal components from the NE and three from the RAM, which explained 73.9% and 89.5% of the total variance for the NE and RAM, respectively (Tables 1 and 2). Four composite variables retained in the NE explained 25.7, 13.4, 21.7 and 13.1 of the variance. The variables that loaded highly and posi-

Table 1: Principal component analyses (PCA) loadings for behaviours in novel environment (NE) test, and repeatability of principal components (behavioural traits)

	NE1	NE2	NE3	NE4
Jumping	0.426	0.404	-0.103	-0.565
Locomotion	0.516	-0.162	-0.458	0.367
Immobility	-0.085	0.093	0.898	0.020
Self-grooming	-0.912	0.270	-0.121	-0.119
Gnawing and scratching	0.136	-0.903	-0.131	-0.047
Rearing	0.741	0.323	-0.312	-0.148
Travelled distance	0.652	-0.016	-0.540	0.088
Latency to enter new environment	0.032	0.035	0.710	-0.069
Defecation	0.181	0.132	-0.064	0.822
% Total variance	25.7	13.4	21.7	13.1
% Repeatability	32.8	24.5	37.5	17.1

PCA scores larger than 0.4 in absolute value are in bold.

Table 2: Principal component analyses (PCA) loadings for behaviours in radial-arm maze (RAM) test, and repeatability of principal components (behavioural traits)

	RAM1	RAM2	RAM3
Mean number of visited arms	0.003	-0.044	0.999
Algorithmic pattern	0.003	0.703	0.028
Nonalgorithmic pattern	0.001	-0.710	-0.034
Number of re-entries	0.999	-0.001	-0.003
% Total variance	39.4	25.1	25.0
% Repeatability	10.4	24.2	34.0

PCA scores larger than 0.4 in absolute value are in bold.

tively on the first principal component (NE1) were the behaviours of activity and exploration (jumping, locomotion, travelled distance and rearing); self-grooming loaded negatively. This component will be called 'activity' hereafter. NE2 was characterized by jumping (positive loading) and gnawing and scratching the vivarium (negative loading) and mirrors 'escape-related behaviours'. Immobility and latency to enter, which were opposed by locomotion and travelled distance, stood out on the third principal component (NE3) and reflect 'anxiety'. The fourth axis (NE4) gave evidence about defecation (positive loading) and jumping (negative loading; Table 1). For the RAM test, we obtained three principal components – RAM1 explained 39.4%, RAM2 25.1% and RAM3 25.0%. On RAM1, number of re-entries loaded positively. RAM2 will be called 'maze-exploring tactic' in further text and was characterized by algorithmic and nonalgorithmic pattern of exploration. The third principal component, RAM3, separated primarily the mean number of arms visited per total time and was labelled 'maze exploration activity' (Table 2).

The first three components of the NE test and maze-exploring tactic (RAM2) and maze exploration activity (RAM3) showed significant individual repeatability ranging from 24.2% to 37.5%. NE4 and RAM1 were not significantly repeatable and thus did not reflect stable personality trait appropriate for further use (Tables 1 and 2; Réale et al. 2007).

BLUP for maze-exploring tactic related positively to the anxiety expressed in the NE (NE3 vs. RAM2, $t = 2.06$, $p = 0.044$, $r = 0.26$; Fig. 1a). Inactive (i.e. not ambulating, jumping, rearing) and anxious (immobile, unwilling to enter NE) individuals tended to perform the algorithmic pattern of exploration. Activity in both NE and RAM correlated positively, i.e. locomotion, jumping and rearing correlated to the total number of visited arms (NE1 vs. RAM3, $t = 3.36$, $p = 0.001$, $r = 0.40$; Fig. 1b). Furthermore, individuals that were anxious in the NE did not explore the RAM actively (NE3 vs. RAM3, $t = -3.55$, $p = 0.001$, $r = -0.42$; Fig. 1c). Together, these correlations indicate trade-offs between fast-random vs. slow-systematic exploration tactics for common voles in a new environment. For the rest of the combinations of BLUP values, respective to the behavioural components, significant correlations were not found ($-0.14 < r < 0.12$, $p > 0.190$; for all correlations, $n = 61$).

Linear mixed effect models showed that no predictor (sex, trial, size of family group and sex ratio) explained NE activity. The number of trials affected

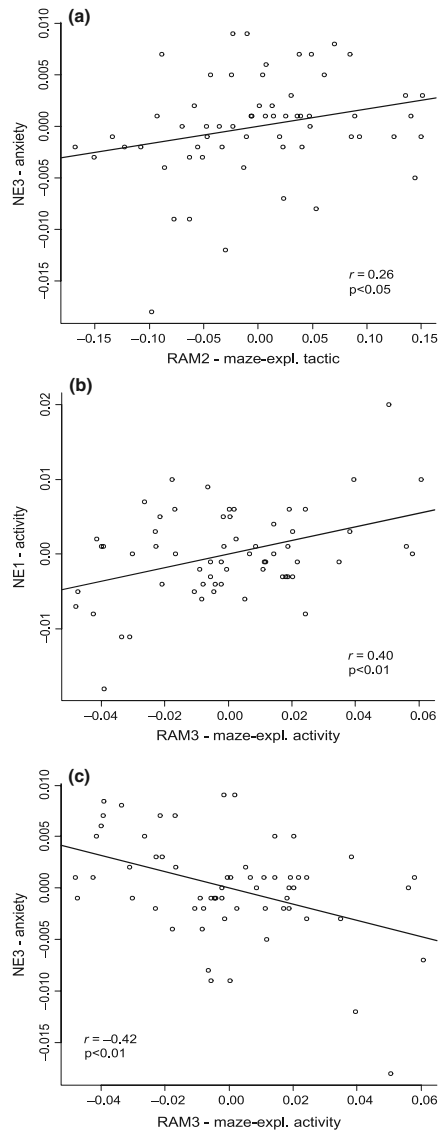


Fig. 1: Correlations between personality traits representing behavioural syndromes. Algorithmic individuals were anxious (a) in the NE. Activity expressed in the RAM correlated positively with the activity in the NE (b) and negatively with anxiety (c). RAM, radial-arm maze; NE, novel environment.

escape-related behaviours, anxiety and maze exploration activity, which reflects animal habituation to repeated testing (Table 3). Individuals from large family groups were less anxious (Table 3). Sex ratio

Table 3: Results of linear mixed effect models of repeatable behavioural components based on novel environment (NE) and radial-arm maze (RAM) tests. Significant values ($p < 0.05$) are in bold. ID nested in family were used as random effects (for random effects see Table 4)

	Estimate	95% HPD interval	p
Activity in NE			
(Intercept)	0.0028	-0.0121, 0.0141	0.6770
Size of family	0.0002	-0.0009, 0.0014	0.7529
Sex ^a	-0.0013	-0.0057, 0.0029	0.6144
Sex ratio	-0.0038	-0.0182, 0.0160	0.6497
Trial	-0.0012	-0.0031, 0.0007	0.1838
Escape-related behaviour			
(Intercept)	-0.0084	-0.0177, 0.0041	0.3457
Size of family	0.0002	-0.0010, 0.0010	0.8635
Sex ^a	-0.0006	-0.0042, 0.0023	0.7525
Sex ratio	0.0070	-0.0089, 0.0201	0.0947
Trial	0.0015	0.0003, 0.0027	0.0199
Anxiety in NE			
(Intercept)	0.0079	0.0004, 0.0175	0.1056
Size of family	-0.0008	-0.0013, -0.0003	0.0481
Sex ^a	-0.0023	-0.0058, 0.0014	0.3364
Sex ratio	0.0048	-0.0064, 0.0125	0.3601
Trial	-0.0015	-0.0033, -0.0004	0.0421
Maze-exploring tactic			
(Intercept)	-0.1027	-0.3580, 0.1489	0.5378
Size of family	0.0138	-0.0100, 0.0372	0.3781
Sex ^a	0.0372	-0.0429, 0.1133	0.4401
Sex ratio	-0.2069	-0.5458, 0.1615	0.3730
Trial	0.0244	-0.0120, 0.0634	0.1522
Maze exploration activity			
(Intercept)	-0.0235	-0.0856, 0.0375	0.4601
Size of family	-0.0008	-0.0051, 0.0043	0.7240
Sex ^a	0.0132	-0.0084, 0.0364	0.3708
Sex ratio	-0.0114	-0.0789, 0.0550	0.7445
Trial	0.0147	0.0046, 0.0262	0.0053

HPD, highest probability density.

^aMale was considered as the reference for sex in the model.

in the family marginally affected escape-related behaviour ($p < 0.1$; Table 3) and was related to the spatial performance in the maze (RAM1; $p = 0.043$). The performance, however, was not repeatable. The effect of affinity to the family group (i.e. similarity within the group) was significant for escape-related behaviours and maze-exploring tactic (Table 4). Variance explained by both ID and affinity to the family group is shown in Table 4.

Discussion

We found consistent inter-individual differences in common voles' behaviour for most of the identified behavioural traits (repeatabilities ranging from 24.2% to 37.5%). This demonstrates that common voles do have personalities and that NE as well as RAM tests are adequate tools capable to discrimi-

Table 4: Likelihood ratio test for random factors (ID; and affinity to family) in linear mixed effect models (DF for both = 1). Significant values ($p < 0.05$) are in bold

	Effect of ID ^a		Affinity to family		Variance explained by ID and family (%)
	LRT	p	LRT	p	
NE1 – activity	14.33	0.0002	1.27	0.2598	51.74
NE2 – escape behaviour	22.52	<0.0001	6.49	0.0109	69.34
NE3 – anxiety	17.45	<0.0001	0.00	0.9900	48.81
NE4	1.35	0.2446	0.00	0.9900	35.00
RAM1	0.00	0.9900	0.00	0.9900	0.50
RAM2 – maze-exploring tactic	10.48	0.0012	13.58	0.0002	60.55
RAM3 – maze exploration activity	12.55	0.0004	0.00	0.9900	47.30

RAM, radial-arm maze; NE, novel environment.

^aVariance explained solely by ID is considered as repeatability in Tables 1 and 2.

nate individual differences. Several behavioural syndromes were identified, because the individual-specific responses to the NE corresponded with the performance in the maze. Active and bold individuals in the NE were also more active in the maze; however, anxious individuals were more systematic in their exploration. One personality trait, anxiety, was negatively related to the size of the family group, and we found significant effect of affinity to family group for escape-related behaviours as well as maze-exploring tactic. Negative relationships between fast-random vs. slow-systematic exploration (Fig. 1) revealed trade-offs between these two tactics. Together, the results give new information about the determinants of behavioural traits in social rodents and demonstrate that the common vole is an appropriate species for further experiments on the fitness costs and benefits of the opposite behavioural tactics in natural circumstances.

Several behavioural traits correlated and presented behavioural syndromes, which reflect inter-individual consistency in behaviour across multiple situations (Sih et al. 2004a; b). We found that activity and anxiety expressed in the NE was a good predictor of spatial behaviour in the maze. The voles, which were active in the NE, visited more arms per total time than the less active individuals. Likewise, the number of visited arms was negatively related to anxiety. The results further showed that anxious animals performed algorithmic strategy of the exploration in the RAM. Gentsch et al. (1987) demonstrated that fearful rodents avoid open spaces (e.g.

the central area of the radial maze), probably because such environment prevents the animals from performing thigmotaxic behaviour. Anxiety and consequent thigmotaxy thus can result in algorithmic strategy of exploration. Similar to Verbeek et al. (1994), who described fast and superficial vs. slow and thorough exploration in great tits, we propose that the speed and the strategy of exploration may create an ecological or evolutionary trade-off. Slower (anxious) but more systematic tactic may be advantageous in unstable environment and/or in an environment with predators, but disadvantageous in stable or predator-free conditions where fast and bold individuals will gain available resources first. Such trade-offs can maintain individual variability in populations and partly clarify the existence of consistent behavioural differences.

How do differences among individuals emerge? The phenotype as an adult arises through complex and dynamic interactions between the environment in which the animal develops and its own genes that regulate its trait development (Pryce & Feldon 2003; West-Eberhard 2003).

We attempted to explain the origin of inter-individual variability by three characteristics of family groups where the individuals developed and lived – by the sex ratio and size of the family group, and relatedness (affinity to the family group). Sex ratios of the family groups significantly affected only spatial performance (RAM1) and marginally escape-related behaviours (NE2). However, as the spatial performance component was not significantly repeatable, this effect should be treated with caution. The size of the family group had significant effect on the level of anxiety so that the voles from larger families were less anxious entering the testing arena sooner and were more explorative. Affinity to the family (relatedness and shared rearing environment), however, played a minor role for anxiety as it explained only 11.3% of the variance and the effect was not significant. Such low value may be surprising because both anxiety and exploration have earlier been shown to have genetic basis and tend to be heritable (e.g. Koolhaas et al. 1999 in a review; Dingemans et al. 2002; Drent et al. 2003). Literature provides ambiguous results about the effect of the size of family. In great tits, in agreement with our findings, individuals from large families were less anxious and had stronger explorative tendencies (Carere et al. 2005); in Sprague–Dawley rats, the size of family similarly affected emotionality (Dimitantos et al. 2007). In contrast, other studies on laboratory rats indicate that in large litters

sib-sib competition and low availability of maternal care and milk in early postnatal phase increase anxiety and decrease exploration in an open field test (Caldji et al. 1998; Macri et al. 2004; Wöhr & Schwarting 2008). We suggest that living in a group where food is not a limiting factor and related individuals (mainly older sisters) can compensate for maternal care could reinforce an individual's confidence in behaviour. This idea, however, needs further testing.

The third proximate factor we studied was the affinity to the family. Out of seven described behavioural traits, we found a significant effect of the family identity for the escape-related behaviour expressed in the NE and the maze-exploring tactic. Significant effect of the family identity arises from either low within-family or high among-family variance. As a result, the effect increases the effectiveness of among-family selection (Wade 1998). Similarity within the family suggests positive covariance between individual's genes and surrounding social environment, which may cause that the expression of the trait is multiplied and per-generation evolutionary change can be greater than for traits with simple Mendelian inheritance (see Wolf et al. 1998 and references therein). On the contrary, negative covariance can constrain the overall response to selection. The expression of escape-related behaviour and maze-exploring tactic were preserved in the families, which confirms what we have observed in our laboratory for a long time – animals in some families are difficult to handle, trying to escape whenever possible, whereas voles from other cages are docile. The among-family variance in both these traits obviously did not diminish in captivity and seems to remain rather stable.

To conclude, we have demonstrated consistent individual differences, personalities, in common voles and identified behavioural syndromes resulting from their responses. Activity, escape-related behaviour, anxiety and maze-exploring tactic showed significant repeatability indicating that these important personality traits may have a genetic background and thus respond to selection. We suggest that the relationships between activity, anxiety and systematicness of exploration create trade-offs, which can present the ultimate mechanisms for maintaining individual variation in the population.

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CHAPTER IV.

*Distinct temporal patterns of behavioural plasticity across repeated trials of the Open Field reflect 'personality' in the common vole, *Microtus arvalis*.*

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(manuscript)

Distinct temporal patterns of behavioural plasticity across repeated trials of the Open Field reflect ‘personality’ in the common vole, *Microtus arvalis*.

Abstract

Consistent individual differences in non-human animal behaviour (‘animal personality’) have been proved in a wide spectrum of taxa. Most frequently, ‘animal personality’ is measured by Open Field test (OFT) where one testing block (two sessions separated by a short time interval) is used. As a consequence, it is not clear if individual values really mirror the long term/multiple testing patterns and if some factors (e.g. social environment) can be related with extent of displayed behavioural plasticity. To address these questions, common voles, *Microtus arvalis*, (a species with well documented personality-linked behavioural variability), were repeatedly subjected to OFT. Two groups were used - individuals single exposed to a testing block (two OFTs separated by 24 h) and voles tested in five blocks conducted every two months over the lifespan. Activity expressed by moved distance was measured and individual characteristics (‘animal personality’ and behavioural plasticity) were extracted using reaction norms approach. At the group level, the effect of previous exposures was found, since repeatedly tested voles displayed significantly lower activity levels than the same aged single exposed ones. Further, the length of inter-sessions interval (24 h vs. two months) did not influence the extent of activity changes and direction of their development (e.g. increase/decrease) did not displayed an uniform pattern (indicating the presence of individual differences in behavioural plasticity). Behavioural plasticity (extracted by fitting of individual activity levels by a linear regression) was affected by neither sex nor affinity to a seasonal

cohort, while number of siblings was positively related to the tendency for session-to-session activity increase. Finally, a relationship between ‘animal personality’ and behavioural plasticity was found when individuals with high average activity levels (fast explorers type) displayed a tendency for session-to-session activity decrease (i.e. exploration reduction caused by habituation), while the opposite pattern was detected in slow explores (i.e. individuals with low average activity). Our study provides additional important step in characterizing individual variation in behaviour, and highlights the importance (at least in the species of vole studied) of taking both of intra- and inter- individual differences into account when OFT is used repeatedly.

Keywords: animal personality, behavioural plasticity, Open Field test, common vole, repeated testing

1. Introduction

Individual differences in behaviour that are repeatable across contexts and time, ‘animal personality’, have been well-documented in a wide array of species including both invertebrates (Mather & Logue, 2013) and vertebrates (Gosling, 2001). This consistent inter-individual variance can involve any type of behaviour condensed into several axes reflecting ‘personality traits’ (broad sense concept, Réale et al., 2010). Commonly, ‘animal personality’ is measured by repeated exposure to Open Field test (hereafter OFT) based on confrontation with open illuminated arena for a predefined time (originally used in rodents, Hall 1974; Walsh & Cummins 1976). Consequently, repeatability of individual patterns can be quantified by several statistical methods including product moment correlation or Spearman rank correlation (Bell et al., 2009), but most commonly intra-class correlation coefficient is used (hereafter ICC;

Hayes & Jenkins, 1997). ICC mirrors the proportion of intra-individual variability divided by its sum with inter-individual variance; the behaviour is highly repeatable (i.e. assumed to reflect underlying ‘personality trait/s’) when individuals behave consistently across the repetitions (i.e. display the low intra-individual variance) and considerable inter-individual differences are presented (i.e. display the high inter-individual variance). Described method has been adopted by predominant number of studies including rodents (e.g. voles: Lantová et al., 2010, 2011; Šíchová et al., 2014; squirrels: Boon et al., 2007; mice: Yuen et al., 2016), birds (e.g. tits: Carere et al., 2005) and fish (e.g. guppies: Budaev 1997; Boulton et al., 2014).

Nevertheless, there are several crucial questions arising from described method those have to be answered by current studies. Most frequently, two OFT sessions separated by a short interval are used (but see e.g. Carere et al., 2005; Kanda et al., 2012). As a consequence, it is not clear if observed tendencies indeed reflect the long-term patterns, since an information about inter-sessions stability of individuals’ expressions (behavioural plasticity) same as its relation to ‘animal personality’ is absent. A very promising approach allows study of both intra- and inter-individual variability, behavioural reaction norms (hereafter BRN), was recently suggested (Dingemanse et al., 2010; Dingemanse & Wolf, 2013). It is based on repeated measurements of individual across particular levels of context (e.g. social, temporal or habitat) followed by comparison of its average behaviour (i.e., ‘animal personality’, quantified statistically as the intercept) with direction and strength of displayed changes (i.e., behavioural plasticity, or statistically, the slope). Several different scenarios of ‘animal personality’ and behavioural plasticity linkage have been suggested (see Dingemanse et al., 2010) and some of them have been also empirically supported

(review in Mathot et al., 2012) - e.g. highly aggressive mice did not adjust their aggressiveness across different social contexts, while the opposite types did (Koolhass et al., 1999; Natarajan et al., 2009). More empirical investigation of consistency-plasticity linkage is needed, since important evolutionary, ecological and methodological consequences are expected (Mathot et al., 2012).

To address this issue, common voles, *Microtus arvalis*, (a species with extensively documented personality-linked variability; Lantová et al., 2011; Herde & Eccard, 2013; Graceva et al., 2014) were repeatedly exposed to OFT. Since the test mirrors an environment free of biologically relevant cues (e.g. food, mates), activity displayed across its repetitions may reflect habituation - the simplest form of learning (Müller et al., 1994). It is likely that, given its heritable basis (Bolivar et al., 2000), habituation patterns may be linked with baseline differences in behavioural reactivity that have endogenous origins such as personality (e.g., shyness-boldness). In line with this, personality related differences in habituation have been documented in humans (e.g. La Rowe et al., 2006; Anderson et al., 2011) and in some non-human animals (review in Mathot et al., 2012).

Habituation can be manifested by inter-session waning of exploration activity (Groves & Thompson, 1970; Thomson & Spencer, 1966). Nevertheless, this prediction is complicated by the fact that OFT behaviour may mirror not only shyness - boldness (Toms et al., 2010) and exploration - avoidance 'personality traits' (Garamszegi et al., 2012), but also general activity may play a role (Carter et al., 2013). As a consequence, inter-session decrease of activity may reflect also aging caused shifts in general activity (Todorovič et al., 2003). To avoid this uncertainty, we compared activity in same aged single and multiple exposed voles; when observed changes are affected by habituation rather than aging,

repeatedly tested voles will display considerable lower levels than single exposed individuals. Further, activity levels may be affected by the length of inter-session interval (e.g. in mice, Paylor et al., 2006). When sessions are separated by a short gap (e.g. 24 h) test-retest activity levels may reflect different underlying tendencies (Whimbey & Denenberg, 1967). On the other hand, activity displayed under a long-term arrangement (e.g. two weeks) may be affected by retention efficiencies (Clemens et al., 2009) and/or shifted states (e.g. hierarchical position, body size; Bell et al., 2009). Ideally, an individual should be tested under both temporal levels to provide complex information about both actual and longitudinal patterns; nevertheless predominantly just a short-term interval is used (but see Carere et al., 2005; Boulton et al., 2014). To bring more light into this field, we compared activity of the same individuals tested under both short- (24 h) and long-term (two months) arrangements.

‘Animal personality’ is shaped by interaction of genetic and environmental factors (e.g. Drent et al., 2005; Stamps & Groothuis, 2010). According to previous studies, social environment and affinity to a seasonal cohort may considerably affect shaping of individual patterns in common voles. In line with another rodent studies (e.g. rats, Dimitsantos et al., 2007), size of rearing groups is negatively related to anxiety level in common voles (measured in OFT; Lantová et al., 2011). Further, winter-born individuals display a more cautious behavioural phenotype (i.e. lower exploration and higher anxiety levels) compared to summer-born ones (Gracceva et al., 2014). To address the question, whether behavioural plasticity can be also affected by social and seasonal factors, the effect of number of siblings and affinity to a seasonal cohort (winter/summer) were tested.

To summarise, common voles were repeatedly exposed to OFT with following aims: at the group level (i) to assess the effect of prior

exposure(s) on actual activity, (ii) estimate the effect of inter-session intervals on extent of test-retest activity changes and (iii) assess the pattern of inter-session activity development (e.g. increase/decrease) and at the individual level (iv) using the behavioural reaction norms approach, extract values reflect ‘animal personality’ and behavioural plasticity and test if they are affected by selected factors (sex, affiliation to seasonal cohort, sex and number of siblings) and (vi) investigate suggested relationship between displayed ‘animal personality’ and behavioural plasticity.

2. Method

2.1. Animals

The sample (N = 132) contained male (n = 51) and female (n = 81) individuals that originated from 34 families that were derived from the first laboratory-bred generation (summer- and winter-born seasonal cohorts) of wild-caught common voles, *Microtus arvalis*. Voles were reared under standard laboratory conditions, with room temperature (18-22 °C) and a 12:12 dark-light arrangement (lights on 06.00 h). After weaning, at 21 days, voles were marked and kept individually in standard polycarbonate cages (31 x 21 x 15 cm) containing wood shavings, hay and plastic tubes as shelters. Water and food were available *ad libitum*, commercial food pellet diet was enriched (three times per week) by fresh grass and vegetables. Voles were weighed weekly (and on the second day of each OFT test block) starting from two days after birth.

All procedures were conducted in accordance with the principles and guidelines of ethical standards. The project was approved by the Departmental Commission for Animal Protection and by the Ethical Commission of the Ministry of Education, Youth and Sports - reference: 7945/2010-30.

2.2. *Design*

Two groups of voles (see Fig. 1) were tested: repeat-exposed (RE group, initial n = 91) and single-exposed (SE group, n = 41). Summer- and winter-born voles were distributed equally across the RE and SE groups, as were males and females. In the RE group (initial n = 91), weanling voles (aged 21 days at first OFT trial) were given a test block of two x 3 min OFT trials (separated by 24 h). Test blocks were then administered every two months until senescence, at nine months, yielding four test blocks of two OFT trials (eight OFT trials in total). In the SE group (n = 41), adult voles aged five months (for direct comparability with the third RE test block) were given a single test block (two x 3 min OFT trials, separated by 24 h). Activity (trajectory length, cm) during each 3 min trial was the primary measure used to calculate the dependent variable(s) used in subsequent multifactorial (e.g., hierarchical analysis of variance, ANOVA) and multivariate (e.g., intra-class correlation) tests of the study hypotheses.

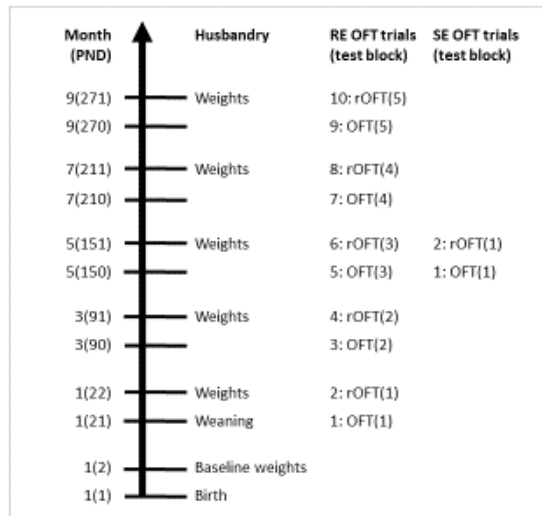


Fig. 1. Husbandry and OFT testing over the full study timeline (birth to nine months). Time is shown by month with post-natal day (PND) shown in parentheses. Each OFT test block consisted of a pair of OFT trials (OFT, rOFT) separated by 24 h (block numbers shown in parentheses). Repeat-exposed (RE) voles received 10 OFT trials in five test blocks; the first test block commenced at weaning (PND 21) and test blocks were repeated every two months until senescence (nine months). Single-exposed (SE) voles received a single OFT test block in adulthood (five months, of PND 150) for comparability with the RE group that also received a test block at five months.

2.3. *Open Field test (OFT) procedure*

All OFT trials were conducted between 9 am and 4 pm under laboratory conditions (as already described). Each OFT trial was initiated by placing a vole in the peripheral zone (approximately 5 cm from the arena wall) of a square non transparent plastic arena (75 x 75 x 50 cm). In each case, the start location was identical. The animal was allowed to explore the arena freely for 3 min, whilst

behaviour was video-recorded from overhead (by a digital camera BASLER acA1300 connected with NUUO recording software 3.3.12, NUUO Inc.). The arena was cleaned between animals with a solution of 30 % ethanol in water. Custom-designed Modular Tracking System version 1.07 (obtained from M. Kučera) was used to extract trajectory length (cm) from the raw videos.

2.4. Statistical analyses

Statistical analyses were performed using Statistica 8 (StatSoft Inc., 2007) and R version 2.15.0 (R Core Team, 2012). Prior to the application of inferential statistical tests, the dependent variables (derived from trajectory data) were evaluated for suitability to the intended test. All statistical outcomes are for two-tailed tests (unless otherwise specified) with alpha set at $p = 0.05$.

2.4.1. Group level patterns:

To ascertain whether the prior OFT trial exposures or simple maturation/aging determined behaviour, the activities (i.e. length of trajectories) of RE and SE voles displayed in OFT and rOFT conducted at the age of five months were compared using hierarchical (nested) ANOVA. Although the RE and SE groups had unequal group sizes ($n = 68$, and $n = 41$, respectively), modified mean squares were not calculated (i.e., corrected using the Scatterthwaite approximation), since such corrections do not necessarily ensure accurate p values. On balance, we chose to use uncorrected values in the knowledge that the resulting p values will be conservative, and more prone to type two error; McDonald, 2014). The hierarchical ANOVA was specified with EXPOSURE (single- *versus* repeat-exposed) as a between-subjects factor, within which individuals'

identities were nested as a random factor subgroup, with both raw OFT and rOFT (non-independent) observations entered per individual.

The effect of inter-trial interval was also analysed using hierarchical ANOVA. Here, activity change between OFTs (delta) with a two month (cross-block) inter-trial interval (RE group only, calculated as: $\text{OFT}(x) - \text{OFT}(x + 2)$, x = the OFT trial number (1, 3, 5, 7, 9, inclusive, see Fig. 1) was compared to activity change between OFTs with a 24 h interval (RE only, calculated as: $\text{OFT}(x) - \text{repetition OFT}(x)$, where x = the OFT trial number, and repetition OFT was the trials' within block counterpart).

To ascertain whether there was significant change in activity in RE voles over their nine month lifespan, activities (trajectory length) across OFT test blocks were compared using one-way ANOVA with OFT blocks as a repeated measures factor (PND 21, 90, 150, 210 and 270).

2.4.2. Individual level patterns:

Repeatability of inter-individual differences (i.e., consistency across OFT trials) was estimated using intra-class correlation coefficient (ICC, τ); Hayes & Jenkins 1997). ICC was calculated as follows - $V_{\text{ind}}/V_{\text{ind}} + V_{\text{residual}}$, when V_{ind} reflects variability within-individuals, V_{residual} reflects variability between-individuals. The aforementioned statistical values were extracted by running a one-way ANOVA (in Statistica 8) with individual identity as independent factor and activity (trajectory lengths) as the dependent variable (between- and within-individuals' MS errors were obtained). ICC was calculated separately for both inter-trial intervals (24 h/two months).

To extract values reflecting ‘animal personality’ and behavioural plasticity, activities (trajectory lengths) of each RE vole were transformed by decadic logarithm and fitted by a linear regression: ‘Animal personality’ was expressed by the intercept (i.e. average level of activity); strength and direction of behavioural change (behavioural plasticity) was mirrored by a regression coefficient beta (i.e., $\beta = 0$ refers to no changes, $\beta > 0$ decrease and $\beta < 0$ increase of activity across repeated trials separated by two months - i.e. 1,3,5,7 and 9). The relationship between ‘animal personality’ and behavioural plasticity was investigated using Spearman’s Rank Correlation.

The effect of factors sex, seasonal cohort and number of siblings on ‘animal personality’ (intercept) and behavioural plasticity (coefficient beta) was determined using a generalised linear mixed model (GLMM); prior to this analysis, inter-correlations between factors was evaluated by Spearman’s Rank Correlation followed by a bivariate regression in the case of a significant result. Since behaviour can vary considerably between families (Lantová et al., 2011), affinity to a family was added as random factor to avoid pseudo-replications. The best model with the lowest AIC value was chosen.

3. Results

3.1. Group level patterns

Effect of previous exposures was found - single exposed voles displayed significantly higher activity levels than repeatedly tested ones ($F(2, 199) = 30.579$, $p < 0.001$; Fig. 2). Overall, no *Effect of inter-session interval* was detected, since activity changes observed within particular inter-session intervals (24 h/two months) did not differ ($F(6,479) = 1.707$, $p = 0.117$) - therefore, only data for two months interval were used in following analyses (except

Repeatability), since they mirror a more relevant part of the lifespan. *Activity development* did not display a uniform pattern (i.e. overall decrease/increase), since activity levels of repeatedly exposed voles did not differ between particular sessions ($F(4, 240) = 2.364$, $p = 0.054$); only a non-significant trend for overall activity decrease was presented (Fig. 3).

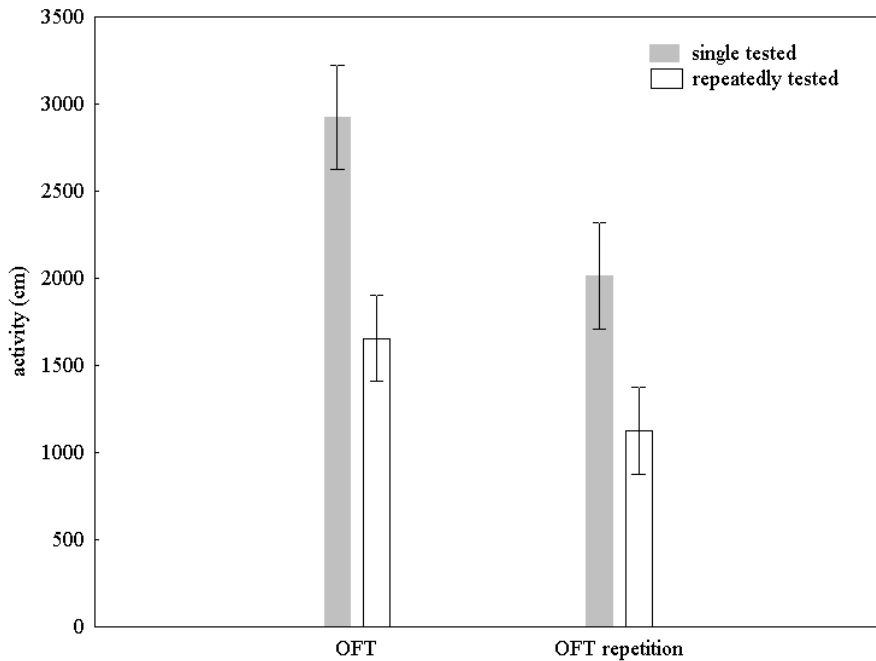


Fig. 2. Comparison of activity levels of single ($n = 41$) and repeatedly ($n = 61$) tested voles within a block (OFT - OFT repetition separated by 24 h). Single exposed ones displayed significantly higher levels compared to repeatedly tested ones (previously exposed to two blocks - i.e. four OFTs). Nested ANOVA, $F(2, 199) = 30.579$, $p < 0.001$; mean and s.e.m. are displayed.

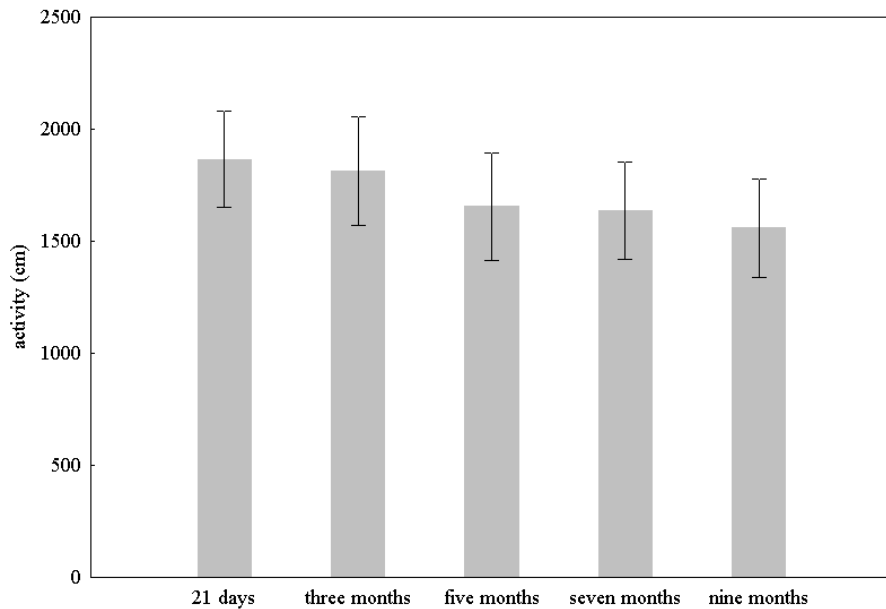


Fig. 3. Comparison of activity levels of repeatedly tested voles ($n = 61$) displayed across the lifespan. Direction of the changes did not displayed a uniform pattern - e.g. overall decrease/increase ($F(4, 240) = 2.364, p = 0.054$), only non-significant trend for inter-session decrease was found; mean and s.e.m. are displayed.

3.2. Individual level patterns

Repeatability was demonstrated, since intra-individual variability in activity levels was lower than inter-individual differences at both 24 h ($F(60, 244) = 3.826, p < 0.001, ICC = 30.9\%$) and two months ($F(60, 244) = 5.157, p < 0.001; ICC = 35.7\%$) inter-session arrangements. Correlation pre-analysis of independent factors revealed a presence of relationship between neonatal weight and the litter size ($p = 0.042$); neonatal weight did not correlate with weights in further ontogenetic phases that were dependent on each other (Spearman coefficients = 0.48 - 0.90). *Effect of selected factors* was partly detected - differences in behavioural plasticity may be predicted by number of siblings ($df = 5, \chi^2 = 4.76, p = 0.029$), since

voles from numerous litters displayed tendency for session-to-session activity increase (beta = - 0.84). Remaining factors did not have any significant effect on neither 'animal personality' nor behavioural plasticity. *Relationship between 'animal personality' and behavioural plasticity* was detected, since individuals displaying high level of average activity (i.e., high intercept values) had significant tendencies for its decreasing across particular sessions (i.e., lower beta values), beta = 0.728, p = 0.046.

4. Discussion

Our results show that repeated exposure to OFT across the life-span lead to session-by-session changes in activity that most probably reflect habituation. Moreover, distinct patterns of change were observed that were dependent on average activity level displayed across the sessions, e.g., low activity tended to be followed by increasing activity over sessions, and *vice versa*. Simple aging did not account for the changes observed (or their direction). The values of detected changes did not differ according the length of inter-session interval (two months vs. 24 h); therefore the development of behavioural patterns session-to-session seems to depend on simple number of exposures, rather than e.g., maturational effects over time. Since the character of activity changes did not display a uniform pattern across individuals this suggests intra-individual variation in habituation rates reflecting behavioural plasticity. The strength and direction of session-to-session activity changes were significantly affected by the number of siblings at birth and by weight in adulthood, either or both of which might, in principle, affect, or reflect personality-related variables. Indeed, we found evidence of personality-related variability, since intra-individual differences in activity were repeatable at both inter-sessions interval arrangements. Taken together, our findings suggest a links between inter- and

intra- individual differences in activity (and pattern) personality and behavioural plasticity.

Studying the (in)stability of personality across the life-span in animals is important for all behavioural researches in order to understand the robustness of experimental effects, nevertheless, this field is still poorly studied (Groothuis & Trillmich, 2011; Stamps & Groothuis, 2010), since its application is limited methodically. Reliability of test/re-test results obtained by repeated exposure to the same situation can be biased by habituation processes (Benus, 2001; Sih et al., 2004; Drent et al., 2003, Carere et al., 2005), thus it is also important to understand their contribution. In our study, observed variation in activity patterns was likely caused by shifts in habituation rather than by ontogenetic causes, such as maturation and aging. The activity levels were considerably higher in the group singly exposed to the OFT, suggesting considerable influence of familiarity (and thus, habituation) on the activity of multiple exposed voles (Chapillon & Roulet, 1997; Leussis & Bolivar, 2006). This is consistent with patterns detected in other non-human animals (e.g. McIlwain et al., 2001; Finger et al., 2016), and likely reflects a novelty response.

Habituation speed is negatively related with the length of inter-session (retention) interval (Bolivar et al., 2000). Different periods are used to study working (3 min), short-term (6 h) or long-term (24 h) memory in rodents (Müller et al., 1994); the effect of longer intervals has been rarely assessed. In OFT, spatial information can be retained for a relatively long periods (e.g. laboratory rats: 8 weeks, Broadbent et al., 2004; marmots (*Marmota marmota*): six months, Clemens et al., 2009); nevertheless, intensity of activity reduction (habituation speed) is higher under the short-term (24 h) than under the long-term (a week) retention interval (inbred mice,

Paylor et al. 2006). We did not find mentioned pattern in voles, since both tested periods (24 h and two months) lead to a similar activity changes; only a non-significant trend for a steeper decline after 24 h than two months was detected. Most probably, predominant part of voles was able to retain spatial information also after two months (i.e. reduced their activity), while the rest did not. The finding is crucial when the length of the species life span is taken into the account (seven months in laboratory conditions; Devevey et al. 2008) and indicates presence of various cognitive phenotypes mirror personality-related variability (Carere & Locurto, 2011).

In voles, individual behavioural patterns can be shaped by non-genetic factors such as social environment and affinity to a seasonal cohort (e.g. Lantová et al., 2011; Graceva et al., 2014). We found a relationship between behavioural plasticity and number of siblings, since voles from large litters displayed considerable session-to-session activity increase. It has been documented that individuals from the large litters may show increased anxiety as a result of intensive sibling competition (Drummond et al., 1999; Hudson et al., 2009; Rödel et al., 2008; Dimitantos et al., 2007). In our study, voles were housed individually with *ad libitum* food access after the weaning; absence of competition pressure might lead to gradual decrease of anxiety expressed by increased activity in OFT. Further, individual patterns may be affected by affiliation to seasonal cohort, since compared to winter born ones summer born voles are more explorative and display higher risk-taking tendencies (e.g. Graceva et al., 2014). Mentioned pattern was absent in our study; nevertheless, we did not arrange neither light timing nor food composition to imitate the natural situation that most probably play an important role.

A linkage between ‘animal personality’ and behavioural plasticity has been detected in several species (reviewed in Mathot et al., 2012)

including rodents (e.g. Wistar rats, Thiel et al., 1999). In line with it, we found a relationship between average activity ('animal personality') and the strength and direction of session-to-session activity changes (behavioural plasticity). Voles with high average activity (bold explores type) decreased their levels across the repetitions, while the opposite pattern was found in shy type (i.e. individuals with low average values). The finding can be explained by individual variation in responsivity to reward and anxiety via behavioural activation (BAS) and behavioural inhibition systems (BIS) associated with dopamine (Gray, 1972). Since dopamine is involved in responses to novelty, as well as locomotor responding, the link between novelty and motor action makes adaptive sense since unfamiliar stimuli may pose a threat or signal reward (e.g. access to food or mates) that require avoidance or approach responses. As a novel stimulus becomes more familiar, the dopaminergic response in nucleus accumbens reduces or shifts to predictors of novelty (Piazza et al., 1991). Our findings are crucial for a wide spectrum of research areas where individuality may play a role and should be followed by further empirical studies.

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CHAPTER V.

Summary

In the present work, we focused on personality in non-human animals - on its 'causes' and stability of individual differences. The findings based on three studies can be summarised as follows:

I. We demonstrated presence of consistent individual differences in reaction to novel stimuli (Open Field test) in bank voles, *Myodes glareolus*. Further, we provided one of the first empirical attentions to the link between personality and energy. A 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 (original or introgressed from *Myodes rutilus*) 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 also showed a negative trend in females, suggesting the alternative compensation model.

II. We demonstrated the presence of stable individual differences in reactions to novel stimuli - Open Filed test and radial maze - in common voles, *Microtus arvalis*. Further, the effect of several social factors (number and sex of siblings and identity of the family group) was investigated. Individual-specific responses to Open Field corresponded with the performance in the maze, which revealed behavioural syndromes and possible trade-offs. Anxiety was determined by the size of the family group, whereas escape-related behaviours and maze-exploring tactic differ between particular family groups. Our study revealed the importance of the social factors in the development of personality and the possible

association between personality and cognitive efficiency in non-human animals.

III. We provided one of the first empirical evidence about the validity of the relationship between inter- and intra- individual stability in common voles, *Microtus arvalis*. Individuals that were repeatedly exposed to the Open Field test displayed stable intra-individual differences in exploratory activity, reflecting distinct temporal patterns of behavioural plasticity. Individuals that can be (according to generally accepted construct of animal personality), characterised as highly explorative/proactive in initial test sessions displayed an elevated habituation rate (expressed by rapid waning of the exploratory activity across repeated exposures to the Open Field) in comparison to neophobic animals with low proactivity in the initial session. Moreover, the shape of temporal activity patterns (individual habituation rate) was affected by number of siblings. Our findings provide an important step in characterizing individual variation in behaviour and should be followed by further empirical studies.

APPENDIX

CURRICULUM VITAE

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WORK EXPERIENCE: ACADEMIC POSITION

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PUBLICATIONS

- **Šíchová K.**, Koskela E., Mappes T., Lantová P., Boratyński Z. (2014). On personality, energy metabolism, and mtDNA introgression in bank voles, *Myodes glareolus*. *Animal Behaviour*. June 2014, vol. 92, pp.229-237.
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