



## Temperament, risk assessment and habituation to novelty in eastern chipmunks, *Tamias striatus*

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An increasing number of studies are focusing on the role of animal temperament in the ecology of wild populations. One important step in these studies is to estimate the repeatability of temperament traits, by replicating measures of an animal's behavioural or physiological reactions to a novel or stressful situation. When studies are performed in the field, several factors can affect repeatability estimates: (1) micro-environmental conditions prior to or during a test may affect the measured behaviour, and spatial heterogeneity in predation risk within the habitat of a population may affect repeatability; (2) a decrease over time in the strength of behavioural reactions as a result of habituation may bias repeatability; and (3) individuals may differ in their habituation. In this study we used a linear mixed-model approach to test for the occurrence of interindividual variation in behavioural reaction and habituation of eastern chipmunks, *Tamias striatus*, to a hole-board test and a handling bag test. We found high intraindividual consistency for the behaviours recorded both in the hole-board and handling bag tests; four temperament traits could be considered (i.e. activity/exploration, reaction to stress, emotionality and docility). Given that we found no phenotypic variation in habituation, chipmunks seem to show a behavioural carryover in activity/exploration and docility, which could have consequences for the evolutionary potential of habituation to novelty.

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The evolutionary and ecological implications of temperament, personality, or coping style have recently experienced a growing interest (Wilson et al. 1994; Wilson 1998; Koolhass et al. 1999; Dingemanse et al. 2003; Réale & Festa-Bianchet 2003; Dall et al. 2004; Sih et al. 2004; Bell 2005; Carere et al. 2005). Temperament and personality are assumed to play an important role in many aspects of an animal's behavioural repertoire including habitat use, predation avoidance, dispersal, or social behaviour (Dingemanse et al. 2003; Dall et al. 2004; Sih et al. 2004; Dingemanse & Réale 2005). Furthermore, recent studies have shown that temperament traits can affect several components of fitness (Dingemanse & Réale 2005). The concept of temperament is defined as consistency of an individual's behavioural responses over time and/or across situations

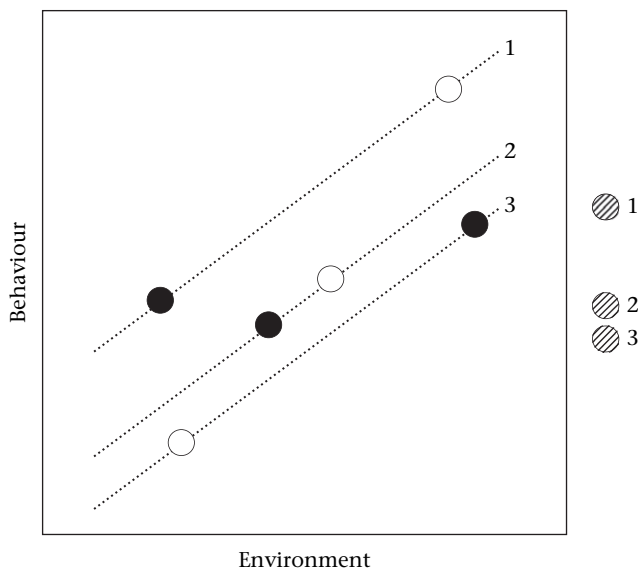
(Réale et al. 2007). To advance in the evolutionary ecology of temperament, it will be necessary to validate the methods by which we measure temperament traits in the field and to understand the components of the phenotypic variation observed in a natural population. Several factors may complicate the study of temperament in the field.

Field experiments often suffer from an inability to control environmental conditions during the test and this may affect our way of estimating an individual's behavioural consistency (i.e. the ranking of individuals across repeated trials). The behavioural response of an individual recorded in experiments used to measure temperament traits, such as open-field tests (Archer 1973), novel object, or startle tests, may therefore differ according to the environmental conditions during the test (Archer 1973; Mettke-Hoffmann et al. 2006; but see Eilam 2003). For example, light intensity or condition of transfer of animals prior to the test can affect behaviour in an open-field (Archer 1973; Walsh & Cummins 1976), and complex objects elicit more exploration than less

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complex objects (Mettke-Hoffmann et al. 2006). An animal may therefore show different responses to a temperament test, only because of the specific microenvironmental conditions prior to or during the test (Henderson 1990). In the absence of information or control of some environmental conditions during a test, an experimenter may obtain spurious results about the rank of behaviour responses amongst different individuals (Fig. 1). In particular, environmental effects may mask consistent differences between individuals when the range of variation in the behaviour across situations is higher than the range of behaviour responses amongst individuals within each particular situation (Hayes & Jenkins 1997). This may be especially problematic for an open-field test performed in field conditions without any knowledge about how animals perceived the risks related to different areas of the habitat.

One way to circumvent this issue is to repeat the test at least twice for each individual and to estimate interindividual variation and behavioural individual consistency (Henderson 1990; Hayes & Jenkins 1997; Réale et al. 2000; Dingemanse et al. 2002). In the case of temperament, this method might still be associated with problems. Many tests of temperament consider novelty as the environmental component at the origin of the stress (Wilson et al. 1994). However, sensitization or habituation to novelty can occur with repeated tests (Groves & Thompson 1970; Archer 1973; van Oers et al. 2005). For example, exploration declines with the number of times the test is



**Figure 1.** Individual behavioural responses (dotted lines) to environmental change for three hypothetical individuals. In the absence of information on a particular environmental condition during a test, an experimenter who collects only one record of the behaviour for each individual (closed circles) may obtain spurious results about the rank of behaviour responses amongst individuals. By averaging the data on replicated measurements of the behaviour on each individual (striped circles), the experimenter reduces the risk of assigning a wrong rank to individuals. Note that interindividual differences may be overestimated if replicated measures of the behaviour for each individual are highly correlated with environment (i.e. presence of individual–environment correlation).

replicated, in open-field tests (Archer 1973; Dingemanse et al. 2002), startle tests (Glowa & Hansen 1994; van Oers et al. 2005), or novel object tests (Mettke-Hoffmann et al. 2006). After estimating consistency of a behavioural trait, one may want to obtain a single value per individual (i.e. behavioural profile) to use in subsequent analyses. The common approach used is to average the values of a trait over repeated tests. Because of habituation, sensitization, or an accumulation of stress with number of tests (Paz-Viveros et al. 1997) averaging the values of repeated tests may provide misleading values about the rank of behaviour responses amongst individuals, particularly when animals have been tested a different number of times. Furthermore, individuals may differ in the way they assess the risk associated with a novel situation (Augustsson & Meyerson 2004) and may differ, not only in their original response to novelty, but also in the speed with which they habituate. For example, experiments on humans have provided some supports to the expectation that extraverts habituate faster than introverts (LaRowe et al. 2006), and rat or mouse strains differ in their habituation to an acoustic startle test (Glowa & Hansen 1994) or to an open-field test (Bolivar et al. 2000). Similarly, differences in habituation can be found between individuals from the two sexes (Elliot & Grunberg 2005; van Oers et al. 2005). Classical methods to estimate repeatability do not consider interindividual variation in habituation, and this omission may underestimate behavioural consistency (Hayes & Jenkins 1997).

Despite the potentially confounding effects of environmental variation and habituation on reaction to novel stimuli, few studies have considered the impact of environmental conditions on exploration in wild animals (Mettke-Hoffmann et al. 2006), and to our knowledge none have investigated interindividual variation in habituation in animals or its effect on the measurement of temperament or personality traits. The absence of interindividual variation in habituation would indicate a behavioural carryover (Sih et al. 2004) in the trait under study (e.g. exploration, activity, stasis, ...). Whether a behavioural trait shows carryover could therefore be important to our understanding of how animals cope with habitat heterogeneity, predation risk, or human disturbance. The existence of a behavioural carryover in a behavioural trait across repeated situations (i.e. no individual variation in rate of habituation) would also have important consequences for our understanding of the behavioural ecology of habituation; the absence of individual phenotypic variation in the rate of habituation would potentially imply an absence of genetic variation for the trait, which would constrain the potential evolution of habituation even in situations favouring it. In the case of discrete environmental situations, people generally use a character state approach (i.e. correlation between the two environmental situations) to illustrate a behavioural carryover (Sih et al. 2004). In the case of continuous environmental variation (i.e. decreasing novelty; population density...), however, such an approach is not the most appropriate and can be replaced by the reaction norm approach (Nussey et al. 2007). In these conditions, the presence of a behavioural carryover can be tested by comparing

individual slopes for the trait as a function of environment; the absence of significant differences in slopes between individuals would reveal limited individual plasticity, as none of these individuals will be able to show the complete spectrum of variation shown in the population.

In this paper we propose to use a mixed-model approach to test, in the field, the effect of environmental conditions on a measure of temperament, and to estimate interindividual variation in both the behavioural reaction to a new stressful situation and rate of habituation to novelty. Having several replicated tests for each individual was essential for this study. We therefore chose to work on the eastern chipmunk, *Tamias striatus*, a sciurid which is easy to observe and to capture on a regular basis. We performed repeated hole-board tests and handling bag tests on 24 wild eastern chipmunks, *T. striatus*. Each chipmunk was tested in two environmental situations differing in their level of predation risk (i.e. low: under canopy; high: open area; McAdam & Kramer 1998). Our objectives were to test whether (1) chipmunks showed consistent differences in their behavioural reaction to a novel or stressful situation (i.e. the hole-board-test or the handling bag test); (2) chipmunks habituated to the experimental test; (3) individuals differed in their speed of habituation to a given test; and (4) reaction and habituation to a hole-board test differed with risk of predation during the test. Finally, we used a mixed linear model to predict individual values for behaviours and habituation that could be used as individual behavioural profiles (Pinheiro & Bates 2000). Using these values we examined phenotypic correlations between the behaviours.

## METHODS

### Study Area and Subjects

Field work was conducted in a deciduous forest in the public area of the McGill University field station at Mont Saint Hilaire, Quebec, Canada, from 2 May to 22 September 2004. The area is covered by a network of public trails. Access is restricted to the trails, and thus the forest is relatively undisturbed by people. Because of frequent contact with humans in the area, chipmunks are habituated to their presence and are often fed by the public. Chipmunks are diurnal, solitary and territorial rodents (Elliott 1978; Snyder 1982). They are active above ground from April to October. During this period, they intensively build up food reserves in their burrow that they use for hibernation in winter.

### Trappings and Markings

Chipmunks were live-trapped with Sherman traps baited with sunflower seeds. We identified the burrows of 24 chipmunks previously trapped, sexed, ear-tagged (National Band and Tag Co., New-Port, KY) and fur-clipped for identification at a distance (Elliott 1978; Giraldeau et al. 1994). Chipmunks were weighed to the nearest gram, using a spring balance, during routine

measurements every 15 days. Some chipmunks were already marked from other studies prior to 2004 (studies have been conducted intermittently in the area since 1984). Because of a lack of long-term information, chipmunks could not be aged precisely. We therefore considered two categories: individuals captured prior to 2004, and individuals captured for the first time in 2004. For each chipmunk, we measured the distance between the burrow and the closest public trail. We differentiated chipmunks with a burrow located more than 5 m from a trail from those whose burrow was located within 5 m of a trail.

### Handling Test

Each time a chipmunk was captured for routine measurements we performed a handling bag test. Tests were performed within 30 min after trapping (mean  $15 \pm 5$  min). The chipmunk was released from the trap into a small mesh handling bag ( $20 \times 15$  cm). The handler suspended the bag containing the chipmunk in the air, and measured the proportion of time the chipmunk spent immobile during 1 min (referred to as static time hereafter). We collected data for an average of six replicated tests (range 2–10) on 29 chipmunks.

### Hole-board Test

The open-field test measures the behavioural reaction of an animal in a novel environment from which escape is prevented by a surrounding wall (Archer 1973; Walsh & Cummins 1976). In the hole-board test, a derivative of the open-field apparatus with holes on the floor, head-dipping (when the animal puts its head in a hole) frequency and proportion are used as measures of exploration that are independent of activity (File & Wardill 1975). The hole-board was constructed from a white rectangular plastic box ( $80 \times 40 \times 40$  cm) with a Plexiglas top and an entrance lock on one of the longer sides. There were four holes on the floor equidistant from the entrance lock, each 5 cm in diameter and 4 cm deep. Two holes were in the centre and two in the corners of the box at 15 and 5 cm from the nearest wall, respectively.

To limit the effects of burrow distance and trapping stress, chipmunks were trapped between 2 and 5 m from their burrow and spent an average of 15 min (range 10–20 min) in the trap before a test. Animals were not manipulated prior to the hole-board test; they were directly placed in the entrance lock for 1 min. We then opened the hole-board door and let the animal enter the arena. After a 10-min period, a chipmunk that had not entered the hole-board was gently pushed in the arena by slowly moving the external door towards the arena. Once the chipmunk was in the arena we closed the door at the entry of the arena to prevent it from returning into the entrance lock. Chipmunk behaviour was then recorded for 5 min with a camcorder. The hole-board was cleaned with water and a cloth between tests.

Video images were later analysed using The Observer 5.0 software (Noldus, Wageningen, The Netherlands). We measured the eight following behaviour variables.

(1) Latency to leave the entrance lock (in seconds; with a maximum of 600 s when animals were pushed in the hole-board after 10 min).

(2) Scanning: proportion of time spent moving head, while the rest of the body is immobile.

(3) Grooming: proportion of time spent grooming.

(4) Locomotion: proportion of time spent walking or running.

(5) Rearing: proportion of time spent rising up on hindlegs.

(6) Climbing: proportion of time spent hanging on top of walls.

(7) Head-dipping: proportion of time spent looking down in a hole. A head-dip was scored when both eyes disappeared into the hole (File & Wardill 1975; Semenova et al. 2001).

(8) Defecation and urination: sum of number of defecation and urination events during the test (Archer 1973).

Hole-board tests were performed in two environmental situations: (1) under forest cover, and (2) in an open area. When attacked by a predator, chipmunks seek cover in holes, under logs, or occasionally climb trees (Clarke et al. 1993; Bonenfant & Kramer 1996). Moving into open areas is therefore likely to increase predation risk because of increased distance from a suitable refuge. Furthermore, chipmunks differ in their locomotion when moving under the forest cover or in an open area, spending more time pausing when moving away from forest cover (McAdam & Kramer 1998). A forested area was thus considered a low predation risk situation compared with an open area. The tests done in the two different predation risk situations were done at equal distances for the burrow entry (i.e. 10 m). Individuals were randomly assigned to one of the two environment types (i.e. forest cover or open area) for the first test, and subsequent tests were alternated between the two environment types. Between one and three tests were performed per environment type and per chipmunk. To limit an effect of stress accumulation with repeated tests (Paz-Viveros et al. 1997), a minimum of 10 days separated two successive tests on the same individual (mean = 16 days, range 10–20). Date, time of the day and order of the test were also noted for each test. Order of the test refers to number of the test done regardless of environment type. Hole-board tests on an individual were never done on the same day as routine measurements and handling bag tests. We collected data for 18 chipmunks with two to three tests in each of the two environmental situations and for another sample of six chipmunks with at least two tests, regardless of the environmental situation.

## Principal Component Analysis

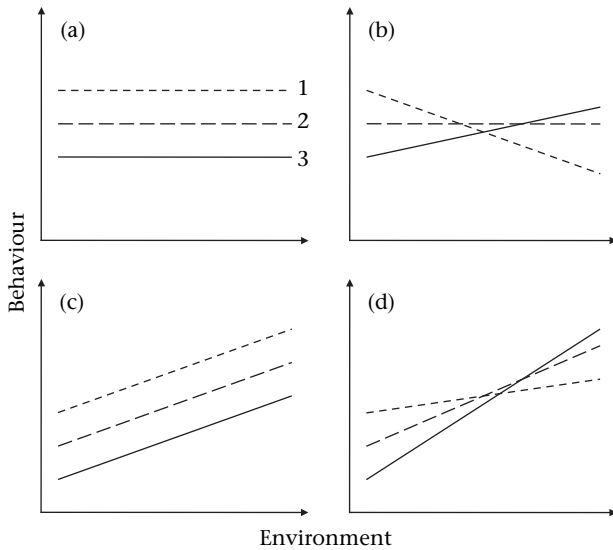
We used a principal component analysis (PCA) on the correlation matrix of behaviour variables in the hole-board test to illustrate the relationship between behaviour

variables, and to reduce the number of behaviour variables prior to additional statistical analyses. The construction of principal components does not require that variables show a multivariate normal distribution (Timm 2002), and thus untransformed variables were used. The Kaiser–Guttman criterion was used to select the number of principal components to retain (Kaiser 1991). Each retained principal component was then used as a composite behaviour variable.

## Linear Mixed Model

Prior to analyses with a linear mixed model, static time in the handling bag was normalized using a square-root transformation. A restricted maximum likelihood linear mixed-model analysis was run on static time from the handling test and on each of the three composite behaviour variables from the hole-board test, using date, hour, environment type (i.e. forested or open area, in the case of hole-board test), trial order, environment type at first trial, duration between two tests, sex, capture year, body mass and distance to the path as fixed effects. Environment type at first trial and duration between two tests showed no significant effects and therefore were excluded from subsequent analyses. Chipmunk identity was included in the model as a random effect. We tested the significance of between-individual variance in behaviour (i.e. between-individual variation in intercepts) by comparing two models, with and without chipmunk ID (Pinheiro & Bates 2000). To test for the significance of between-individual variation in phenotypic plasticity (i.e. between-individual variation in slopes of the value of a behavioural trait), we compared models with and without the interaction between chipmunk ID and date, hour, environmental situation, and trial order, respectively. The additional explanatory power of adding one random effect to a model, provided that the fixed-effect structure remains the same, can be measured using a log-likelihood ratio test (Pinheiro & Bates 2000). We calculated the log-likelihood ratio as  $2[\log\text{-likelihood of model B} - \log\text{-likelihood of model A}]$ , where model B is a more general model than model A (i.e. model A has fewer random effects). The resulting log-likelihood test statistic distribution approximates a chi-squared distribution with  $k_B - k_A$  degrees of freedom, where  $k_i$  is the number of parameters to be estimated in model  $i$  (Pinheiro & Bates 2000). In the mixed-effect model including chipmunk ID as a random effect, the fixed-effect estimate of an environmental covariate provides an estimate of the overall plastic response of individuals to that variable. A significant fixed-effect indicates that on average individuals are plastic (see Fig. 2c or 2d). A significant interaction between chipmunk ID and one environmental variable indicates some between-individual variation in the plastic response of individuals to the variable (see Fig. 2b or 2d). Interaction between chipmunk ID and an individual fixed trait, such as sex, could not be tested. Because observations were made over the same year, age and distance to the nearest path were also fixed within an individual, and interaction with chipmunk ID could not be tested.





**Figure 2.** Individual behavioural response to environmental change for three hypothetical individuals (each line represents an individual response), illustrating the main patterns of plasticity (adapted from Pigliucci 2001): (a) variation in elevation (trait means) but no average plasticity or variation in plasticity; (b) no overall plastic response but variation in plasticity; (c) average plastic response without variation in plasticity; (d) average plastic response with variation in plasticity. (a and c) The potential for a behavioural syndrome is illustrated (i.e. no individual by environment interaction).

### Correlation between Temperament Variables

Best linear unbiased predictors (BLUPs) for random effects (i.e. chipmunk ID) within the mixed models were used as estimates of individual behaviour profiles. The BLUP method provides estimates of random effects independent of other terms within a model, standardized to a mean of zero (Kruuk 2004). They also have the advantage of being less sensitive to extreme values within data than separate regression estimates (Pinheiro & Bates 2000). Therefore, they provide more appropriate estimates of the behaviour profile of an individual than the mean of all the measures for that individual. The model with only the significant effects was used to calculate the BLUPs. We estimated phenotypic correlations between hole-board behaviour profiles and handling bag profiles. Statistical analyses were performed using R 2.1.0 (Ihaka & Gentleman 1996).

## RESULTS

### Principal Components Analysis on the Hole-board Test

All the analyses were first conducted on the 18 chipmunks for which we had data from at least two tests in each of the two environmental situations (i.e. forest cover and open area). Behavioural components obtained from the PCA (see below) were analysed using a mixed model with date, time of the day, order of the test, body mass, sex, first capture year, distance to trail and environmental situation as fixed effects. Neither environmental situation

nor the interactions between chipmunk ID and environmental situation had significant effects on the three behavioural components of the hole-board test ( $P > 0.44$  in all cases). We thus present analyses from a database of 24 chipmunks for which we had data on at least two tests, regardless of the environmental situation.

Three variables, explaining 66% of the total variance, were retained in the PCA (Table 1). The first component represented activity and exploration, with locomotion, climbing, rearing and head-dipping mainly contributing to the left-hand side of axis 1 (Table 1). The second component was bipolar, opposing scanning and grooming behaviour with the urination–defecation index (Table 1). The third component was dominated by latency to enter and the urination–defecation index (Table 1).

### Mixed Models on the Hole-board and Handling Bag Tests

None of the interactions between chipmunk ID and date, hour or trial order significantly improved the model fit for any of the behavioural variables tested (Table 2). This indicates that individuals did not differ in their behavioural responses to change in these environmental variables (similar to the patterns shown in Fig. 2a, c). We therefore considered chipmunk identity as the only random effect in the next model (Table 2). Chipmunk identity explained a significant proportion of the variance in the three principal components (PC1: 42.99%; PC2: 23.39%; PC3: 34.39%) and in static time (23.83%; Table 2). We then tested for the effects of state or of environmental variables on behavioural variables (Table 3).

Chipmunks significantly decreased their activity/exploration (i.e. PC1) with the number of trials in the hole-board (Table 3 and Fig. 3a). Activity/exploration was less for individuals living farther from the path (Table 3 and Fig. 3b) and also decreased with year of first capture (Table 3 and Fig. 3c). Animals first captured before 2004 urinated and defecated more, and groomed and scanned less (i.e. PC2) than animals captured for the first time in 2004 (Fig. 3d). Behavioural stress response (i.e. PC3) to the hole-board decreased with the number of trials (Fig. 3e). In the handling bag, static time decreased significantly with repeated tests and with body mass (Table 3). Date, time of the day and sex had no significant effect on the three behavioural components or on static time (Table 3). Distance to trail had no effect on PC2, PC3 or static time, and year of first capture did not significantly affect PC3 or static time (Table 3). No significant correlations were found between BLUP values of the three principal components of the hole-board test and the BLUP value of static time in the handling bag ( $r < 0.25$  and  $P > 0.42$  for all correlations).

## DISCUSSION

Individual chipmunks were consistent in their reaction to repeated hole-board tests and to repeated handling bag tests. Exploration and activity in the hole-board were strongly related to each other and could not be separated

**Table 1.** Summary of principal component analysis of the hole-board test data for 24 chipmunks at Mont St-Hilaire

| Variables                               | Components  |               |             |       |       |       |       |       |
|---|-------------|---------------|-------------|-------|-------|-------|-------|-------|
|   | 1           | 2             | 3           | 4     | 5     | 6     | 7     | 8     |
| Locomotion                              | <b>0.54</b> | −0.13         | 0.10        | −0.12 | −0.06 | −0.07 | −0.08 | 0.80  |
| Rearing                                 | <b>0.45</b> | 0.05          | 0.13        | 0.39  | 0.07  | −0.18 | −0.67 | −0.33 |
| Climbing                                | <b>0.43</b> | 0.10          | −0.03       | −0.17 | 0.73  | −0.13 | 0.41  | −0.19 |
| Head-dipping                            | <b>0.43</b> | 0.13          | 0.11        | 0.31  | −0.56 | 0.01  | 0.56  | −0.22 |
| Scanning                                | 0.19        | − <b>0.55</b> | 0.34        | −0.57 | −0.16 | 0.21  | −0.07 | −0.35 |
| Grooming                                | −0.10       | − <b>0.61</b> | 0.20        | 0.60  | 0.28  | 0.29  | 0.17  | 0.11  |
| Urination and defecation                | −0.04       | <b>0.52</b>   | <b>0.61</b> | −0.01 | 0.12  | 0.56  | −0.05 | 0.08  |
| Latency to enter                        | −0.27       | −0.02         | <b>0.64</b> | −0.01 | 0.01  | −0.70 | 0.11  | 0.05  |
| Standard deviance                       | 1.67        | 1.12          | 1.09        | 0.95  | 0.79  | 0.76  | 0.66  | 0.42  |
| % of total variance                     | 0.35        | 0.15          | 0.14        | 0.11  | 0.07  | 0.07  | 0.05  | 0.02  |
| Cumulative proportion of total variance | 0.35        | 0.51          | 0.66        | 0.77  | 0.84  | 0.92  | 0.97  | 1.00  |

Components retained for further analysis (using Kaiser–Guttman criterion) and coefficients larger than 0.4 in absolute value (in retained components only) are in bold.

by the PCA. In contrast, we could not show any correlation between static time in the handling bag test and activity/exploration, reaction to stress or emotionality in the hole-board test. Chipmunks showed a decline in intensity of behaviours measured over repeated trials, in both the hole-board and the handling bag test. Rate of habituation, however, did not differ between individuals. Finally, chipmunks did not differ in their behaviour in the hole-board when the test was performed in a more (i.e. open area) or less risky (i.e. forest cover) situation.

From the PCA of behaviours in the hole-board we extracted three behavioural components. Locomotion, rearing, climbing and head-dipping behaviours were all grouped within PC1, which reflects variation in activity/exploration. [File & Wardill \(1975\)](#) proposed the hole-board test to circumvent the inability of the open-field test to separate activity from exploration in small rodents. However, despite these experimental precautions, locomotion and head-dipping remained strongly and positively associated in chipmunks. PC2 contrasted scanning,

**Table 2.** Explanatory power and significance of random effects in linear mixed models of behavioural variables (i.e. the first three components of the principal component analysis for the hole-board test data and static time for the handling bag test), for chipmunks at Mont St-Hilaire

|             | ID | ID*D | I*H | I*O | Model | K  | Loglik. | Test    | LRT   | df | P                |
|-------------|----|------|-----|-----|-------|----|---------|---------|-------|----|------------------|
| PC 1†       |    |      |     |     | 1     | 9  | −159.26 |         |       |    |                  |
|             | x  |      |     |     | 2     | 10 | −151.56 | 1 vs. 2 | 15.40 | 1  | <b>&lt;0.001</b> |
|             | x  | x    |     |     | 3     | 12 | −151.55 | 2 vs. 3 | <0.01 | 2  | 0.99             |
|             | x  |      | x   |     | 4     | 12 | −151.55 | 2 vs. 4 | <0.01 | 2  | 0.99             |
|             | x  |      |     | x   | 5     | 12 | −151.55 | 2 vs. 5 | <0.01 | 2  | 0.99             |
| PC 2‡       |    |      |     |     | 1     | 9  | −138.78 |         |       |    |                  |
|             | x  |      |     |     | 2     | 10 | −136.53 | 1 vs. 2 | 4.50  | 1  | <b>0.033</b>     |
|             | x  | x    |     |     | 3     | 12 | −135.67 | 2 vs. 3 | 1.73  | 2  | 0.42             |
|             | x  |      | x   |     | 4     | 12 | −135.88 | 2 vs. 4 | 1.31  | 2  | 0.52             |
|             | x  |      |     | x   | 5     | 12 | −136.17 | 2 vs. 4 | 0.73  | 2  | 0.69             |
| PC 3§       |    |      |     |     | 1     | 9  | −141.26 |         |       |    |                  |
|             | x  |      |     |     | 2     | 10 | −135.98 | 1 vs. 2 | 10.56 | 1  | <b>&lt;0.001</b> |
|             | x  | x    |     |     | 3     | 12 | −135.95 | 2 vs. 3 | 0.04  | 2  | 0.97             |
|             | x  |      | x   |     | 4     | 12 | −135.51 | 2 vs. 4 | 0.92  | 2  | 0.63             |
|             | x  |      |     | x   | 5     | 12 | −135.83 | 2 vs. 5 | 0.30  | 2  | 0.86             |
| Static time |    |      |     |     | 1     | 9  | −276.53 |         |       |    |                  |
|             | x  |      |     |     | 2     | 10 | −271.59 | 1 vs. 2 | 9.86  | 1  | <b>&lt;0.001</b> |
|             | x  | x    |     |     | 3     | 12 | −271.59 | 2 vs. 3 | <0.01 | 2  | 0.99             |
|             | x  |      | x   |     | 4     | 12 | −271.59 | 2 vs. 4 | <0.01 | 2  | 0.99             |
|             | x  |      |     | x   | 5     | 12 | −271.59 | 2 vs. 5 | <0.01 | 2  | 0.99             |

'X's indicate random effects fitted in respective models. Significant differences between models, based on log-likelihood tests, are in bold. The analysis of variance component of the models indicated that identity counted for 42.99%, 23.39%, 34.39% and 23.83% of the variance of the variables PC 1, PC 2, PC 3 and static time, respectively. D = date; H = hour of day; O = trial order; LRT = log-likelihood ratio test.

\*ID = identity; D = date; H = hour of day; O = trial order; LRT = log-likelihood ratio test.

†Activity/exploration.

‡Reaction to stress.

§Emotionality.

**Table 3.** Estimates of fixed effects produced by a linear mixed model of behavioural indexes with chipmunk ID as random effect (for random effects see Table 2)

| Variables           | Behavioural components |                  |                  |
|---------------------|------------------------|------------------|------------------|
|                     | PC 1*                  |                  |                  |
|                     | Coefficient            | F test           | P                |
| Intercept           | 3.10±1.02              | $F_{1,62}=0.04$  | 0.83             |
| Date                | 0.00±0.01              | $F_{1,62}=0.19$  | 0.67             |
| Time                | -0.00±0.06             | $F_{1,62}=1.18$  | 0.28             |
| Order               | -0.78±0.24             | $F_{1,62}=45.2$  | <b>&lt;0.001</b> |
| Body mass           | 0.05±0.05              | $F_{1,19}=0.95$  | 0.34             |
| Sex†                | -0.39±0.56             | $F_{1,19}=0.11$  | 0.74             |
| First capture year‡ | -1.46±0.49             | $F_{1,19}=4.93$  | <b>0.039</b>     |
| Distance to trail§  | -1.19±0.47             | $F_{1,19}=5.84$  | <b>0.026</b>     |
| PC 2**              |                        |                  |                  |
| Intercept           | 1.60±0.84              | $F_{1,62}=0.00$  | 0.99             |
| Date                | 0.00±0.01              | $F_{1,62}=0.36$  | 0.55             |
| Time                | -0.08±0.05             | $F_{1,62}=1.69$  | 0.20             |
| Order               | 0.12±0.20              | $F_{1,62}=0.03$  | 0.86             |
| Body mass           | 0.00±0.03              | $F_{1,19}=0.00$  | 0.97             |
| Sex†                | 0.45±0.38              | $F_{1,19}=2.00$  | 0.18             |
| First capture year‡ | -0.93±0.33             | $F_{1,19}=10.1$  | <b>0.005</b>     |
| Distance to trail§  | 0.17±0.32              | $F_{1,19}=0.29$  | 0.59             |
| PC 3††              |                        |                  |                  |
| Intercept           | -0.3±0.84              | $F_{1,62}=0.02$  | 0.89             |
| Date                | 0.01±0.01              | $F_{1,62}=0.61$  | 0.44             |
| Time                | 0.02±0.05              | $F_{1,62}=0.16$  | 0.69             |
| Order               | -0.44±0.20             | $F_{1,62}=5.27$  | <b>0.025</b>     |
| Body mass           | 0.05±0.03              | $F_{1,19}=2.21$  | 0.15             |
| Sex†                | 0.20±0.42              | $F_{1,19}=0.28$  | 0.60             |
| First capture year‡ | -0.03±0.37             | $F_{1,19}=0.01$  | 0.92             |
| Distance to trail§  | -0.09±0.35             | $F_{1,19}=0.00$  | 0.96             |
| Static time         |                        |                  |                  |
| Intercept           | 5.18±0.92              | $F_{1,122}=535$  | <b>&lt;0.001</b> |
| Date                | 0.00±0.01              | $F_{1,122}=1.01$ | 0.31             |
| Time                | -0.0±0.06              | $F_{1,122}=1.29$ | 0.25             |
| Order               | -0.14±0.06             | $F_{1,122}=10.1$ | <b>0.002</b>     |
| Body mass           | -0.07±0.03             | $F_{1,24}=5.11$  | <b>0.033</b>     |
| Sex†                | 0.51±0.40              | $F_{1,24}=0.02$  | 0.88             |
| First capture year‡ | -0.01±0.39             | $F_{1,24}=0.00$  | 0.98             |
| Distance to trail§  | -0.65±0.36             | $F_{1,24}=2.92$  | 0.10             |

Significant values are in bold.

\*Activity/exploration.

†Female was considered as the reference for sex in the model.

‡Year 2004 was considered as the reference for first capture year.

§<5 m was considered as the reference for distance between burrow and the nearest trail in the model.

\*\*Reaction to stress.

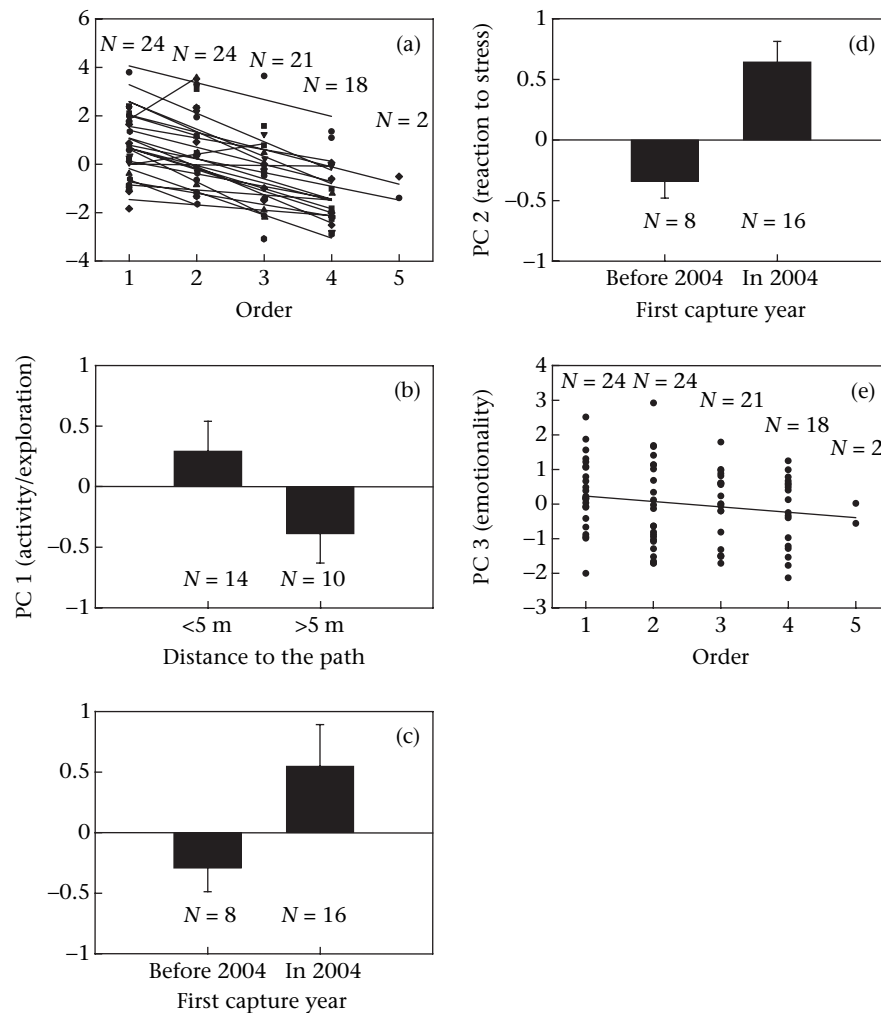
††Emotionality.

grooming and the urination–defecation index, which have all been identified as reactions to a stress (Daniels et al. 2004; Kalueff & Tuohimaa 2004; Koya et al. 2005). During the hole-board test, chipmunks groomed by short bouts of paw licking, and nose and face washing. The absence of complete cephalocaudal progression of grooming reflected a typical stress response to novelty shown in rodents (Kalueff & Tuohimaa 2005). A urination/defecation index was also associated with latency to enter the hole-board, within PC3. These two behaviours have often

been considered as measures of emotionality, or of the activity of the sympathetic nervous system in response to stress stimuli (Archer 1973; Walsh & Cummins 1976). The handling bag test was performed to measure the behavioural response to human presence and manipulation. The term docility has been defined as the reaction of an animal towards humans (e.g. Boivin et al. 1992; Leneindre et al. 1995; Réale et al. 2000; Korhonen et al. 2002). Thereafter, we consider static time in the handling bag as a measure of docility for chipmunks, with high static time reflecting high docility.

Behaviours were significantly repeatable over the summer, with  $r$  ranging between 0.23 and 0.43 (see Table 2). This consistent behaviour difference amongst individuals indicates variation in temperament between chipmunks (Clark & Elhinger 1987; Réale et al. 2000; Dingemanse et al. 2002; Carere et al. 2005). Sex, body mass, age (measure as year of first capture), date, and time of the day were accounted for by the model and therefore individual behavioural differences could not be explained by differences in morphology, body condition, individual experience or seasonal effects at the time of the test. A high repeatability guarantees that the variation observed at the phenotypic level is not caused by microenvironmental effects at the moment of the measurement alone. This high repeatability could also reflect potential high heritability of the traits studied (Boake 1989). However, permanent environmental effects related to a specific chipmunk's home-range that alter its temperament early in life, such as microhabitat difference in resource abundance or human perturbation, may still explain a portion of the individual differences in behaviour observed in this study.

Chipmunks showed a decrease in activity/exploration and emotionality with trial order, which reflects habituation or sensitization (Groves & Thompson 1970) to the hole-board apparatus. Sensitization would be detected by an increase in flight attempts (i.e. jumping and climbing) with test order. In our experiment, flight attempts were associated positively with exploration behaviour and activity and part of PC1. A decrease in PC1 with test order suggests a stronger effect of habituation than of sensitization. However, our results do not allow us to test for the relative importance of habituation versus sensitization. Habituation is one of the most widespread forms of behavioural plasticity (Shettleworth 1998). Habituation has been implicated in several aspects of the behavioural ecology of wild animals (e.g. predator recognition: Rainey et al. 2004; territoriality: Bee & Schachtman 2000; wildlife conservation: McDougall et al. 2006) and has already been observed on other species in open-field tests (Archer 1973; Dingemanse et al. 2002; Elliott & Grunberg 2005), novel object tests (Mettke-Hoffmann et al. 2006), restraint tests (Heblum et al. 1998) and startle tests (Glowa & Hansen 1994; van Oers et al. 2005). Reaction to stress (PC2), however, did not show any evidence of habituation. Habituation to a hole-board or an open-field is often considered as a measure of learning (File 2001; Dukas 1998) or of information processing (Elliott & Grunberg 2005). Habituation may thus provide a good index of some simple form of individual cognitive abilities. Using



**Figure 3.** Significant effects on behavioural indexes obtained from the principal component analysis of hole-board data in the chipmunk population of Mont St-Hilaire. Effects of (a) trial order for each individual on PC1; (b) distance between the burrow entry and the closest trail on PC1; (c) first year capture on PC1; (d) first year capture on PC2; and (e) trial order on PC3. PC1, PC2 and PC3 can be interpreted as activity/exploration, reaction to stress and emotionality, respectively. *N* equals number of individuals observed.

a mixed-effects model allowed us to test for the presence of between-individual differences in habituation. Chipmunks did not differ in their rate of habituation to either hole-board or handling bag tests. Contrary to other studies (Glowa & Hansen 1994; LaRowe et al. 2006), our results did not support the hypothesis of individual variation in habituation to a hole-board test or to handling caused by genetic or environmental influences. Thus, individual differences in chipmunk temperament may not be affected by interindividual differences in learning or by information-processing capabilities. Based on our results we cannot say that chipmunks will never show individual variation in rate of habituation. However, the hole-board test and the handling bag test, two tests performed in different contexts, consistently showed an absence of individual variation in chipmunks. We have not found any studies reporting an absence of individual (or strain) variation in habituation, and to our knowledge, no other studies have tested for the presence of individual variation in habituation to novelty in wild animals. Given the rarity

of studies on habituation in wild animals, it is not possible, at this stage, to generalize to other species or situations. Our knowledge of the ecology of habituation will improve by testing for the presence of individual variation in the rate of habituation in different animals and by linking that variation to various ecological contexts.

Several of our results provided information on the potential for behavioural syndromes (Sih et al. 2004) in chipmunks. First, exploration was strongly and positively associated with activity, which indicates the presence of a behavioural syndrome (Sih et al. 2004) if it could be shown that these two measures represented two distinct traits. Exploration and activity may thus be subject to antagonist selection pressures, a necessary condition for the maintenance of a behavioural syndrome (see Sih et al. 2004). Exploration probably plays an important role in this species, which depends on food resources that are distributed heterogeneously through space and time. Moreover, above-ground activity is associated with high predation risk (Bowers et al. 1993). Second, the absence



of individual difference in rate of habituation suggests the presence of a behavioural carryover (Sih et al. 2004) for activity/exploration, emotionality and docility. A behavioural carryover implies that animals show consistent variation in behaviour across environmental situations (Sih et al. 2004). Highly exploratory chipmunks may suffer higher risk of predation, but may also increase their chance of finding new resources or new burrows, and may cope more easily with changes in their environment than less exploratory individuals. However, the presence of a behavioural carryover in exploration (i.e. absence of interindividual variation in habituation) probably constrains any evolution towards a chipmunk that could show both high exploration and fast habituation. Finally, we could not find any association between chipmunk docility and exploration/activity, stress response or emotionality. These results, and those of other studies (Heblum et al. 1998; Réale et al. 2000; Bell 2005), support the idea that temperament traits may not always be associated with a behavioural syndrome (Wilson et al. 1994; Wilson 1998; Sih et al. 2004).

Contrary to our expectation, chipmunks did not show higher exploration/activity or faster habituation in the low risk environment (i.e. forest cover) than in the high risk environment (i.e. open area). Chipmunks may not perceive the difference between a hole-board placed under the forest cover and in an open area, or the stress related to novelty may be overwhelming. Each chipmunk was successively tested in the two conditions, leading to a possible overall habituation and hiding the differences between the situations. This result seems to contradict findings from previous laboratory studies where exploration was affected by the characteristics of the apparatus (Archer 1973; Walsh & Cummins 1976), and highlights a potential limitation for the use of the open-field test to evaluate how animals assess risks in their natural environment. To generalize our results, further studies assessing relationships between temperament traits and other ecological situations (predator avoidance, foraging, exploration, ...) are needed. The strong behavioural consistency, independent of the general environmental conditions, however, indicates that the hole-board test could be a robust approach to measure animal temperament in wild animals.

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### References

- Archer, J. 1973. Test for emotionality in rats and mice: a review. *Animal Behaviour*, **21**, 205–235.
- Augustsson, H. & Meyerson, B. J. 2004. Exploration and risk assessment: a comparative study of male house mice (*Mus musculus musculus*) and two laboratory strains. *Physiology and Behavior*, **81**, 685–698.
- Bee, M. A. & Schachtman, T. R. 2000. Is habituation a mechanism for neighbour recognition in green frogs? *Behavioural Ecology and Sociobiology*, **48**, 165–168.
- Bell, A. M. 2005. Behavioural differences between individuals and two populations of stickleback (*Gasterosteus aculeatus*). *Journal of Evolutionary Biology*, **18**, 464–473.
- Boake, C. R. B. 1989. Repeatability: its role in evolutionary studies of mating behavior. *Evolutionary Ecology*, **3**, 173–182.
- Boivin, X., Le Neindre, P., Chupin, J. M., Garel, J. P. & Trillat, G. 1992. Influence of breed and early management on ease of handling and open-field behaviour of cattle. *Applied Animal Behaviour Science*, **32**, 313–323.
- Bolivar, V. J., Caltarone, B. J., Reilly, A. A. & Flaherty, L. 2000. Habituation of activity in an open field: a survey of inbred strains and F-1 hybrids. *Behavior Genetics*, **30**, 285–293.
- Bonenfant, M. & Kramer, D. L. 1996. The influence of distance to burrow on flight initiation distance in the woodchuck, *Marmota monax*. *Behavioral Ecology*, **7**, 299–303.
- Bowers, M. A., Jefferson, J. L. & Kuebler, M. G. 1993. Variation in giving-up densities of foraging chipmunks (*Tamias striatus*) and squirrels (*Sciurus carolinensis*). *Oikos*, **66**, 229–236.
- Carere, C., Drent, P. J., Privitera, L., Koolhaas, J. M. & Groothuis, T. G. G. 2005. Personalities in great tits, *Parus major*: stability and consistency. *Animal Behaviour*, **70**, 795–805.
- Clark, A. B. & Ehlinger, T. J. 1987. Pattern and adaptation in individual behavioral differences. In: *Perspectives in Ethology* (Ed. by P. P. G. Bateson & P. H. Klopfer), pp. 1–47. New York: Plenum.
- Clarke, M. F., Burke da Silva, K., Lair, H., Pocklington, R., Kramer, D. L. & McLaughlin, R. L. 1993. Site familiarity affects escape behaviour of the eastern chipmunk, *Tamias striatus*. *Oikos*, **66**, 533–537.
- Dall, S. R. X., Houston, A. I. & McNamara, J. M. 2004. The behavioural ecology of personality: consistent individual differences from an adaptive perspective. *Ecology Letters*, **7**, 734–739.
- Daniels, W. M. U., Richter, L. & Stein, D. J. 2004. The effects of repeated intra-amygdala CRF injections on rat behavior and HPA axis function after stress. *Metabolic Brain Disease*, **19**, 15–23.
- Dingemanse, N. J. & Réale, D. 2005. Natural selection and animal personality. *Behaviour*, **142**, 1159–1184.
- Dingemanse, N. J., Both, C., Drent, P. J., van Oers, K. & van Noordwijk, A. J. 2002. Repeatability and heritability of exploratory behaviour in great tits from the wild. *Animal Behaviour*, **64**, 929–938.
- Dingemanse, N. J., Both, C., van Noordwijk, A. J., Rutten, A. L. & Drent, P. J. 2003. Natal dispersal and personalities in great tits (*Parus major*). *Proceedings of the Royal Society of London, Series B*, **270**, 741–747.
- Dukas, R. 1998. Evolutionary ecology of learning. In: *Cognitive Ecology. The Evolutionary Ecology of Information Processing and Decision Making* (Ed. by R. Dukas), pp. 129–174. Chicago: University of Chicago Press.
- Eilam, D. 2003. Open-field behaviour withstands drastic changes in arena size. *Behavioural Brain Research*, **142**, 53–62.

- Elliott, B. M. & Grunberg, N. E. 2005. Effects of social and physical enrichment on open field activity differ in male and female Sprague-Dawley rats. *Behavioural Brain Research*, **165**, 187–196.
- Elliott, L. 1978. Social behavior and foraging ecology of the eastern chipmunk (*Tamias striatus*) in the Adirondack Mountains. *Smithsonian Contributions to Zoology*, **265**, 1–107.
- File, S. E. 2001. Factors controlling measures of anxiety and responses to novelty in the mouse. *Behavioural Brain Research*, **125**, 151–157.
- File, S. E. & Wardill, A. G. 1975. Validity of head-dipping as a measure of exploration in a modified hole-board. *Psychopharmacologia*, **44**, 53–59.
- Giraldeau, L.-A., Kramer, D. L., Deslandes, I. & Lair, H. 1994. The effect of competitors and distance on central place foraging eastern chipmunks, *Tamias striatus*. *Animal Behaviour*, **47**, 621–632.
- Glowa, J. R. & Hansen, C. T. 1994. Differences in response to an acoustic startle stimulus among forty-six rat strains. *Behavior Genetics*, **24**, 79–84.
- Groves, P. M. & Thompson, R. F. 1970. Habituation: a dual-process theory. *Psychological Review*, **5**, 419–450.
- Hayes, J. P. & Jenkins, S. H. 1997. Individual variation in mammals. *Journal of Mammalogy*, **78**, 274–293.
- Heiblum, R., Aizenstein, O., Gvoryahu, G., Voet, H., Robinson, B. & Snapir, N. 1998. Tonic immobility and open field responses in domestic fowl chicks during the first week of life. *Applied Animal Behaviour Science*, **60**, 347–357.
- Henderson, N. D. 1990. Quantitative genetic analysis of neurobehavioral phenotypes. In: *Developmental Behavior Genetics: Neural, Biometrical, and Evolutionary Approaches* (Ed. by M. Hamn, J. Hewitt & N. Henderson), pp. 281–297. New York: Oxford University Press.
- Ihaka, R. & Gentleman, R. 1996. R: a language for data analysis and graphics. *Journal of Computational and Graphical Statistics*, **5**, 299–314.
- Kaiser, H. F. 1991. Coefficient alpha for a principal component and the Kaiser–Guttman rule. *Psychological Reports*, **68**, 855–858.
- Kalueff, A. V. & Tuohimaa, P. 2004. Contrasting grooming phenotypes in C57Bl/6 and 129S1/SvImJ mice. *Brain Research*, **1028**, 75–82.
- Kalueff, A. V. & Tuohimaa, P. 2005. The grooming analysis algorithm discriminates between different levels of anxiety in rats: potential utility for neurobehavioural stress research. *Journal of Neuroscience Methods*, **143**, 169–177.
- Koolhass, J. M., Korte, S. M., De Boer, S. F., Van Der Vegt, B. J., Van Reenen, C. G., Hopster, H., De Jong, I. C., Ruis, M. A. W. & Blokuus, H. J. 1999. Coping styles in animals: current status in behavior and stress-physiology. *Neuroscience and Biobehavioral Reviews*, **23**, 925–935.
- Korhonen, H. T., Jauhainen, L. & Rekila, T. 2002. Effect of temperament and behavioural reactions to the presence of a human during the pre-mating period on reproductive performance in farmed mink (*Mustela vison*). *Canadian Journal of Animal Science*, **82**, 275–282.
- Koya, E., Spijker, S., Homberg, J. R., Voorn, P., Schoffemeer, A. N. M., De Vries, T. J. & Smit, A. B. 2005. Molecular reactivity of mesocorticolimbic brain areas of high and low grooming rats after elevated plus maze exposure. *Molecular Brain Research*, **137**, 184–192.
- Kruuk, L. E. B. 2004. Estimating genetic parameters in natural populations using the ‘animal model’. *Philosophical Transactions of the Royal Society of London, Series B*, **359**, 873–890.
- LaRowe, S. D., Patrick, C. J., Curtin, J. J. & Kline, J. P. 2006. Personality correlates of startle habituation. *Biological Psychology*, **72**, 257–264.
- Le Neindre, P., Trillat, G., Sapa, J., Ménissier, F., Bonnet, J. N. & Chupin, J. M. 1995. Individual differences in docility in Limousine cattle. *Journal of Animal Science*, **73**, 2249–2253.
- McAdam, A. G. & Kramer, D. L. 1998. Vigilance as a benefit of intermittent locomotion in small mammals. *Animal Behaviour*, **55**, 109–117.
- McDougall, P. T., Réale, D., Sol, D. & Reader, S. M. 2006. Wildlife conservation and animal temperament: causes and consequences of evolutionary change for captive, reintroduced, and wild populations. *Animal Conservation*, **9**, 39–48.
- Mettke-Hoffmann, C., Rowe, K. C., Hayden, T. J. & Canoine, V. 2006. Effects of experience and object complexity on exploration in garden warblers (*Sylvia borin*). *Journal of Zoology*, **268**, 405–413.
- Nussey, D. H., Wilson, A. J. & Brommer, J. 2007. The evolutionary ecology of individual plasticity in wild populations. *Journal of Evolutionary Biology*, **20**, 831–844. doi:10.1111/j.1420-9101.2007.01300.x.
- van Oers, K., Klunder, M. & Drent, P. J. 2005. Context dependence of personalities: risk-taking behavior in a social and a nonsocial situation. *Behavioral Ecology*, **16**, 716–723.
- Paz-Viveros, M., Martin, S. & Martin, M. I. 1997. Methodological aspects about the combination of holeboard, plus-maze and open-field within a test battery, in ethopharmacological studies. *Sciences et Techniques de l'Animal de laboratoire*, **22**, 171–175.
- Pigliucci, M. 2001. *Phenotypic Plasticity*. Baltimore: Johns Hopkins University Press.
- Pinheiro, J. C. & Bates, D. M. 2000. *Mixed-effects Models in S and S-Plus*. New York: Springer-Verlag.
- Rainey, H. J., Zuberbühler, K. & Slater, P. J. B. 2004. Hornbills can distinguish between primate alarm calls. *Proceedings of the Royal Society of London, Series B*, **271**, 755–759.
- Réale, D. & Festa-bianchet, M. 2003. Predator-induced natural selection on temperament in bighorn ewes. *Animal Behaviour*, **65**, 463–470.
- Réale, D., Gallant, B. Y., Leblanc, M. & Festa-bianchet, M. 2000. Consistency of temperament in bighorn ewes and correlates with behaviour and life history. *Animal Behaviour*, **60**, 589–597.
- Réale, D., Reader, S. M., Sol, D., McDougall, P. & Dingemanse, N. 2007. Integrating animal temperament within ecology and evolution. *Biological Reviews*, **82**, 291–318.
- Semenova, T. P., Anoshkina, I. A., Khomut, B. M. & Kolaeva, S. G. 2001. Seasonal peculiarities of behavior of ground squirrel *Citellus undulatus* in holeboard and open field tests. *Behavioural Processes*, **56**, 195–200.
- Shettleworth, S. J. 1998. *Cognition, Evolution, and Behaviour*. New York: Oxford University Press.
- Sih, A., Bell, A. M., Johnson, J. C. & Ziemba, R. E. 2004. Behavioral syndromes: an integrative overview. *Quarterly Review of Biology*, **79**, 241–277.
- Snyder, D. P. 1982. *Tamias striatus*. *Mammalian Species*, **168**, 1–8.
- Timm, N. H. 2002. *Applied Multivariate Analysis*. New York: Springer-Verlag.
- Walsh, R. N. & Cummins, R. A. 1976. The open-field test: a critical review. *Psychological Bulletin*, **83**, 482–504.
- Wilson, D. S. 1998. Adaptive individual differences within single populations. *Philosophical Transactions of the Royal Society of London, Series B*, **353**, 199–205.
- Wilson, D. S., Clark, A. B., Coleman, K. & Dearstyne, T. 1994. Shyness and boldness in humans and in other animals. *Trends in Ecology and Evolution*, **9**, 442–446.