



Pilot Study on the Breeding, Activity and Behaviour of the European Ground Squirrel *Spermophilus citellus* (Linnaeus, 1766) (Rodentia: Sciuridae) in Zoo Conditions

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Abstract: The European ground squirrel *Spermophilus citellus* is listed as "Endangered" on the IUCN Red List. Around the world, the species is bred in zoos to improve its conservation status. Until now, sousliks have not been bred in zoos in Bulgaria, despite this goal being included in the Action Plan for the Conservation of the European Ground Squirrel (*Spermophilus citellus*) in Bulgaria (2023-2031). We present the pilot activities for souslik husbandry at Sofia Zoo. Seven individuals were captured during a rescue operation in the area of Pazardzhik, within the Natura 2000 site "Reka Maritsa". The animals were placed in the quarantine facility of Sofia Zoo. During the first week, their behaviour was recorded with video cameras. Two behavioural tests – the open field test and novel object exploration test were used to assess their locomotor and exploratory behaviour, as well as risk-taking in response to novelty, based on the recorded video. Two female sousliks were selected based on their behaviour to be kept in the Zoo's exhibition area, while the others were released into a suitable habitat. The initial results show that the sousliks build nests from the provided hay and spend more time inside them. In the open field test, the sousliks spent significantly more time in the peripheral zone of the experimental arena than in the centre. In the novel object exploration test, only one female and one male approached the object and made contact with the object. The two individuals that remained in the exhibition enclosure showed an afternoon peak in activity during August, which corresponds to observations from other regions in Bulgaria. In conclusion, this is the first study to apply the open field and novel object exploration tests with *S. citellus*, and sousliks have been housed in captivity and displayed to the public in Bulgaria. This is an important step toward establishing captive breeding for conservation purposes.

Key words: husbandry, novel object exploration test, open field test, souslik

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Introduction

The European ground squirrel *Spermophilus citellus* (Linnaeus, 1766) is a diurnal rodent inhabiting open, uncultivated, treeless habitats in Central Europe and the Balkan Peninsula. It lives in colonies (Millesi et al. 1999, Koshev 2010), in underground burrow systems that it excavates itself. Each adult individual occupies its own burrow, which has a complex structure consisting of an entrance, tunnels, nesting chambers, galleries, and an emergency exit. Chernozemic soils, but also some shallow soils like leptosols and regosols, are preferred by the species, as long as these are loosely compacted to allow easy excavation of burrows and the underlying rock formations (most often limestone) have good drainage properties (Koshev & Kocheva 2008, Martinková et al. 2026). The European ground squirrel is primarily herbivorous, feeding on all parts of herbaceous plants, as well as seeds and bulbs. However, it also consumes food of animal origin, such as insects, worms, eggs, and sometimes even small birds (Straka 1961, Herzig-Straschil 1976, Kachamakova et al. 2022). Rare cases of food storing for winter have been documented (Hristov 1974, Koshev 2022, Hoffmann & Haberl 2023).

European ground squirrels are true hibernators and remain underground from August or September until March (Millesi et al. 1999, Koshev & Kocheva 2008). The duration of hibernation ranges from 180 to 240 days (Matějů 2008). At the southern edge of the species' range, females typically emerge from hibernation around March 22 (± 9 days), while males emerge around March 9 (± 8 days). Entry into hibernation may occur as early as July 21 (± 6 days) for females and August 6 (± 10 days) for males (Youlatos et al. 2007). During warmer winters, individuals may emerge earlier, even in mid-February (Straka 1961). Populations in Northern Greece (Youlatos et al. 2007), Bulgaria, North Macedonia, and European Turkey (Kryštufek & Vohralík 2005, Özkurt et al. 2005) enter hibernation earlier than those in Central Europe (Millesi et al. 1999), likely due to higher ambient temperatures, which make food harder to find and reduce its water content (Schwanz 2006). Females hibernate longer and lose more body mass than males (Koshev 2022). In the lowland regions of Bulgaria, emergence from hibernation occurs between March 16 and April 4 (Straka 1961). The maximum hibernation duration in Bulgaria is 7-8 months, recorded in the area of Belmeken Peak in the Rila Mountains (Koshev 2022).

The daily aboveground activity of ground squirrels in their natural habitats is influenced by

photoperiod, temperature, humidity, cloudiness, and the reproductive status of individuals (Everts et al. 2004, Koshev & Kocheva 2008). In Hungary, peak activity occurs in the morning, followed by a midday rest and lower activity in the afternoon (Katona et al. 2002). In northwestern Bulgaria, however, a higher activity peak is observed in the afternoon (Koshev & Kocheva 2008). During summer, individuals typically emerge about two hours after sunrise, with an active period lasting around 11 hours per day. In autumn, activity begins about four hours after sunrise and lasts approximately seven hours. The strongest influence on activity patterns is the time of the year, as it is linked to the successive stages of the species' yearly activity (Koshev & Kocheva 2008).

The European ground squirrel is classified as "Endangered" on the IUCN Red List (Ćosić et al. 2024), which necessitates multiple conservation measures across its range. As mentioned by Bárdos et al. (2024), its protection cannot be ensured solely by protecting its habitat. It is also necessary to protect the animals *ex situ*. The species is bred in zoos in Poland, Switzerland, Hungary, Ukraine, Germany, Austria, the Czech Republic, the United Kingdom, and at the zoological laboratory in Haren (the Netherlands), among others (Górecki et al. 2012, Koshev 2022, Schneiderová et al. 2026, Nazarizadeh et al. 2026, Frascchetti et al. 2026). In the Poznań zoo, Poland, behavioural responses of European ground squirrels to visitor presence and varying noise levels have been studied (Górecki et al. 2012). These findings are applied in decision-making for reintroduction programmes (Schneiderová et al. 2026). In addition to zoo settings, the species has also been studied under laboratory conditions (Millesi et al. 2001, Németh et al. 2010). Gedeon et al. (2010) investigated the functional significance of nest material selection and preferences in relation to nest insulation. They found that fresh fescue grass (*Festuca* spp.) is the most preferred material due to its superior insulating properties. Laboratory studies have also examined the effects of hibernation on memory by testing spatial memory in mazes and operant task performance using feeding devices (Millesi et al. 2001). Compared to females, males are more likely to take risks and expose themselves to predators when farther from their burrows (Hoffmann et al. 2004). Based on the available information, it can be said that no studies have been conducted on the diurnal activity and behaviour of the European ground squirrel in zoos in the southern part of its range, which includes Bulgaria, and knowledge about its needs and behaviour is essential for captive housing.

Some of the most commonly used ethological tests for studying rodent behaviour are the open field test and the novel object exploration test. The open field test is used to analyse locomotor activity, as well as exploratory and emotional behaviour in rodents (Hall 1934, Walsh & Cummins 1976, Prut & Belzung 2003, Tatem et al. 2014, Simeonovska-Nikolova 2016). This test is based on the concept that most rodents avoid brightly lit, open areas (Hall 1934, Prut & Belzung 2003). As mentioned by Prut & Belzung (2003), the open field test is now one of the most popular procedures in animal psychology. Different versions are available, differing in the shape of the environment (circular, square or rectangular), lighting, presence of objects within the arena such as platforms, columns, tunnels, etc. The procedure usually involves forced confrontation of a rodent with the situation. The animal is usually placed in the centre or close to the walls of the apparatus, and behaviours like locomotion, rearing, grooming etc. are recorded for a period ranging from 2 to 20 min (usually 5 min). In such a situation, rodents spontaneously prefer the periphery of the apparatus to activity in the central parts of the open field. Behavioural differences observed in this test across rodent species are interpreted in the context of their ecological strategies and adaptations (Frynta 1994, Simeonovska-Nikolova 2000). Using the open field test, Semenova et al. (2001) found that locomotor and exploratory activity in the long-tailed ground squirrel (*Citellus undulatus*) is higher in summer and decreases during autumn before hibernation. The novel object exploration test is an increasingly popular experimental procedure in the behavioural sciences. Behavioural endpoints such as general activity, reactivity to novelty, and learning can be rapidly obtained (Heyser & Chemero 2012). Similar to the open field test, it is used to assess exploratory behaviour and is based on rodents' natural tendency to investigate unfamiliar objects, much like their tendency to explore new environments. This test has been used to study the relationship between age and risk-taking behaviour in wild rodents (Lafaille & Féron 2014). Although the open field test and object exploration tests are widely established to measure locomotor activity, exploration, and anxiety-like tendencies, increasingly, conservation scientists map these assays to animal personality frameworks (such as boldness and exploratory tendencies) to predict post-release survival and habitat integration in reintroduction programs (Allard et al. 2019).

The European ground squirrel is not well known to the general public in Bulgaria, and especially to residents of large cities, even though it is a

diurnal member of our fauna. The Action Plan for the Conservation of the European Ground Squirrel (*Spermophilus citellus*) in Bulgaria 2022-2031, approved by the Ministry of Environment and Water on June 17, 2022 (Koshev 2022), states that this attractive species can be bred and reared in zoos in Bulgaria. Animals bred in captivity can be reintroduced into areas where the species has become extinct. Reviews in conservation science explicitly advocate for incorporating standardised behavioural tests like the novel object test and open field paradigms into captive breeding protocols. Profiling stress resilience, risk assessment, and exploratory drive helps managers filter or train candidates, thereby optimising the phenotypic quality of cohorts destined for endangered species restoration (de Azevedo & Young 2021). In this context, the present study aims to investigate the behaviour and activity of the European ground squirrel under zoo conditions in order to expand knowledge on its husbandry and its application in conservation programs.

Materials and Methods

Animals studied and housing conditions at the time of accommodation

The study on the activity and behaviour of the European ground squirrel at Sofia Zoo was conducted between April 2021 and July 2024. Additionally, it was noted when the animals entered hibernation in August 2024. Observations were carried out in three settings: (1) in the Zoo's quarantine unit immediately after the animals' arrival, (2) during laboratory tests (open field test and novel object exploration test), and (3) in the exhibition enclosure.

A total of seven adult, sexually mature individuals were included in the study – six females and one male. The females weighed between 136 and 236 g, while the male weighed 274 g. The number of individuals included varied across the different observation settings.

The ground squirrels were wild-caught using live traps from a colony threatened by urban development in the town of Pazardzhik (42.187131, 24.311212) in Natura 2000 site BG0000578 "Reka Maritsa". Upon capture, the animals were measured (head, ear and foot length (mm) and weighed (g)), examined by a veterinarian, and treated with Ivermectin against internal and external parasites. According to the regulations of Sofia Zoo's quarantine unit, the animals were housed in a specially designated room. Each individual was placed in a separate metal rodent cage (Fig. 1) in a room with natural daylight and a temperature ranging between

17°C and 25°C. The bedding in the cages consisted of wood shavings and nesting material–hay–as well as cardboard shelters. Food was offered ad libitum in a metal bowl and consisted of seeds (wheat, sunflower), dandelion leaves, cucumber, zucchini, carrot, and apple. Since the species also consumes animal-based food in the wild, boiled egg was included in the diet once per week. Water was provided via rodent drinking bottles attached to the cages. The animals were handled only by members of the research team once daily for feeding and for initiating video recording of behaviour.

Behaviour and activity of the ground squirrels upon housing in the quarantine unit

During the first 7 days of their stay in the quarantine unit, three of the female ground squirrels (FM1, FM2, FM3) were housed in separate observation cages with a glass front panel and the possibility of mounting video cameras on the top of the cage. This setup allowed monitoring of their behaviour during the adaptation period and in the absence of human presence. The animals were recorded in their cages for 1 hour per day between 10:00 and 12:00 using an ACME VR06-4K Ultra HD camera. A total of 1 260 minutes of video recordings were collected.

Behaviour and activity of the ground squirrels in the open field test and the novel object exploration test

After the seventh day of the ground squirrels' stay in the quarantine unit, two ethological tests were con-

ducted with all seven individuals: the open field test and the novel object exploration test. The tests were scheduled according to the animals' activity patterns and were carried out in the morning between 10:00 and 12:00. The two tests were conducted on separate days, with the open field test always performed before the novel object exploration test.

Open field test. For the purposes of the test, a specialised grey opaque plexiglass square experimental arena with dimensions of 100 × 100 cm and a height of 50 cm was used (Fig. 2A). The arena was conditionally divided into the following sections (Fig. 2B):

- “Arena” – representing the total area of the experimental arena (1 m²)
- “Central zone” – the area located in the mid-



Fig. 1. European ground squirrel in a metal rodent cage in the quarantine unit of Sofia Zoo.

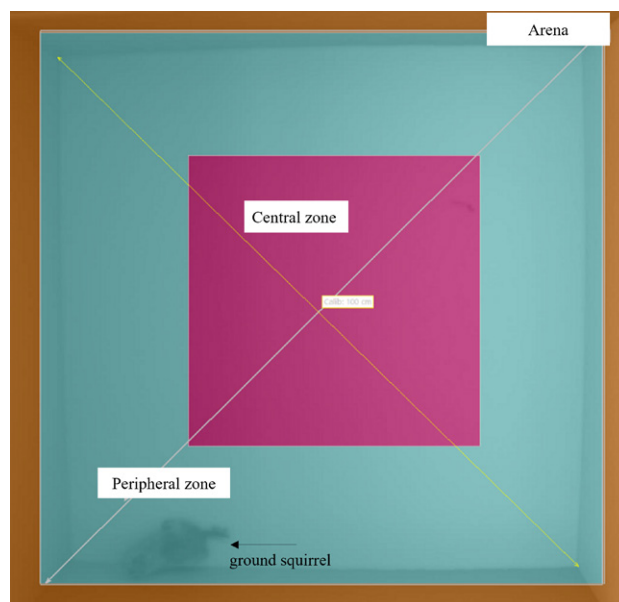


Fig. 2. Open field arena. A) A moment during the open field test. B) Division of the experimental arena into a “Central zone” and a “Peripheral zone”, which are located within the “Arena” area when configuring the open field test settings using EthoVision XT13 software.

dle of the arena, measuring 40×40 cm (1600 cm²). The dimensions of the central zone were defined in EthoVision XT, following the configuration used by Gajdošová et al. (2024).

- “Peripheral zone” – the area situated between the central zone and the outer boundaries of the arena (8400 cm²)

According to the common procedure, each individual was placed in the centre of the empty arena and allowed to explore the unfamiliar environment for 5 minutes (Prut & Belzung 2003). During the open field test, the following parameters of the software were included in the analysis:

- distance moved by the animal in the arena
- distance moved in the central zone
- velocity in the arena
- velocity in the central zone
- mobility in the arena
- mobility in the central zone
- immobility in the arena
- immobility in the central zone
- duration of time spent in the central zone
- duration of time spent in the peripheral zone

To compare the variables “distance travelled” and “velocity,” “mobility” (the movement detected within the observation area based on changes in the animal’s position, including locomotion, jumping, rearing, and other movements) and “immobility” (defined as the animal not moving), as well as “duration of time spent in the central zone” and “duration of time spent in the peripheral zone,” a paired t-test was applied at a significance level of $p < 0.05$. The normality of the paired differences was assessed using the Shapiro-Wilk test, and normality was confirmed for all variables. Comparisons were made between the distance travelled in the arena and in the central zone, the velocity in the arena and in the central zone, the mobility in the arena and in the central zone, the immobility of the animals in the arena and in the central zone, as well as the duration of time spent by the animals in the central zone and in the peripheral zone.

Novel object exploration test. This test was conducted in the same experimental arena used for the open field test, but on the following day. Like the open field test, the animals were placed in the empty arena for 5 minutes to habituate to the environment (training phase), after which they were briefly removed so that the novel object could be introduced without being seen. The ground squirrels were then returned to the arena, and their behaviour was video recorded. The novel object consisted of a green rubber rodent-shaped figure, 10 cm in length. It was selected in accordance with the size of the ground

squirrels and due to its relatively natural shape. Studies in mice and rats have demonstrated that rodents possess colour vision, with enhanced sensitivity to the green part of the spectrum. The colour green plays an important role for rodents in urine marking, because they can identify the marks visually, seeing them in green thanks to the UV-green colour channel of the retina (Joesch & Meister 2016).

During the analysis of the novel object exploration test, the following variables were considered:

- latency to first movement (the duration in seconds from placing the animal in the arena until its first movement)
- latency to approach the object (the duration in seconds from placing the animal in the arena until its first approach to the object)

After each experiment in both behavioural tests, the experimental arena was cleaned with 70% ethyl alcohol to remove any odours left by the tested individual. The behaviour of the animals in both tests was video-recorded using a specially mounted Basler acA1920-155um USB 3.0 camera (164 frames per second and 2.3 MP resolution), connected to a computer. Behavioural analysis was carried out using the specialised software EthoVision XT13 with the Multiple Body Module and Social Interactions Module, developed by Noldus Information Technology. The software allows tracking of movement (Track Visualisation option) and location within the experimental arena (Heatmap Visualisation option, Fig. 3) for each individual. Various observed behaviours, such as rearing, self-grooming, jumping, defecation, and others, were coded and annotated during the analysis of the recordings.

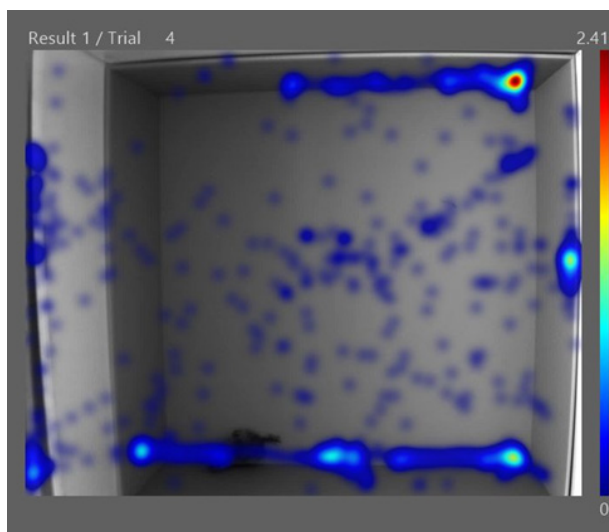


Fig. 3. Tracking of the individuals’ location within the arena using EthoVision XT (Heatmap Visualisation option).

Behaviour and activity of the ground squirrels in the exhibition enclosure

For long-term monitoring of behaviour and activity, two female ground squirrels, who showed greater activity and boldness in behavioural tests, were transferred to a specially designed exhibition enclosure. The number of individuals in the group was consistent with the ecological requirements of the species and the dimensions of the enclosure. The enclosure measured 2.5×2.5 m with a height of 4 m and was filled with chernozemic soil to a depth of 1.5 m. To allow observation of part of the underground tunnels excavated by the animals, three windows made of tempered glass were installed in the outer walls of the enclosure. Grass was planted in advance, shelters were created from logs and ceramic pipes, and dry hay mix was provided as nesting material. The hay originated from high-mountain pastures and had a heterogeneous composition dominated by *Poa* spp., *Festuca* spp. Food and water were supplied once a day through a special hatch in the enclosure wall. The animals' diet included seeds (sunflower and wheat), carrot, cucumber, courgette, apple, willow branches, dandelion leaves and flowers, grass, and boiled egg once a week. The behaviour and activity of the two female ground squirrels housed in the enclosure were monitored using a Game/Surveillance Camera WK 8A1 (Video: 1920×1080 px; Photo: 8-16 MP), mounted on the inner side of the enclosure. The analysis of image and video data obtained from the camera was performed using Timelapse Image Analyzer 2.0. For each frame, the date, time, temperature, lunar phase, number of individuals in the frame, and, in some cases, notes on observed behaviour were recorded. The observation period extended from 2021 to 2024 (Fig. 4).



Fig. 4. Photo by the camera trap installed in the enclosure, showing the two ground squirrels.

Results

Behaviour and activity of the ground squirrels upon housing in the quarantine unit.

From the very first days after being housed in the quarantine unit, the animals were active – they were feeding, attempting to climb the walls of the cages, and digging, mainly in the corners. They were also jumping and building nests from the hay provided. The animals spent varying amounts of time feeding, staying immobile, and attempting to dig and climb (Fig. 5). The behaviour with the longest duration observed in the three female ground squirrels was locomotion within the cage, followed by staying in the shelter.

The results showed that differences were also observed between individuals. Active behaviours predominated in the first two female ground squirrels, whereas passive behaviours predominated in the third female (Fig. 6).

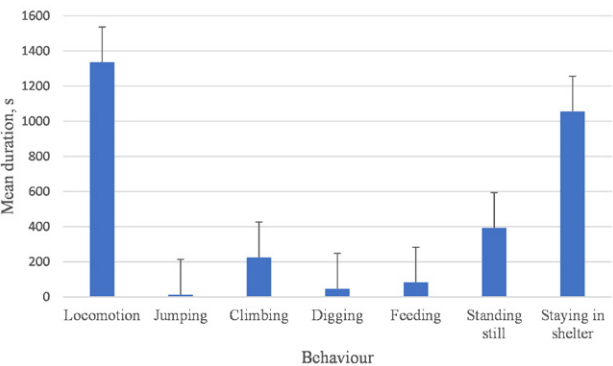


Fig. 5. Mean duration (s) of behaviours exhibited by the three female ground squirrels in the quarantine enclosure (mean $\pm$ SD).

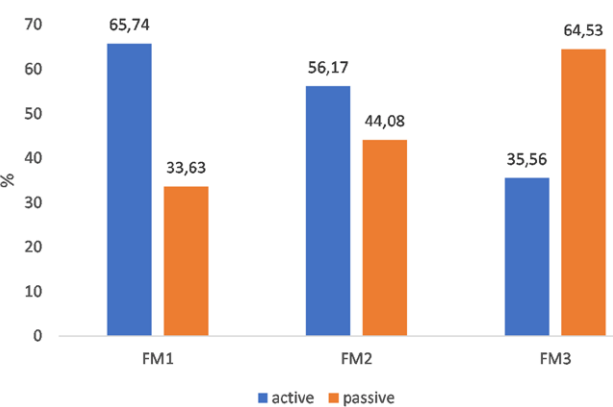


Fig. 6. Comparison of active behaviours- locomotion, jumping, climbing, digging, feeding, and passive behaviours – standing still and staying in shelter (%) in the three observed female ground squirrels (FM1, FM2, and FM3).

Table 1. Mean values (Mean) and standard error (SE) of the investigated variables in the open field test for the seven individuals. The results of the paired t-test are presented. A significance level of $p < 0.05$ in the paired t-test is indicated in bold. Abbreviations: A – arena; CZ – central zone; PZ – peripheral zone.

	Distance moved (cm)		Velocity (cm/s)		Mobility (s)		Immobility (s)		Duration of time spent in CZ (s)		Duration of time spent in PZ (s)	
	A	CZ	A	CZ	A	CZ	A	CZ	CZ	PZ	CZ	PZ
	9641,12 ±1124,17	2513,98 ±315,64	127,17±20,01	468,40±75,18	3,25±0,34	0,35±0,10	72,59±9,76	3,85±1,48	7,14±1,62	56,77±9,03		
	$t = -7.79$, $p = 0.00023$		$t = 5.45$, $p = 0.00159$	$t = -10.56$, $p = 0.00004$	$t = -7.86$, $p = 0.00022$	$t = -6.32$, $p = 0.00073$						

Behaviour and activity of the ground squirrels in the open field test and the novel object exploration test.

Open field test. During the open field test, the following behaviours were observed in the seven studied ground squirrels: jumping, rearing, locomotion or standing still in the peripheral zone of the arena and crossing the centre, vocalisations, defecation, and urination. A statistically significant difference was found in the total distance moved in the arena compared to the distance moved in the central zone ($t = -7.79$, $p = 0.00023$), in velocity between the arena and the central zone ($t = 5.45$, $p = 0.00159$), and in the duration of time spent in the central and peripheral zones ($t = -6.32$, $p = 0.0007$) (Table 1). No statistically significant difference was observed between the mobility and immobility of the animals in the central zone ($t = 2.42$, $p = 0.052$).

Novel object exploration test. During this test, the ground squirrels exhibited the following behaviours: standing still, jumping, attempting to escape from the arena, approaching the object, and contact with the object, as well as vocalisations, defecation, and urination. The animals displayed caution towards the novel object. The latency to first movement in all animals ranged from 0 to 245 s. Five of the females did not approach the object at all. Only the male and one female approached and interacted with it through sniffing and physical contact – latency to approach for male 220 s; latency to approach for female 285 s.

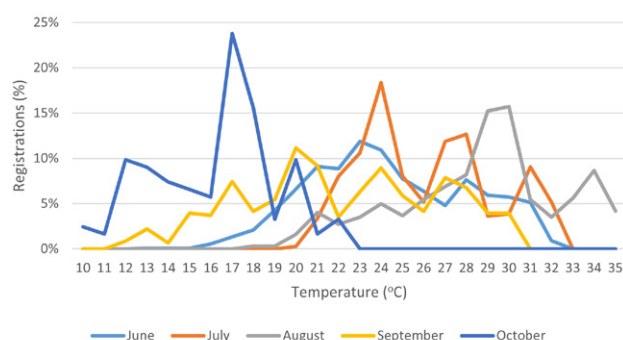
Following the completion of observations in the quarantine unit and in the two ethological tests, two female ground squirrels were selected from the seven studied individuals for housing in an exhibition enclosure.

Behaviour and activity of the ground squirrels in the exhibition enclosure.

The two selected female ground squirrels (FM1 and FM2) actively explored their new enclosure—they climbed the mesh up to the roof, moved along the corners, sniffed, and began digging burrows in the soil. They excavated three burrows as far as possible from the mesh and the outer boundaries of the enclosure. The animals began carrying hay into the burrows with their mouths on the sixth day after their introduction. They frequently adopted the typical upright “sentinel” posture to survey their surroundings, and alarm calls were heard relatively rarely. When people approached from outside the enclosure, when the door was opened for handling the camera trap, or during feeding, the animals retreated into their burrows. They continued to hide in their

Table 2. Earliest and latest registration of daily activity during the months; mean, maximum, and minimum temperatures during the period June–October 2021.

Month	Earliest registration of daily activity	Latest registration of daily activity	Temperature, °C (mean, min-max)
June	09:19	21:10	24,4°C (13°C – 32°C)
July	09:22	19:28	26,1 °C (20°C – 32°C)
August	09:09	20:09	28,6 °C (18°C -35°C)
September	09:29	17:11	22,1°C (12°C – 30°C)
October	10:20	17:50	16,2°C (10°C – 22°C)

**Fig. 7.** Activity of the two ground squirrels in the exhibition enclosure in relation to ambient temperature, recorded by the camera trap during the period June–October 2021.**Table 3.** Seasonal activity of the ground squirrels in the exhibition enclosure for 2021 – 2024.

Year	Date of emergence	Date of entry into hibernation
2021	-	10.10.2021
2022	31.03.2022	10.09.2022
2023	17.03.2023	16.06.2023
2024	03.03.2024	11.08.2024

burrows when disturbed throughout the observation period. They were observed drinking water from a shallow metal bowl, which was consistently kept filled. During feeding, they showed a preference for dandelion leaves and flowers. The two animals were active over the longest time intervals in June and August 2021 (Table 2). The relationship between daily activity and ambient temperature is presented in Fig. 7.

The two female ground squirrels hibernated during the winter periods of 2021/22, 2022/23 and 2023/24. The dates of entry into hibernation and emergence, as determined from the camera trap recordings, are presented in Table 3.

Discussion

For rare and endangered animal species, behavioural observation plays a crucial role in conservation practices. The observations suggest that the ground squirrels were able to acclimatise to the captive conditions and exhibited behaviour like that observed in their natural environment. During the open field test, all animals spent significantly more time in the peripheral zone of the experimental arena than in the central zone. In addition, their velocity was significantly higher when crossing the central zone compared to their movement within the arena as a whole. These findings confirm that ground squirrels, like most rodents, are cautious and prefer the peripheral rather than the central zone of the experimental arena (Walsh & Cummins 1976, Lafaille & Féron 2014, Sturman et al. 2018, Kraeuter et al. 2019). Expressions of anxiety in rodents are often accompanied by reduced exploratory behaviour, seeking shelter, escape, burying, or defecation (Bailey & Crawley 2009, Simeonovska-Nikolova 2000). Many species avoid open microhabitats, which are perceived as areas of high predation risk (Dickman 1992, Jacob & Brown 2000, Mandelik et al. 2003, Fanson et al. 2010). Furthermore, reduced locomotor activity and immobility are among the typical anti-predator behavioural responses in rodents (Dielenberg et al. 2001, Edut & Eilam 2003, Taraborelli et al. 2008), and these were also observed in the studied ground squirrels. In both tests, all animals spent part of the time remaining immobile in one of the corners of the arena. Nevertheless, inter-individual differences in behaviour were observed. The results showed that only one female and the male approached and interacted with the novel object, whereas another female did not leave the corner it had occupied and showed no response to the object. Differences in individual behavioural traits, particularly traits such as boldness and shyness, may be of considerable importance when making decisions about the inclusion of

individuals in reintroduction programmes. According to some authors, a greater exploratory activity may indicate higher ecological plasticity and better adaptive capacity (Richard et al. 2008, Herborn et al. 2010, Allard et al. 2019). Thus, in a study on zoo-hatched Blanding's turtles (*Emydoidea blandingii*), a modified open-field test was used to score individual exploration and boldness prior to release. The analysis mapped individual phenotypic variation directly to post-release monitoring, demonstrating that individuals with higher exploration scores in structured arenas experienced significantly higher multi-year survival rates in the wild (Allard et al. 2019). In this context, the more active and bolder female individuals in the ethological tests were selected for housing in the exhibition enclosure. As in many other animal species, male ground squirrels tend to take greater risks compared to females (Hoffmann et al. 2004). During the novel object exploration test, the male individual also displayed higher exploratory activity. The application of the open field and novel object tests in the present study demonstrated their potential as tools for selecting animals with specific individual traits for reintroduction programmes.

During observations in the exhibition enclosure at the Sofia Zoo, the two female ground squirrels successfully acclimatised and socialised after their release. In the absence of people, the ground squirrels were more active, which has also been observed in zoos (Górecki et al. 2012). They began digging burrows from the first day, and by the sixth day were already using three entrances, which is consistent with their behaviour in the wild. In colonies within natural habitats, ground squirrels inhabit individual burrows up to 2 m deep (Ružić 1978), where they hide, rest, reproduce, and hibernate (Kachamakova et al. 2019). The system of underground tunnels typically consists of a nesting chamber and a second chamber used as a latrine (Ružić 1978, Hoffmann & Haberl 2023). In the exhibition enclosure, the two females, likely due to limited space, occupied the same tunnel system; however, it remains unclear whether each individual maintained a separate nesting chamber for resting and hibernation. Cases have been reported in which two females, most often sisters, may share a burrow and even hibernate together (Matějů 2008). Between hay and newly grown grass (ryegrass) in the enclosure, the animals showed a clear preference for hay as nesting material, carrying it into the burrows with their mouths. Similar results were reported by Bárdos et al. (2024). However, other researchers, such as Gedeon et al. (2010), found that animals preferred fresh *Festuca pseudovina* over

dry material. In the present study, the hay provided in the enclosure originated from high-mountain pastures and had a heterogeneous composition. The animals not only transported it into the burrows but were also observed eating it. Bárdos et al. (2024) also reported that, among three types of food – commercial rabbit food, complete rabbit food and natural feed mixture – the animals preferred the grain feed mixture. The authors suggest that this preference may be due to its similarity to natural diet composition and the presence of animal protein. In addition, in the husbandry of spotted ground squirrels (*Spermophilus suslicus*) under outdoor aviary conditions for breeding and subsequent reintroduction, animals were also observed to use hay composed of fine-stem grasses as nesting material, filling the entire nesting chamber with it (Sapelnikov & Sapelnikova 2021). Considering all these studies, it may be suggested that under aviary conditions, preferences for nesting material are determined not only by its type but also by its availability and quantity. For instance, studying the species' diet and seasonal changes in food consumption of European Ground Squirrels in an anthropogenic area of Central Macedonia, Greece (Klagkou et al. 2025) revealed that *Cynodon*, *Carex*, *Cyperus*, *Salvia*, *Solanum*, and *Plantago* were the dominant plant genera in the species' diet. Rhizomes and leaves were the most frequently consumed plant parts. The authors found seasonal dietary shifts, with ground squirrels selecting specific plants and plant parts based on availability. Under resource-limited conditions, they adapted by consuming nutritious rhizomes or the less common, but toxic, *S. elaeagnifolium*. In the present study, the ground squirrels showed the strongest preference for dandelion leaves and flowers. It is well established that ground squirrels feed on the green parts of plants throughout their active season, which may fully satisfy their water requirements (Straka 1959, 1961, Koshev 2022). From the camera trap recordings, the animals were repeatedly observed drinking water from a metal bowl provided during the summer months. It is generally recommended that fruits and vegetables be consistently included in the animals' diet as a source of water. Although the animals in the present study had daily access to fresh fruits and vegetables, they were occasionally observed drinking water, which may be attributed to the high temperatures during the summer months and the relatively dry environment in which they were kept. Furthermore, in their natural habitats, ground squirrels have also been observed drinking water from puddles and natural water sources (Wade 1930).

Ambient temperature has a significant influence on the daily activity of ground squirrels (Váczí et al. 2006). Within their system of underground tunnels, ground squirrels can avoid extreme high or low temperatures by selecting the more stable and favourable thermal conditions inside their burrows (Hut et al. 1999). Data from the camera trap in the enclosure showed that in 2021, the animals were most active (i.e. exhibited the longest daily activity intervals) in June at temperatures between 16 and 19 °C, in July between 20 and 26 °C, and in August between 27 and 31 °C (Fig. 7). This is likely related to the longer daylight periods and higher temperatures during these months. These data are difficult to compare directly with field studies, as the two individuals were maintained under semi-natural conditions in a covered enclosure, which limited direct solar exposure across the entire area. Nevertheless, the observed activity patterns correspond to those reported by Koshev & Kocheva (2008) for the daily above-ground activity of a free-living colony. Fraňová (2013) also demonstrated that captive ground squirrels exhibit activity patterns similar to those in the wild, typically showing two daily peaks: the first between 09:00 and 11:00 and the second between 14:30 and 17:00. During the summer of 2023, temperatures were unusually high. Mean monthly temperatures in July ranged between 20 and 28 °C (with deviations of +0.5 to +3.5 °C above the monthly norm). Moreover, July 2023 was warmer than July 2022 and ranked among the three warmest Julys in the past 25 years (National Institute of Meteorology and Hydrology, 2023). The disappearance of the animals in mid-July 2023, therefore, suggests that they may have an alteration of their endogenous circannual rhythm (MacCannell & Staples 2021). Although camera trap monitoring continued until the end of October 2023, the animals were not observed again. It is possible that the summer torpor transitioned into winter hibernation. Overall, the results indicate that the captive ground squirrels entered and emerged from hibernation at approximately the same periods of the year as wild populations at similar latitudes (Koshev & Kocheva 2008). Climate change factors, including droughts, floods, severe storms, and rising temperatures in recent decades, are among the main threats to the declining populations of the European ground squirrel (Zidarova & Popov 2022, Čosić et al. 2024). In conclusion, despite the small sample, ground squirrels have been housed in captivity and exhibited to the public in Bulgaria for the first time, representing an important step towards the development of captive breeding programmes for conservation purposes. Further research is needed to deepen behavioural

studies on the European ground squirrel and shed more light on its personality characteristics, which would be important in conservation practice.

Ethics in research. The experiments were conducted in a manner that did not cause distress or injury to the animals, in accordance with ethical guidelines for working with animals. During the capture, transport, and housing of the ground squirrels, modern professional equipment was used, and all necessary precautions were taken to ensure the animals' health and welfare.

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