Capture-mark-recapture reduces habituation to



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repeated behavioral test in the common vole







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Introduction

Originally we investigated the repeatability of exploratory behavior of the common vole (Microtus arvalis) (Fig. 1) in an open field test. However we got knowledge on relation between CMR metod and habituation.



Fig. 1 Common vole (*Microtus arvalis*)

Methods

Animals for the test were obtained using the capture-mark-recapture (CMR) method. Previous study did not show any selective effects for the used live-traps (Fig. 2). Wild individuals were caught in their natural habitat (Fig. 3), immediately tested in the laboratory (Fig. 4-7) and then placed into the breeding box (Fig. 8) and breeding room (Fig. 9) or released at the capture site (Fig. 10). We evaluated single behavioral parameters (see vectors in Fig. 11 a 14) as well as synthetic variables created by Principal Component Analysis (PCA). We have estimated the repeatability of exploratory activity and other comparative parameters for the recaptured individuals as well as laboratory kept ones as the intraclass correlation coefficient ICC.



Fig. 2 Live-trap for small mammals

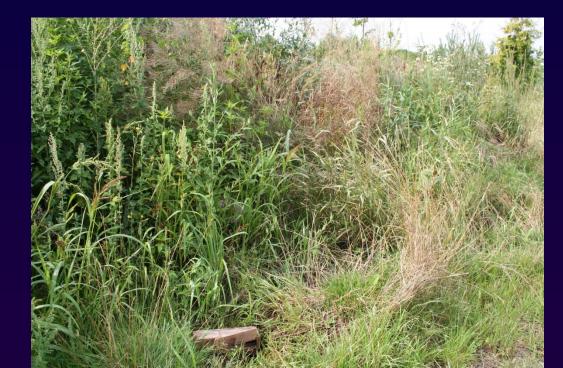


Fig. 3 Trapping of the common vole



Fig. 4 Small bag for the resistance test



Fig. 5 Vole in the Open Field test



Fig. 6 Vole tested for the start latency

Fig. 7 "Freezing" behavior after acoustic stimulus



Fig. 8 Vole in a monotone environment of the breeding box



Fig. 9 Breeding room



Fig. 10 Vole is released into its home range with structured vegetation

Results The intraclass correlation coefficient ICC for the total number of explored squares in recaptured voles ranged from 0.53 to 0.75 (Fig. 15), however, higher values were obtained for the comparative parameter "Freezing" ICC = 0.78 (Fig. 16). Data based on CMR method were compared with data obtained from animals that were not returned to their natural environment, but spent all the experiment under laboratory conditions in breeding boxes: in the total number of explored squares the ICC = 0.25–0.38 (Fig. 12) and in "Freezing" the ICC = 0.83 (Fig. 13). Generally, the CMR values were markedly higher than the data from laboratory conditions. The difference of exploratory activity between the second and the first capture/measurement was negatively correlated with the time (in days) between captures (r = -0.741, p < 0.001) - Fig. 15. Based on this relationship it is possible to assess the interval for the second measurement of exploratory activity that shows the same activity values as the first measurement. This interval reducing habituation takes 3-4 weeks. Under laboratory conditions the comparative interval exceeded 10 weeks (Fig. 12).

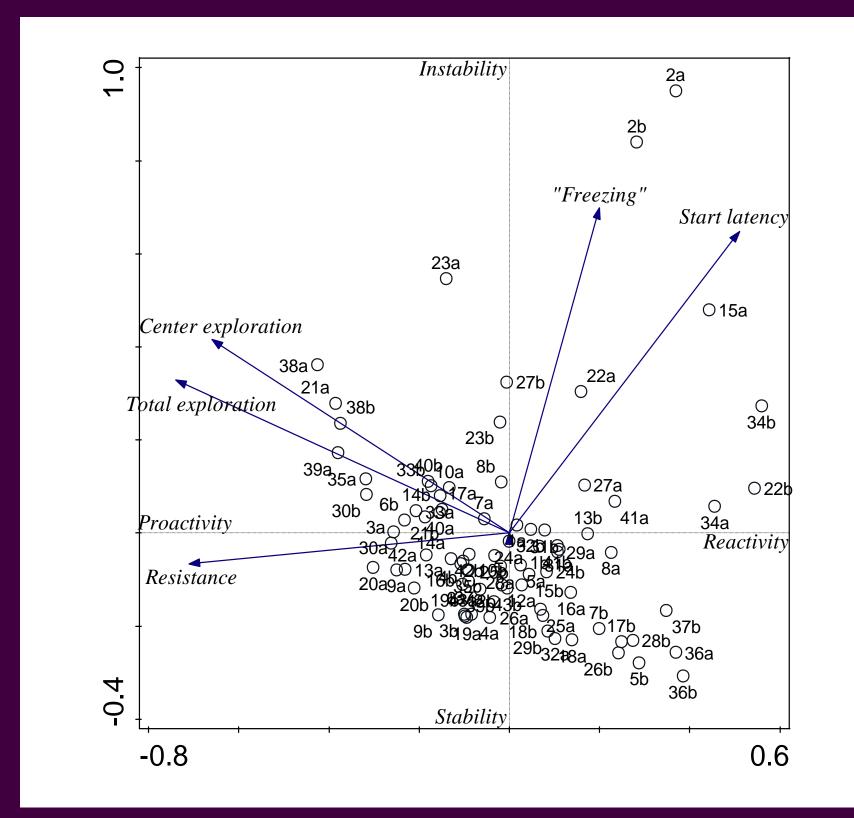


Fig. 11 PCA of data obtained in voles (circles with number) that spent time between personality tests in the laboratory. The first test (a) and the second test (b) created space with two gradients: axis 1 (x) – proactivity-reactivity and axis 2 (y) stability-instability of running behavior. See e.g. points 2a and 2b above - their position means that animal no. 2 behaved under both tests very similarly. "Freezing" was a little bit shorter in the second test (b).

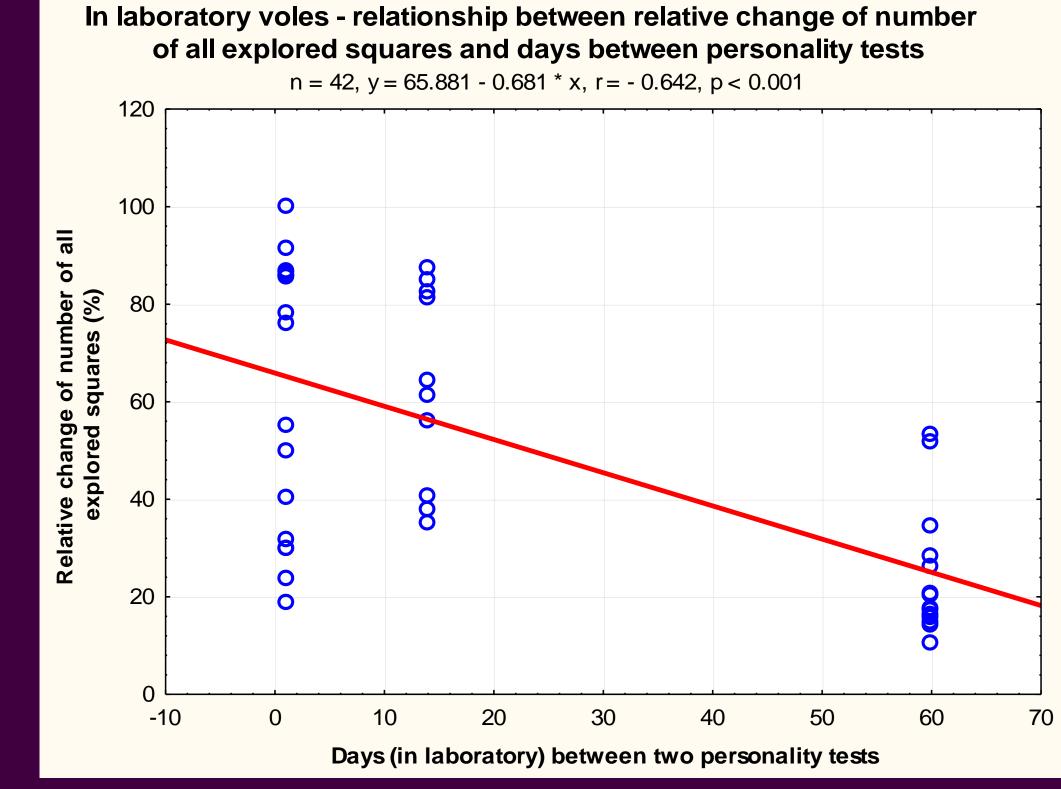


Fig. 12 Relationship between relative changes of total number of explored squares and days between personality tests – in the laboratory. After one day the change was very high and the repeatability was low – ICC = 0.25 (25 %). After 60 days the differences were lower and the repeatability was higher – ICC = 0.38 (38 %). Return to the original values could last more than 10 weeks.

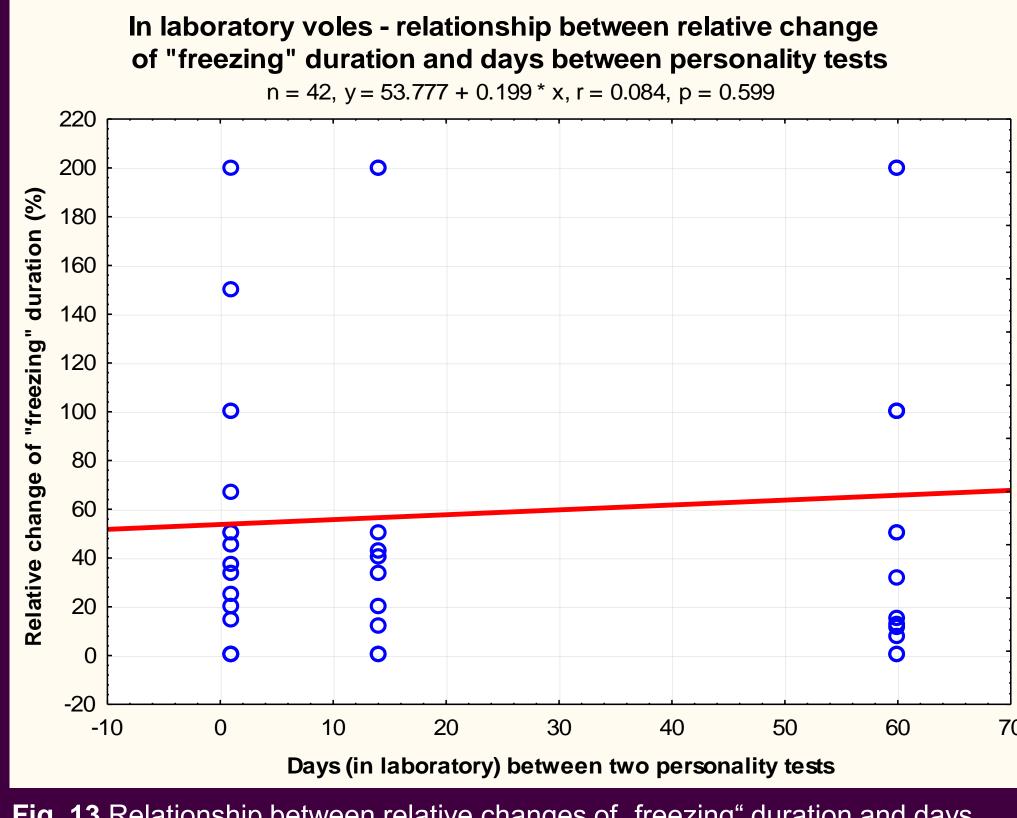


Fig. 13 Relationship between relative changes of "freezing" duration and days between personality tests. We can see a broad range of relative changes which stay relatively stable during the all terms of tests. After 60 days the repeatability was very high - ICC = 0.83 (83 %). It means that the comparative (more reflex) parameter "freezing" was influenced by habituation only a little.

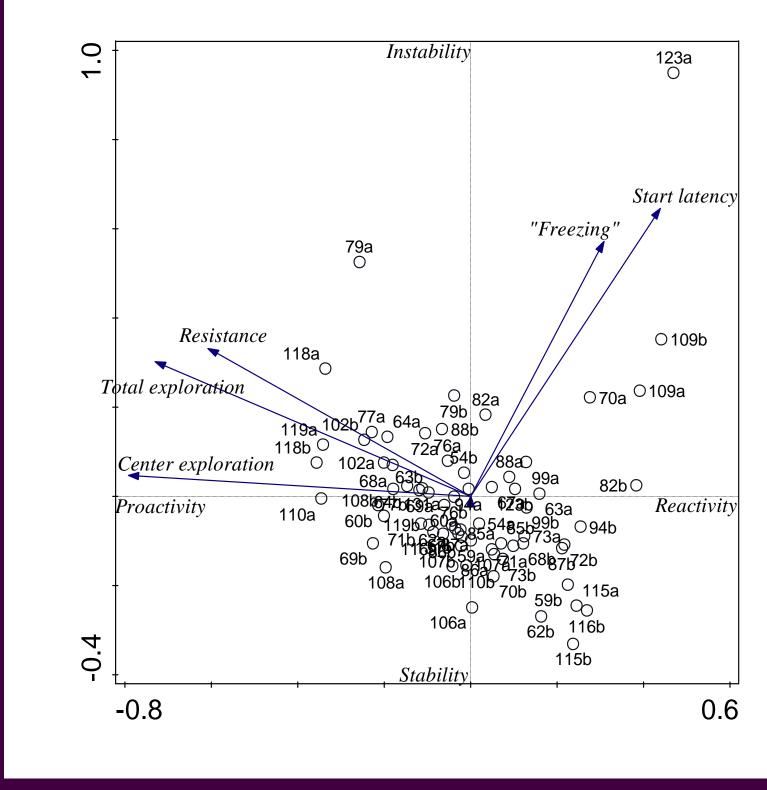


Fig. 14 PCA of data obtained in voles (circles with number) that spent time between personality tests in their home habitat. The first test (a) and the second test (b) created space with two gradients: axis 1 (x) – proactivity-reactivity and axis 2 (y) stability-instability of running behavior. See e.g. points 109a and 109b on the right edge – their position means that animal no. 109 behaved under both tests very similarly. "Freezing" and Start latency were a little bit longer in the second test (b).

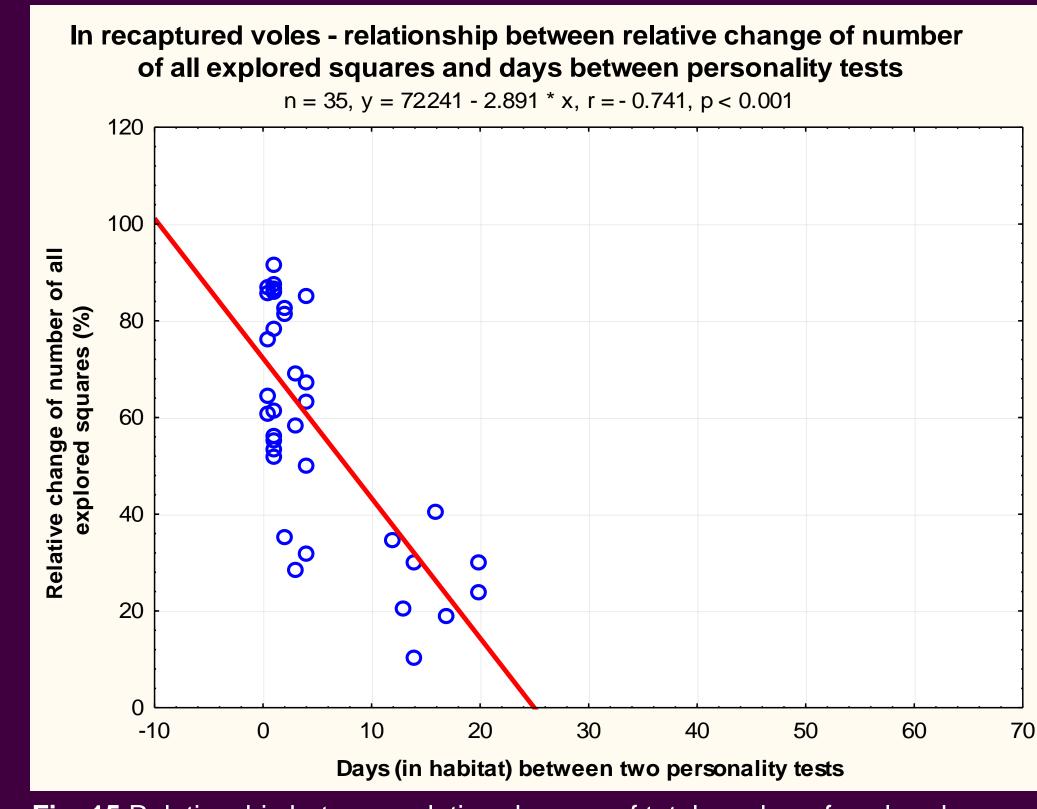


Fig. 15 Relationship between relative changes of total number of explored squares and days between personality tests – in natural habitat. After one day the change was very high and the repeatability ICC = 0.53 (53 %). After 20 days the differences were markedly lower and the repeatability was much higher – ICC = 0.75 (75 %). Return to the original values could last 3-4 weeks.

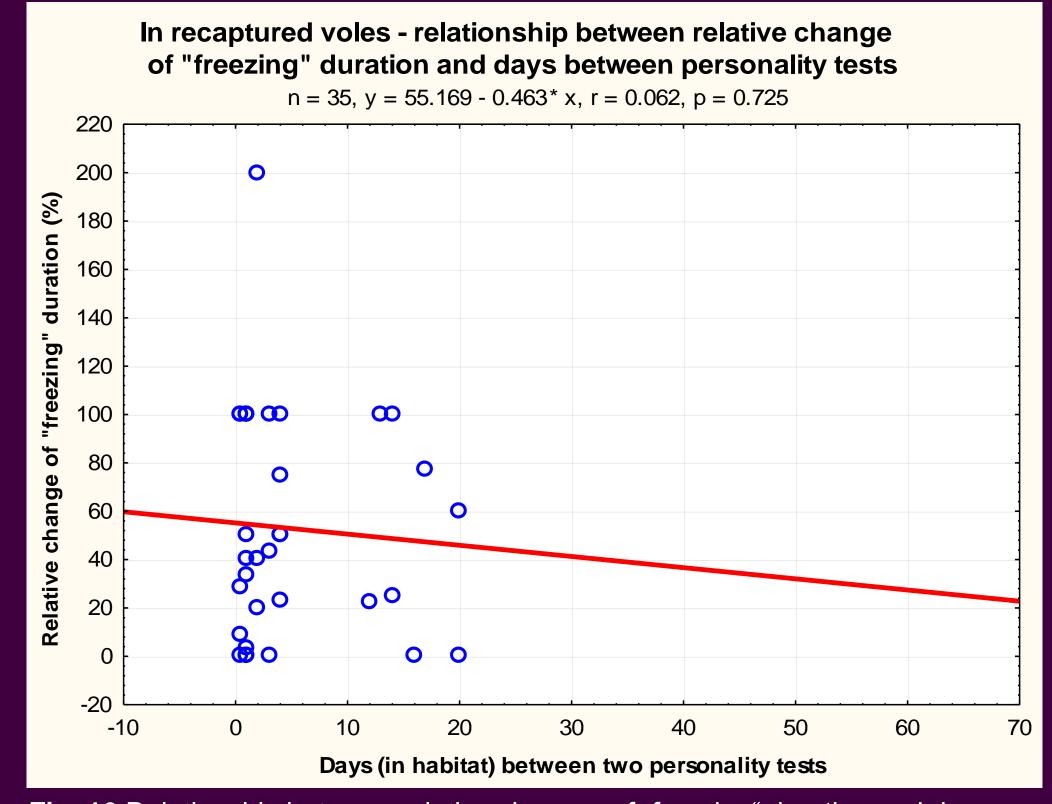


Fig. 16 Relationship between relative changes of "freezing" duration and days between personality tests. We can see a broad range of relative changes which stay relatively stable during the all terms of tests. After 20 days the repeatability was very high – ICC = 0.78 (78 %). It means that the comparative (more reflex) parameter "freezing" was influenced by habituation only a little.