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COMPARATIVE ETHOLOGICAL STUDY OF WILD AND LABORATORY MEMBERS OF THE MUS GENUS

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1. Background of Research, Objectives

Laboratory mice (*Mus musculus domesticus*) have become one of the most important model organisms in scientific research over the past century. Due to their ease of care, rapid reproduction, and extensively mapped genome, they play a fundamental role in medical, pharmacological, and behavioral studies (Silver, 1995). The laboratory mice used as model organisms originate from various species of the *Mus* genus, among which genetic analyses have identified four primary ancestral components: *Mus musculus domesticus*, *Mus musculus musculus*, *Mus musculus castaneus*, and *Mus musculus molossinus* (Frazer et al., 2007). This mixed ancestry provides significant genetic diversity, influencing the physiological and behavioral traits of laboratory mice. The domestication and selective breeding of mice began in Japan and Europe during the 18th and 19th centuries, but the use of mice for scientific research dates back to the mid-19th century. The long process of domestication not only induced morphological and physiological changes in laboratory mice but also had profound effects on their behavioral patterns (Trut et al., 2009). Domesticated animals are generally less aggressive, exhibit reduced activity levels, and are more adaptable to human presence. These changes are not solely the results of directed selection but also adaptations to new environments, such as captive living and frequent human interactions. The laboratory environment imposed unique challenges that have shaped the genetic and behavioral characteristics of laboratory mice over many generations (Benjamini et al., 2001).

The study of wild *Mus* species provides a valuable baseline for understanding behavioral changes induced by domestication. The aim of the present study was to compare the behavioral patterns of wild *Mus* species (namely, the mound-building mouse and the house mouse) with those of commonly used inbred laboratory strains such as BALB/c, C3H, and C57BL/6. Previous studies have shown that these three strains display distinct behavioral profiles.

The C3H strain is particularly notable as it is often used in studies examining learning and memory functions (Bryan et al., 2008). BALB/c mice are characterized by higher anxiety levels and lower exploratory activity, while the C57BL/6 strain is generally more exploratory and exhibits lower anxiety (O'Leary et al., 2013). The differing behavioral profiles of these strains provide an opportunity to investigate the genetic and environmental bases of behavioral variation, as well as to assess how far laboratory mouse behavior has diverged from that of their wild ancestors. Findings from this research could offer insights into refining the housing conditions of laboratory mice.

1.1. Aims of the Study

In this research, I examined the behavior of mound-building and house mice under laboratory conditions, as these species have not previously been studied in captive environments. Additionally, I compared their behavior with that of laboratory strains. The second part of my thesis focused on the behavioral examination of the most commonly used laboratory mouse strains.

The specific aims were:

1. To investigate the climbing ability of two closely related species: the mound-building mouse and the house mouse.
2. To compare the exploratory behavior of the mound-building mice and house mice with that of two commonly used laboratory strains, BALB/c and C57BL/6.
3. To compare the behavior of two laboratory strains, the albino BALB/c and the pigmented C57BL/6 mice, under different lighting conditions.

4. To examine the climbing ability of three laboratory strains: BALB/c, C57BL/6, and C3H.

2. MATERIAL AND METHODS

2.1. Housing of Mouse Stocks Used for the Study

The experiments were conducted at the Rodent Facility of the Kaposvár Campus, Hungarian University of Agriculture and Life Sciences. The facility maintained its own breeding colony, with individuals of known age, sex, and origin. The *Mus spicilegus* (mound-building mouse) and *Mus musculus* (house mice) populations consisted of laboratory-born offspring of wild-captured parents from various regions of Hungary. The laboratory strains (BALB/c, C57BL/6, and C3H) were descendants of breeding stocks obtained from Akronom Ltd.

Mice were housed in standard T4 laboratory cages (600 × 200 × 380 mm) in small groups (one male with two to three females), with deep bedding (SAFE® 3-4 S fibrillated fibers) and nesting material (SAFE® crinklets natural and SAFE® nesting small). Water and SAFE® 132 laboratory mouse feed were provided ad libitum. Animals were kept in a darkened room under a reversed light cycle: 10 lux red light from 8 a.m. to 8 p.m., followed by 220 lux yellow light from 8 p.m. to 8 a.m., at 20–22°C and 50–55% relative humidity. Daily animal care monitoring and annual veterinary inspections were performed.

2.2. Assessment of Climbing Ability in *Mus spicilegus* and *Mus musculus*

Outside the testing sessions, mice were group-housed (3–4 individuals per T4 cage) under standard laboratory conditions at 21°C and a reversed light cycle, with ad libitum food and water.

A total of 88 individuals were selected: 44 mound-building mice (22 juveniles and 22 adults) and 44 house mice (22 juveniles and 22 adults). Juveniles were aged between 28–35 days, and adults were approximately 500 days old. Males and females were equally represented across groups.

The climbing apparatus consisted of a hand-made setup: a 27 cm tall, 0.9 cm diameter untreated wooden rod attached to a 10 cm diameter wooden base at a

90° angle. Five climbing rods were placed inside a circular red open-field arena (65 cm in diameter, 55 cm in height).

Each mouse was tested individually. After each session, the arena and climbing rods were wiped with alcohol to eliminate scent traces. Testing consisted of a 10-minute habituation phase followed by a 5-minute observation phase. Recorded parameters included whether the mouse climbed the rod, the number of climbing events, the latency to first climb, and tail use during climbing (following the method of Earl, 1976).

Data were grouped by species and age group. Statistical analyses were performed using SAS 9.4 software, employing LIFEREG and PHREG procedures (SAS Inst. Inc., 2013, 2014). Survival analysis (Kaplan and Meier, 1958) was applied to assess latency to first climb. Log-rank tests determined significant differences between survival curves, and the Cox proportional hazards model (Cox, 1972) assessed the effects of species and age on climbing behavior.

2.3. Comparison of Exploratory Behavior Between *Mus spicilegus*, *Mus musculus*, and Laboratory Strains

For this study, 32 individuals per species/strain (16 males and 16 females, subdivided into 8 juveniles and 8 adults) were randomly selected.

Testing was conducted using a 36.5 × 21 × 18 cm white plastic box equipped with bedding, nesting material, food, and water. A 10 cm long, 5 cm diameter tube attached to the side wall provided access to an open-field arena, simulating the outside environment.

Each mouse was tested individually. After each session, bedding and nesting materials were replaced, and the box was disinfected to prevent olfactory cues. The test included a 10-minute habituation phase with the tube blocked, followed by a 5-minute observation phase where the tube was opened. Behavioral recording focused on whether the mouse exited the box, and if so, at what latency.

Statistical analysis was conducted using IBM SPSS Statistics 29.0. Survival analysis assessed differences between wild and domesticated groups, and between species/strains, using Log-rank tests. The influence of sex and age was further analyzed using Cox proportional hazards models.

2.4. Comparison of Two Laboratory Strains Under Different Lighting Conditions

An open-field test was used to describe the behavior of BALB/c and C57BL/6 mice under two lighting conditions: bright (220 lux yellow light) and dim (10 lux red light). A total of 64 mice were tested: 32 under bright light (16 BALB/c, 16 C57BL/6) and 32 under dim light.

Outside of testing, mice were housed in standard T4 cages under reversed 12:12 h light cycles, at 20–22°C, with ad libitum food and water. Illumination was automated, with OSRAM L36W/640 tubes during the light phase and Philips TL-D 36W/10 red tubes during the dark phase.

Behavioral testing was conducted individually in a 36.5 × 21 × 18 cm white plastic container. Each test lasted 300 seconds, during which behaviors were recorded: number of approaches to the observer's hand, latency to first approach, and number of fecal boli.

Latency to first approach was analyzed using survival analysis (Kaplan and Meier, 1958), and differences between lighting conditions and strains were tested using the Log-rank test. Effects of lighting intensity, pigmentation (albino vs pigmented), and age were analyzed using Cox proportional hazards models.

The number of approaches, following a Poisson distribution, was analyzed using Poisson regression. The relationship between fecal boli production and exploratory behavior was assessed via Pearson correlation. All statistical analyses were performed using IBM SPSS Statistics 29.0.

2.5. Assessment of Climbing Ability in Three Laboratory Strains

A total of 60 mice (20 BALB/c, 20 C57BL/6, and 20 C3H) were individually tested. Each mouse was placed in a red open-field arena equipped with five climbing rods, and the arena and rods were cleaned with alcohol between tests. The test protocol consisted of a 10-minute habituation phase followed by a 5-minute observation phase. Measurements included the occurrence and frequency of climbing, and latency to first climb.

Mice were categorized by strain, sex, and age group (juveniles: 28–35 days; adults: ~500 days). Statistical analyses included survival analysis (Kaplan and Meier, 1958), Log-rank tests, and Cox proportional hazards models for latency data. Climbing frequency was analyzed using Poisson regression. All analyses were performed using IBM SPSS Statistics 29.0.

3. RESULTS

3.1. Assessment of Climbing Ability in *Mus spicilegus* and *Mus musculus*

According to the log-rank test, both species and age had a significant effect on climbing ability ($p<0.05$). For mound-building mice (Group 1), the first climbing event occurred at 101 seconds, whereas for house mice (Group 2), the first climb occurred at 32 seconds.

Among the mound-building mice, 28 individuals failed to climb during the test, meaning that over 63% of them showed no inclination to climb. In contrast, only 10 house mice did not climb, indicating that fewer than 23% of them did not exhibit climbing behavior.

In both species, juvenile individuals (Group 2) climbed earlier than adults (Group 1). Among adults, 25 individuals did not climb during the test (over 56%), compared to only 11 juveniles (25%).

The estimated Cox regression coefficients were significant ($p<0.005$), indicating that both species and age had significant effects on climbing behavior. The estimated hazard ratios showed that house mice were four times more likely to climb within a given time unit compared to mound-buildign mice, and juveniles were three times more likely to climb than adults.

3.2. Comparison of Exploratory Behavior Between Wild and Domesticated Mice

The survival curves for wild and laboratory mouse groups showed a significant difference based on the log-rank test ($p<0.001$). In wild mice, exploration occurred well before 50 seconds, while in laboratory mice, the first exploration was observed around 70–75 seconds.

When comparing within groups (*Mus spicilegus*, *Mus musculus*, BALB/c, and C57BL/6), Mound-building mice explored the external environment the earliest, followed by house mice, with BALB/c and C57BL/6 mice exploring later at similar rates.

The log-rank test showed no significant difference between BALB/c and C57BL/6 ($p=0.328$), but there was a significant difference between mound-building mice and house mice ($p=0.002$). Significant differences were also found between mound-building mice and the two laboratory strains ($p<0.001$), and between house mice and the laboratory strains ($p=0.041$).

In the four-group comparison, mound-building mice were the earliest explorers (~35 seconds), followed by house mice (~50 seconds), C57BL/6 mice (~75 seconds), and BALB/c mice (~100 seconds).

The Cox regression coefficient for the wild vs. laboratory mice was significant, indicating a significant effect on the time to first exploration. No significant effects were found for sex or age on exploration time.

3.3. Comparison of Two Laboratory Strains Under Different Lighting Conditions

At 10 lux, the first approach occurred well before 50 seconds, while at 220 lux, it occurred around 140–150 seconds.

Survival analysis revealed that under 220 lux yellow light, only 14 out of 32 mice (44%) approached the experimenter's hand. Under 10 lux red light, 27 out of 32 mice (84%) approached.

For BALB/c mice, the survival curves showed that under 10 lux, most mice approached earlier (~70–80 seconds), while under 220 lux, the first approach occurred around 150 seconds.

Among BALB/c mice, only 6 out of 16 approached under 220 lux (37.5%), while 13 out of 16 approached under 10 lux (81%).

For C57BL/6 mice, the first approach under 10 lux occurred before 50 seconds, while under 220 lux it was around 150 seconds. At 220 lux, 10 out of 16 C57BL/6 mice approached (62.5%), while under 10 lux, 14 out of 16 approached (87.5%).

Log-rank tests indicated significant differences in first approach times between the two lighting conditions. The Cox proportional hazards model showed that lighting intensity and age significantly affected ($p<0.005$) exploration time, while strain (and thus pigmentation) did not ($p=0.148$).

Poisson regression showed a significant difference in activity (number of approaches) between lighting conditions ($p<0.001$), with higher activity under red light. Age also significantly affected activity ($p=0.022$), with juveniles being more active than adults. No significant difference in activity was found between strains ($p=0.784$).

Pearson correlation analyses showed a positive correlation between fecal boli count and age ($p<0.001$, $r=0.470$), older mice defecated more during the test. A positive correlation between fecal boli count and light intensity ($p<0.001$, $r=0.688$): more feces were produced under higher light intensity. A negative correlation between fecal boli count and activity ($p<0.001$, $r=-0.484$), less active mice defecated more.

3.4. Assessment of Climbing Ability in Three Laboratory Strains

The survival curves for the three laboratory mouse strains (BALB/c, C57BL/6, and C3H) indicated no significant differences according to the log-rank test ($p=0.118$). The first climbing attempt occurred around 50 seconds for both BALB/c and C57BL/6 mice, whereas for C3H mice, the first climb was delayed and occurred approximately at 130 seconds. During the 5-minute test period, 55% of BALB/c mice and 65% of C57BL/6 mice successfully climbed the rod, while only 35% of C3H mice exhibited climbing behavior.

Regarding sex differences, no significant effect was observed on climbing performance ($p=0.869$), with the first climbs occurring around 50 seconds in both males and females. The climbing percentages were comparable between the sexes, with 53% of males and 50% of females successfully climbing during the test.

In contrast, significant differences were detected between juvenile and adult mice ($p<0.001$). Juveniles climbed earlier, typically before 50 seconds, whereas adults showed a delayed climbing response, with the first climbs occurring around 190 seconds. Moreover, a higher proportion of juveniles (63%) climbed the rod compared to adults (36%).
Cox regression analysis confirmed that age had a significant effect on the latency to first climb, while strain and sex did not influence this parameter. Similarly, Poisson regression analysis revealed no significant differences in the number of climbs between strains ($p=0.417$) or sexes ($p=0.363$). However, age again showed a significant effect ($p=0.004$), with juvenile mice climbing more frequently than adults during the test period.

4. Conclusions and Suggestions

4.1. Assessment of Climbing Ability in *Mus spicilegus* and *Mus musculus*

In this study, I compared the climbing ability of house mice (*Mus musculus*) and mound-building mice (*Mus spicilegus*) under laboratory conditions. The results confirmed that house mice generally exhibited a significantly stronger inclination to climb than mound-building mice. Juvenile individuals of both species showed a higher tendency to climb compared to adults. According to Cox regression analysis, the probability of a successful climbing event within a given time unit was four times higher in house mice than in mound-buidling mice, and three times higher in juveniles than in adults.
These findings highlight that habitat characteristics and morphological traits (such as tail length) substantially influence climbing ability. The superior climbing ability of house mice is likely an adaptation to anthropogenic environments, where vertical movement is often crucial. In contrast, mound-building mice, typically associated with agricultural landscapes dominated by herbaceous vegetation, have less need for climbing behavior.

It is important to note that this study was conducted under laboratory conditions. Further field observations could provide additional evidence regarding the role of young mound-building mice in mound-building activities and their use of climbing in natural habitats. To deepen our understanding of the relationship between habitat and climbing ability, comparative studies with rodent species living in diverse ecological contexts would be valuable. Given the mound-building mouse's close association with agricultural areas and the impacts of human activities on their habitats, further research is necessary to support the conservation of their populations.

4.2. Comparison of Exploratory Behavior Between Wild Mice and Two Laboratory Strains

In this part of the study, I compared the exploratory behavior of wild mouse species (*Mus musculus* and *Mus spicilegus*) with that of domesticated laboratory strains (BALB/c and C57BL/6). The results revealed that wild mouse species were significantly more active, leaving their familiar environment faster and in higher proportions than laboratory mice. The study confirmed that domestication is associated with a decrease in activity levels and a delay in the initiation of exploratory behavior.

Differences were also observed among wild species: mound-building mice explored the external environment faster and more frequently than house mice. This is likely due to differing habitat requirements and behavioral adaptations. Among the laboratory strains, no significant differences were found in exploratory behavior, which is probably due to their close genetic similarity. These results support the idea that behavioral changes during domestication are primarily quantitative rather than qualitative.

To better understand the genetic basis of domestication-induced behavioral changes, it would be worthwhile to conduct comparative studies involving other rodent species. Exploring the genetic background of activity levels and stress responses could be particularly informative. Increasing the genetic diversity of laboratory mouse stocks by incorporating wild-derived strains could improve the ecological relevance of behavioral tests. These findings may also contribute to the refinement of laboratory mouse models, especially in the study of behavioral disorders linked to reduced activity levels or altered stress responses.

4.3. Comparison of Two Laboratory Strains Under Different Lighting Conditions

The results of this investigation demonstrated that light intensity has a significant impact on the behavior of laboratory mice. Strong illumination (220 lux) considerably reduced the mice's activity levels, whereas under low-intensity red light (10 lux), mice were significantly more active. This difference was observed not only in activity levels but also in stress responses: a higher amount of fecal boli was recorded under strong illumination, suggesting elevated stress levels. C57BL/6 mice, with pigmented eyes, appeared somewhat less sensitive to strong light compared to the albino BALB/c mice; however, a negative effect of strong light on activity was evident in both strains.

Albino mice are particularly sensitive to light, likely due to light-induced photoreceptor degeneration. Our findings also highlighted the influence of age, as younger mice were more active and displayed lower stress sensitivity compared to older individuals, likely due to their greater curiosity and resilience.

In laboratory mouse husbandry, the use of low-intensity red light is recommended, as it minimally disrupts animal behavior and circadian rhythms. Avoiding strong illumination may help reduce stress levels and thus improve the reliability of experimental results. The use of a reversed light/dark cycle enables behavioral testing during the animals' active phase, increasing the biological relevance of the studies. Age should be carefully considered when designing behavioral tests, as younger animals may display greater activity and lower anxiety levels, potentially influencing experimental outcomes. Further research into the effects of different light spectra and intensities on mouse behavior and physiology is also recommended.

4.4. Assessment of Climbing Ability in Laboratory Mouse Strains

Behavioral differences between laboratory mouse strains, when assessed using simple tests such as the climbing test, proved to be minimal. Our results confirmed that strain differences were not significant, and no notable differences were observed between sexes either. However, age was found to be a major factor significantly affecting both the latency to the first climb and the overall climbing activity. Juvenile mice were significantly faster and more active climbers than adults.

Therefore, it is advisable to control for age when designing experiments, using animals of the same age to minimize variability related to age differences. Although climbing ability differences between strains were not significant in this study, further research employing more complex behavioral paradigms (such as anxiety, memory, or learning tests) may reveal subtler effects of genetic differences.

To ensure comparability between studies, standardized housing and experimental conditions are essential, as laboratory environments can also influence behavioral outcomes. Particular phenotypes, such as the albinism of BALB/c mice, may affect performance in tests involving strong illumination, thus requiring adapted experimental setups for different strains.

5. NEW SCIENTIFIC RESULTS

1. Our investigations demonstrated that there is a difference in climbing ability between two closely related mouse species, the mound-building mouse (*Mus spicilegus*) and the house mouse (*Mus musculus*). The house mouse exhibited a greater tendency to climb than the mound-building mouse, which is likely due to the fact that the two species occupy different habitats in nature.

2. In examining the exploratory behavior of wild mouse species and laboratory mouse strains, we demonstrated that wild mouse species were more likely to leave the familiar environment, and did so earlier, than the laboratory strains.

3. We confirmed that there were no behavioral differences in climbing ability among the three laboratory mouse strains (BALB/c, C57BL/6, and C3H). Age had a greater influence on climbing behavior than the strain to which the tested animals belonged.

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7. PUBLICATIONS ON THE SUBJECT OF THE DISSERTATION

Peer-reviewed papers published in foreign scientific journals

Bárdos, Boróka; Altbacker, Vilmos; Szabó, András; Török, Henrietta Kinga; Nagy, István: Study of climbing ability for two closely related mouse species. EUROPEAN ZOOLOGICAL JOURNAL 90: 1 pp. 395-400., 6 p. (2023)

Bárdos, Boróka; Török, Henrietta Kinga; Nagy, István: Comparison of the exploratory behaviour of wild and laboratory mouse species. BEHAVIOURAL PROCESSES 217 Paper: 105031 (2024)

Török, Henrietta Kinga; Bárdos, Boróka; Hoffmann, Orsolya Ivett: Comparison of the behaviour of three strains of laboratory mice in the climbing test. ANIMAL WELFARE, ETHOLOGY AND HOUSING SYSTEMS 21: 1 (2025)

Török, Henrietta Kinga; Bárdos, Boróka: Ethological Constraints and Welfare-Related Bias in Laboratory Mice: Implications of Housing, Lighting, and Social Environment. ANIMALS 16(2) 314 (2026)