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Establishing and Maintaining an Etruscan Shrew Colony

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The Etruscan shrew (*Suncus etruscus*) is one of the smallest mammals on earth and is used in many fields of research, including physiology, behavioral science and neuroscience. However, establishing and maintaining a breeding colony of this species in the laboratory can be challenging, as it requires specific husbandry conditions that greatly differ from those of more common laboratory species such as mice or rats. Over the past 15 y, we have successfully established a long-term thriving colony of 150 to 200 animals originating from 36 founders. The colony shows longer life expectancy and larger litter sizes than wild conspecifics. Breeding occurs year-round, independent of seasons, and a breeding pair can regularly produce 2 to 6 offspring with an average life expectancy of more than 3 y. The shrews are housed in glass or plastic enclosures on a specific soil-sand-mixture bedding and are provided with hideouts and nesting material consisting of moss, wood, or bark. Due to their high basal metabolic rate, the shrews require food intake greater than their body weight per day, can hunt arthropods as large as themselves, and cannot survive more than a few hours without food. Live feed such as crickets or mealworms is crucial and must be provided daily or, at the very least, every 2 d. Although our husbandry practices have constantly been adapted and refined, shrew husbandry remains challenging, and great care is necessary to meet the specific needs of this species. Here, we describe the establishment of a long-term stable colony of Etruscan shrews in a research animal facility and the specific husbandry requirements for animal wellbeing.

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The Etruscan shrew *Suncus etruscus* (SAVI 1822), also known as the white-toothed dwarf shrew, is a member of the shrew family (*Soricidae*) in the insectivorous order (*Eulipotyphla*).[5] *Suncus etruscus* occur in regions of southern Europe such as the Apennines, Balkans, and Iberian Peninsula and also in parts of North Africa and Asia[27,33] and live very seclusively in richly structured biotopes.[5,29] Together with the bumblebee bat (*Craseonycteris thonglongyai*) from Thailand, Etruscan shrews are the smallest known mammalian species. The shrews (Figure 1) are only 2 to 3 grams (g) in weight[18,24,29] and their body measures around 3 to 4 centimeters (cm) excluding the tail.[29] The shrews must feed up to 25 times per day, with daily consumption of more than their body weight in food. The heart, respiratory system, and skeletal muscles of this species are functionally and structurally adapted to meet the enormous metabolic needs,[19,29] as demonstrated by their heart or respiratory rate of up to 1500 or 900 beats or breaths per min, respectively.[19] In resting condition and at room temperature, their oxygen consumption rate is 67 times higher than that of humans.[18] These extraordinary metabolic characteristics render the shrew particularly interesting for research. In addition, Etruscan shrews display seasonal variations in cell architecture and neuronal activity.[28] Therefore, they are of particular interest to the field of neuroscience.[4,5,25,26,28] For example, a shrew's tactile object recognition shares many characteristics of human visual object recognition, although proceeding much faster and in a brain 20,000 times smaller.[2] However, the unique physiologic features of the Etruscan shrew also pose challenges regarding colony establishment and long-term animal husbandry. Previously published reports on husbandry conditions, however, only covered brief periods of time.[14,15,32] Furthermore, breeding shrews is subject to special requirements.[14] Etruscan shrews are monogamous breeders and require specific breeding conditions such as the provision of cave stones[27,32] or temperature and light cycles.[4,27] They have a short gestation period of 11 wk and produce 2 to 6 young per litter.[4,27] As an exception in the genus of *Suncus*, the Etruscan shrew lives in family groups.[15]

To the authors' knowledge, current and detailed literature on successful long-term husbandry and breeding of Etruscan shrews, especially under laboratory conditions, is lacking. Therefore, this manuscript presents an overview of the management strategy we have used for successful breeding, husbandry, and wellbeing of these shrews in a colony that has been maintained for over 15 y.

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Materials and Methods

Animal Husbandry. ***Origin of colony.*** The Etruscan shrews (Figure 1) of this colony originate from a breeding group established at Erasmus University Rotterdam (Netherlands), which had initially captured 36 animals (20 male, 16 female) in various regions of north-central Italy (San Marino, Castagneto Carducci and Cecina, Italy) with official permission in 2005 and 2006 (permit no. N 6085T-A31, Firenze, Italy). The shrews were acquired from Rotterdam in 2006 and have since been

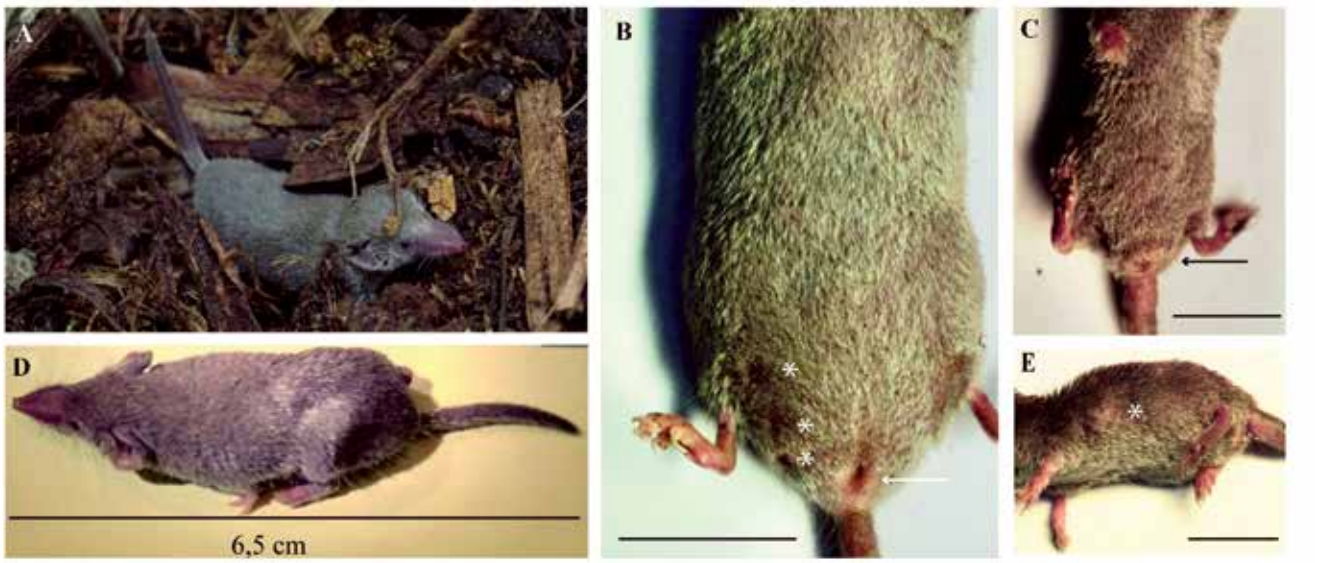

Figure 1. Images of *Suncus etruscus*. A) Female Etruscan shrew in one of our housing devices. Please note the use of bark enrichment for hiding. B) Gender determination of a female Etruscan shrew. Please note the flat anus close to the tail base (white arrow) and the 3 teats on each side of the abdomen between the hind leg and anus (white asterisks). Scale bar 1 cm. C and E) Gender determination of a male Etruscan shrew. C) A male Etruscan shrew can be distinguished by the slightly visible internal testicles posterior of the anus (black arrow). Scale bar 1 cm. D) Typical size of an Etruscan shrew. Scale bar 6,5 cm. E) Especially notable are the male-specific lateral glands (white asterisk) in the region of the lateral thorax, the greasy secretion of which is responsible for the distinct smell of male Etruscan shrews. Scale bar 1 cm.

successfully bred at Humboldt University zu Berlin, with a current population of about 153 animals. Since their capture in Italy, no other shrews from the wild or other colonies have been introduced into the existing breeding group. Accurate breeding planning and documentation are necessary to avoid mating of closely related animals. To our knowledge, the colony at Humboldt University zu Berlin is one of the few colonies maintained in German research facilities.

Animal facility. Breeding and husbandry of shrews at Humboldt University zu Berlin has been approved by the local governmental authorities (ZH 149, State Office for Health and Social Affairs, Berlin, Germany) and follows local, national, and international guidelines and regulations, including the European Directive 2010/63 (2010/63/EU, available at https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32010L0063&from=EN).

Housing, substrate, and enrichment. Shrews communicate via ultrasonic vocalization which at the lower end of the spectrum can be perceived by humans as chirping sounds.[5] Therefore, artificial ultrasonic sources (e.g., fume hoods, humidification vaporizers, centrifuges, radio transmitters or receivers, portable CD players) and the presence of other animal species that communicate in the ultrasonic range (e.g., rats) must be excluded from husbandry units that house shrews.

The shrews are housed in either light-colored, slightly transparent plastic containers (Figure 2, Clipbox, 38 L., Heidrun, Europlastic, Italy; minimum dimensions 52 × 37 × 27 cm) or glass terrariums (Figure 3, minimum dimensions 42 × 60 × 38 cm). The minimum floor area for a breeding pair should be 1800 cm^2. In addition, the cage should have sufficient height (based on our experience, at least 25 cm) to insert the labyrinth stone (Figure 4). As shrews are very good jumpers and climbers, plastic containers or glass terrariums must be equipped with an entirely shrew-proof cover such as a plastic lid containing an area of tight stainless-steel mesh of sufficient size and a mesh width of 2 to 3 mm to allow sufficient air supply. Shrews can squeeze through gaps of only a few millimeters in width, and escape must be prevented as this would quickly lead to death by starvation or dehydration.

Bedding for shrews must be a soil-sand-mixture substrate that is at least 4 to 6 cm deep to allow burrowing. Any natural bedding material such as the soil (Blumenerde, 40 L, BASIC, Hellweg, Dortmund, Germany) and sand (Spielsand, SAHARA, WECO GmbH and KG, Leer, Germany) or enrichment items such as moss, wood or bark (Rinden-Mulch, 50 L, BASIC, Hellweg, Dortmund, Germany) should be autoclaved prior to use (Figure 2 C). The substrate in areas of defecation or urination must be replaced regularly, at least every 7 d. A complete substrate change is performed every 4 wk. Depending on the number of animals housed per container, the presence of visible soiling or a circumstance such as a water bottle leakage, the substrate may require complete replacement sooner than 7 d. Care should be taken to remove all dead feeder insects directly before the next live feeding to prevent mite infestation. The bedding used for rodents is absolutely unsuitable for shrews. In our experience, shrews may injure themselves on the rodent bedding with fine wooden shavings or splinters. Furthermore, conventional rodent substrates are not suitable for the natural burrowing behavior of shrews, which serves to clean the fur. A mixture of rodent bedding with soil is likewise unsuitable.

At least 3 hideouts per animal are needed in each container or terrarium and may consist of pieces of bark, flat stones, brick fragments, or small clay flower pots (Figure 3). Because shrews tend to hide in narrow crevices or blend into a richly structured environment, great care is necessary when manipulating the enclosure during routine husbandry to avoid accidental harm.

Porous stones or plaster blocks with a preformed labyrinth of paths and caves are used for rearing the young.[14,34] A litter of 2 to 6 pups are born in these labyrinths, typically enriched by the mother with moss as nesting material (Figure 4), and in which the pups remain during the first few days after birth. The labyrinth is oriented toward the transparent wall of the

Figure 2. A) Plastic container with removable and foldable but shrew-proof lid with 2 ventilation insets. The lid needs to tightly seal the container since shrews are very good jumpers and climbers and may squeeze through gaps only a few millimeters in width and thus may easily escape, which would quickly lead to death by starvation and dehydration. B) The container for general husbandry (not breeding) with bedding, enrichments and 2 separate water sources – bowl and bottle. It is important to create many retreats for the animals. Scale bars, 5 cm. C) Open container. The lid is folded back in its centerline and shows the stainless-steel mesh insets for ventilation (mesh size between 2 and 3 mm). Of note, the mesh is soldered into the plastic to prevent escape or injuries, for example during handling or cleaning.

breeding container or terrarium to allow for daily observation. Only one breeding pair is kept per container. After weaning, 3 to 5 littermates, separated by sex, can be kept together. Because shrews are naturally social animals, single-housing should be avoided whenever possible; single, sexually mature animals can be paired again, and litters with only one pup can be housed with other pups of the same sex and, if possible, of the same age after weaning.

Light, temperature, and humidity. A 12:12h light:dark cycle, each with a dawn and twilight phase of approx. 60 min, is suitable for shrews. Fluorescent tube light (L36 W/840 or 860, LUMILUX Cool White, Osram, München, Germany) with a daylight-similar spectrum is recommended. In our experience, the light intensity should range from 50 to 300 lx within which no preferences have been noted. Ballasts should be operated at high frequency (approximately 50 kHz) to avoid flickering. The room temperature is monitored daily and maintained between 19 and 24 °C. Temperature and lighting cycles are kept constant throughout the year with no adjustments required for breeding, which occurs year-round. The relative humidity is also monitored daily and maintained between 45 and 60% by use of a fogger (HumSpot, DRAABE, Condair Systems GmbH, Norderstedt, Germany).

Behavior and handling. Traditionally, shrews (including *Suncus etruscus*) have been considered nocturnal animals. However, perhaps due to their constant food requirements, they show a circadian activity pattern with frequent bursts of activity spread over a 24-h period.[29] During the light phase, shrews sleep for longer periods in their hideouts. Shrews cannot maintain their high metabolic rate during these sleeping periods. Therefore, their body temperature and vegetative and sensory functions are greatly reduced during sleep. They do not or cannot react to disturbances and, therefore, may appear ill or dead to an untrained observer. To avoid disturbing the shrews during their sleep periods, husbandry should be performed during late afternoon, when almost all shrews are observed to be active. In case of food shortage or at low temperatures, they may fall into a torpor,[5,16,29,35] a physiologic adaptation characterized by short periods of reduced metabolic rate and body temperatures, which is similar to but mechanistically distinct from hibernation.[30,36]

Depending on the time of day, the shrews quickly become very active and hunt and kill insects immediately after the live

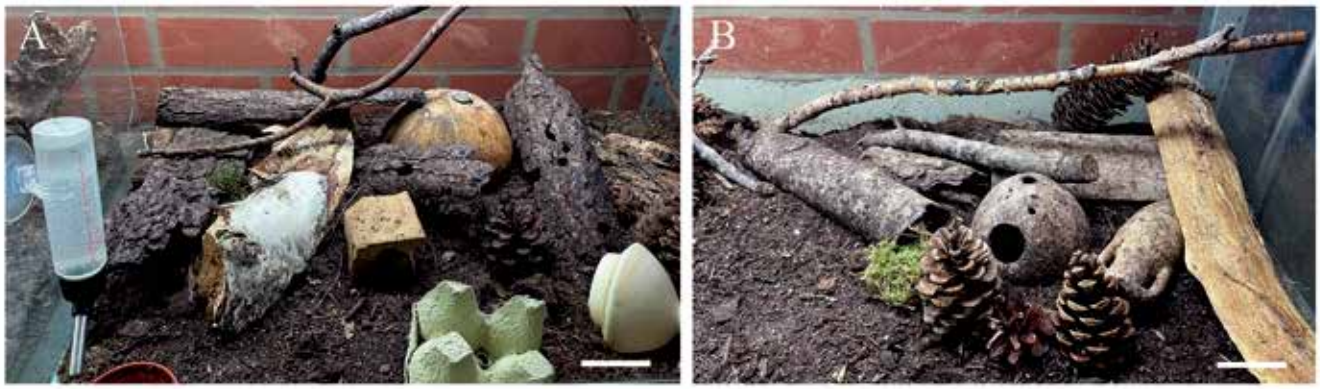

Figure 3. Various enrichment possibilities in a glass terrarium, such as A) wood, bark, egg packaging, clay pots beside water bottle and dish and B) pine cones, moss, and coconut shells. Scale bar, 5 cm.

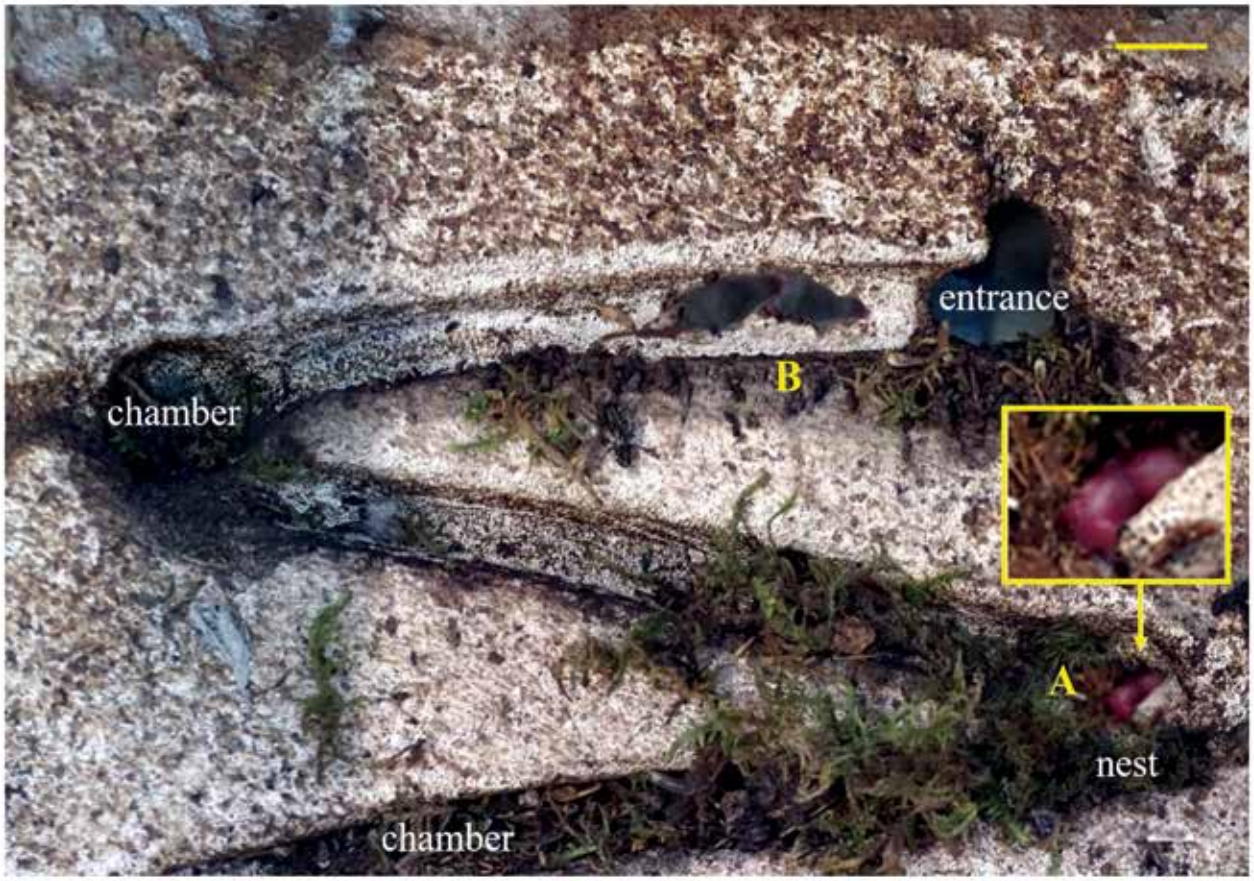

Figure 4. Labyrinth stone with young (A) in nest and 2 shrews (B). The stone is made of plaster and measures 30 × 25 × 6 cm similar as described by others.[14] Passages and chambers were manually scratched into the stone. Scale bar, 3 cm.

feed is provided. Behavioral observations are only possible during this time. Shrews move very quickly and jerkily, with their head and tactile hairs constantly in motion, and they can deliver a painful bite that easily pierces human skin.

Insectivores are the only order of mammals in which certain species produce venomous saliva, including several solenodon, short-tailed shrew (*Blarina*), and water shrew (*Neomys*) species.[9] However, to the authors' knowledge, whether the saliva of Etruscan shrews is venomous is currently unknown. Two toxins produced by venomous species have been identified as soricidin[7] and Blarina toxin.[20] The 2 venomous insectivore species have enlarged and granular submaxillary salivary glands that produce the venomous saliva, which in turn collects in a concavity on the first incisors.[9] In the Northern Short-Tailed Shrew *(Blarina brevicauda)*, soricidin is hypothesized to be used for "live hoarding" behavior, in which invertebrates, such as mealworms, may remain paralyzed but alive for several days as reserve feeding source;[17] Blarina toxin is functionally toxic even in mice.[17,20] If shrews must be picked up, this is best done before or several hours after feeding by handling them at the tail root using soft silicone tube-padded tweezers. Restraint by hand is not possible due to the small size of the animal and their speed of movement.[14,34]

Prey species. Live feed such as crickets are best obtained weekly from a specialized breeder (fauna topics GmbH,

Marbach/N.-Rielingshausen, Germany) to avoid any contamination via contact with other small mammals at the wholesaler's or in pet shops. The crickets are then briefly maintained inhouse and fed with carrot slices prior to feeding the crickets to shrews. Mealworms used for feeding purposes are bred exclusively inhouse. Shrews are not picky in the choice of food insect and eat between 8 to 12 adult crickets of different genus (*Achaeta*, *Gryllus*, *Gryllodes*) per day. This corresponds to about 6 times their body weight.[5] Shrews use their sense of touch to detect and hunt prey in a highly precise and rapid fashion[2,6,29] and strongly prefer to feed on live insects. The provision of a continuous supply of live food in sufficient quantity is vital for the colony.[14,34] Shrews must be fed live crickets at least once a day. However, not all prey is eaten immediately after killing. Some is hidden as a food resource and eaten later. In case of a shortage of live prey, shrews may accept frozen insects for a limited time. Any substitution, such as with cat or dog food, is unsuitable. In our experience, at least a weekly requirement of frozen feed insects should be stored to bridge unexpected supply shortages of live insects.

Certain individuals (for example, aged animals that require a higher nutrient and fat content) should be supplemented with meal beetle larvae (*Tenebrio* sp.). Mealworms should be fed to shrews that cannot hunt the large and quick crickets, which would include animals that are over a year old, lactating females, and juvenile shrews that have just started hunting. Although feeder insects provide an excellent source of most nutrients, they may provide inadequate calcium, fat-soluble vitamins, thiamine, and omega 3-fatty acids to the shrews.[3,8,10-13,22] To correct any nutritional imbalance, feed insects can be dusted or gut loaded. While dusting with calcium and multivitamin powders is simple, it is rather unreliable due to variables such as poor adhesion (e.g., *Tenebrio* larvae), or the quick removal of the dust by grooming seen in crickets.[1,22] Therefore, gut-loading may be preferable.[1] However, its efficacy depends on the quality and palatability of the gut loading diet, the duration of gut loading, and developmental cycle of the insects, which can also show increased mortality due to nutritional toxicosis.[12,23]

The authors found that feeding 10 insects dusted with a maximum of 500 mg Micro Minerals Rodents (cdVEt (Schweiz) GmbH, Speicher, Switzerland) was effective as prophylaxis, when indicated, and for shrews of 2 y of age and above. Indications of dietary deficiency include a change in the animals' coat (dull, unkempt or scanty) and age-related changes in locomotor activity.

Water. Fresh drinking water with a median concentration of 2.52 mmol/l Calcium and 0.47 mmol/l Magnesium ions (DIN EN ISO 11885-E22) was supplied ad libitum. The pH of the water was 7.5 (DIN 38404-C05). If very soft tap water is being used, calcium or magnesium chloride can be added or additional minerals can be provided via feeder insects. Water is best provided fresh daily in small, shallow water bowls[14,34] with a diameter of 3 to 5 cm and a water depth of about 8 to 15 mm placed at the bottom of the cage. In addition, small drinking bottles (Living World, Hagen Group, Holm, Germany) attached to the cage wall with a suction cup can be provided on a weekly basis (Figure 2, B and C). However, the acceptance of drinking bottles should be monitored closely.

Behavioral and animal welfare monitoring. The colony is monitored daily by specialized husbandry and scientific personnel, including on weekends and holidays. Furthermore, the facility Attending Veterinarian and Animal Welfare Officers should also monitor the shrews' wellbeing. A monitoring guideline – also termed "Care Sheet" – can provide the basis for a detailed and systematic assessment of animal wellbeing and should be used to record any deviations from the norm and to indicate task completion.

We have established a set of possible parameters for monitoring shrew behavior in the enclosure during feeding and during manipulation of the hideouts. These parameters include a description of the condition of interest, assessment, and a subsequent action plan that may be modified with respect to individual experience with the colony and the given conditions of the facility.

Normal behavior of healthy, acclimatized shrews that does not require intervention includes exploratory behavior shortly after introduction of a shrew to the enclosure, hiding or startling behavior in reaction to movement in front of and above the enclosure, and loud acoustic communication by high frequency chirping. After addition of prey animals to the enclosure, exploratory behavior, focused hunting and killing of prey, and transfer of prey to hideouts should occur. During manipulation of the hideouts, escape behavior and subsequent disappearance into a different hiding place is expected. Manipulation of hideouts should only be done for monitoring purposes under justified circumstances in order to avoid disturbing the animals unnecessarily.

Shrews chasing each other is a behavior that requires observation and increased frequency of monitoring. Because this behavior is not necessarily associated with aggression, further observation is necessary to assess the potential for aggression that may require further action. If the shrew cohort in which this behavior arises includes both adults and offspring, separation of the offspring from the parents may be necessary. Conflicts may also occur within a group of young shrews, which may also require separation of the animals. Lack of escape behavior, lack of interest in foraging despite general active behavior, or remaining motionless in a hideout may be due to torpor. If the behavior continues for a prolonged period (> 10 min), the animal should be checked again to see if it reacts normally and forages after a few minutes. If the behavior returns to normal after a second inspection, a score is not necessary. If the behavior has not improved or has worsened, the next higher category or score should be used.

Behavior requiring recording of the observation, at least one more check on the same day, and the notification of the veterinarian and the scientist in charge include lying motionless inside the hiding place despite manipulation or remaining outside the hiding place for a prolonged period of time (> 20 min). The behavior may be due to an unusually long torpid state. A torpid state in an animal located outside the hiding place is unusual but may occur rarely. In this case, the animal must be checked after a few minutes to determine whether its behavior has normalized. If necessary, the animal may be removed from its hiding place and examined for injuries. If an animal lacks interest in live feed and shows decreased or absent general active behavior, it should be placed in a separate container for a brief period of time and monitored several times throughout the day. The period of separation depends on the improvement or deterioration of active behavior. Bedding consisting of white absorbent tissue may help detect any bleeding or abnormal secretions or excretions. Appropriate treatment of the animal should be determined by the veterinarian. If the behavior has not improved within an established timeframe, the guidelines should indicate what immediate action is required. If the animal quickly reaches a humane endpoint based on the guidelines, it must be humanely euthanized via isoflurane anesthesia overdose, as also stated in the guidelines.

Animal behavior requiring immediate action may include worsening of the behaviors mentioned above, or finding the

shrew immobile in its hideout for over 60 min. If the shrew's condition does not improve, it should be removed from the enclosure and closely examined for vital signs and injuries. If the animal's general condition or food intake is greatly impaired or if it shows injuries, immediate euthanasia may be indicated, depending on the severity of impairment. Otherwise, the shrew should receive immediate treatment based on veterinary assessment. Physical impairment and reduced food intake are mostly age-related and will inevitably progress to the need for euthanasia. Injuries due to fighting seldom occur. In cases of repeated aggressive behavior toward conspecifics, the aggressor should be removed from the group immediately and placed in another container. Reassociation or remating should be possible, as general aggressive behavior by an individual has not been observed.

In aged shrews, escape behavior may be severely reduced, particularly in shrews over 30 mo old. Therefore, the age of the animal should be considered when lack of escape behavior is observed. Age-related reduction in locomotor activity is normal and may be tolerated if the animals are in good general health and able to hunt prey insects or consume larvae provided as a supplement. Old shrews may also show reduced grooming. A lack of continuous wear on the claws of older animals may lead to overgrowth and impaired agility, perhaps requiring special attention.

In addition to a descriptive monitoring guideline, a numeric scoring scheme can be used to delineate specific actions for certain scores (Figure 5). Our experience to date has shown that shrews can die from one day to the next with no evidence of disease due to their high metabolic rate.

Environmental monitoring. Environmental parameters should also be included in the daily monitoring records and require immediate action and technical support if outside their normal range. Regular maintenance of all technical systems and

Category		Condition/ Description	Score	Action
Feeding behavior (A)	A1	Exploratory behavior (searching around and touching enrichment items) when feed animals are introduced.	0	0
	A2	Targeted capture and killing of feed animals.	0	0
	A3	Carrying feed animals into hiding places.	0	0
	A4	General activity without interest in feed animals.	1	A
	A5	No activity during the addition of feed animals. No feed intake.	2	B

The animals need to be checked daily, also on weekends and holidays. Only deviations from the norm must be recorded.

Scoring column rating, here the sum of the numbers is relevant

Score = 0 No reaction necessary.

Score ≥ 1 In case the sum is 1 or above (at least one conspicuous sign), another control is to be performed < 8 h.

Score ≥ 3 If the sum repeatedly equals 3 or above, the veterinarian must be informed and/or appropriate countermeasures are to be taken. The frequency of the controls is to be increased.

General Action Instruction:

0 No reaction necessary.

A Note observation and check enclosure at least one more time within < 8 h. If the score no longer applies after the second inspection, it must be deleted. If there is no improvement, increase the score to B!

B Note observation, check enclosure at least one more time on the same day, and, if in doubt, consult veterinarian.

Figure 5. Example category of a score sheet: feeding behavior. Please note: when using a scoring scheme, especially with a numeric addition as above, please be aware to group parameters or symptoms to one specific corresponding behavioral, appearance, or organ complex. For example, excretions in the nose area may be grouped to outward appearance or to symptoms of the respiratory tract. If this specific criterion is, however, stated twice, that is once each in both complexes, it would be scored twice, leading to a higher score than actually present, which should be avoided in order not to falsify the scoring. Categories of 0 delineate the normal behavior of the shrew. This may be omitted. However, particularly concerning exotic species, this may facilitate the aim of the scoring sheet, that is every observer scoring equally.

equipment by specialized personnel is mandatory. A daily check and recording of the room temperature, humidity, and light are essential and can be completed manually or automatically, depending on equipment availability. Ventilation and air change rate, if technically assessable, should also be checked on a daily basis. A deviation from normal parameters may be due to malfunctioning of cooling, heating or ventilation systems and require immediate attention.

Microbiologic monitoring. The shrews are housed in 3 rooms of the Central Animal Husbandry Biology (ZH 149) of the Humboldt University zu Berlin, Germany. No experiments with infectious or zoonotic agents are performed in these rooms. The animal colony is monitored by a specialized veterinarian. Periodically and when indicated, animals are sent for diagnostic testing according to FELASA recommendations by a certified laboratory (GIM Gesellschaft fuer innovative Mikrooekologie mbH, Michendorf, Brandenburg, Germany). The shrews can be screened for a specific subset of mouse pathogens by polymerase chain reaction (PCR) or, after culture, by Gram staining and growth on selective media by Matrix Assisted Laser Desorption Ionization — Time of Flight Mass Spectrometry (MALDI-TOF). For this, we use a highly sensitive detection protocol for immunocompromised mice. This protocol detects Mouse Norovirus (MNV) and Mouse Parvovirus (MPV) by PCR and several additional bacteria that are not monitored routinely in mice, such as *Pasteurellaceae*, *Klebsiella*, *Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Pneumocystis murina*.

Infections that are routinely detected in mice by enzyme-linked immunosorbent assay (ELISA) cannot be analyzed in shrews because shrew-specific ELISAs have not been developed to date. Screening is not performed for some common mouse viral infections, including Mouse Hepatitis Virus (MHV), Minute Virus of Mice (MVM), Mouse Rotavirus (EDIM), Mouse Adenovirus 1 and 2 (MAV), Reovirus Type 3 (Reo), Ectromelia Virus (ECTV), Sendai Virus (SeV), Lymphocytic Choriomeningitis Virus (LCMV), and Theiler's Murine Encephalomyelitis Virus (TMEV). Whether the primers used to bind to and amplify DNA sequences of bacteria, fungi, and viruses of mice can also be used to amplify the pathogen strains infecting shrews is unknown. Likewise, shrews may be infected with murine pathogens at an undetectable level and thus provide a potential reservoir for pathogens that may infect mice housed in the same animal facility. Therefore, in addition to diagnostic testing of randomly chosen shrews from the colony, the use and routine testing of bedding sentinel mice, such as SWISS mice, is advisable; these should be tested routinely, at least once a year. The sentinels are kept on standard mouse bedding mixed with littered sand-earth-mix shrew bedding for least 6 mo prior to diagnostic testing. Parasitic pathogens can be detected for both mice and shrews, since these are frequently detected via direct preparation, fecal flotation, and light microscope analyses and do not require species-specific measures.

Statistical analysis For statistical analysis of life expectancy, body weight, body size, the number of litters and progeny, IBM SPSS Statistics (version 25, IBM, Armonk) was used. Results are presented as median (minimum - maximum).

Results

Colony development. Of the animals (n = 36; 20 male, 16 female) originally captured, 15 founder breeding pairs were established in Berlin. Since 2006, a total of 3,041 animals (1,511 or 49.7% females, 1,488 or 48.9% males, and 42 or 1.3% ambiguous have been registered during the time period between February 2007 and February 2021.

Natural life expectancy, body weight and size. The median lifespan of the 557 animals that died spontaneously due to natural causes was determined to be 454 d (1 to 1,375 d). Of the 557 animals that died of natural causes, 26 reached the age of 1,192 d (3 y, 3 mo, 13 d).

For analysis of life expectancy, body weight and body size, a random sample of 76 animals was analyzed by IBM SPSS Statistics (version 25, IBM, Armonk). Results are presented as median (minimum - maximum). Of these 76 animals, 36 were male (47.4%) and 40 female (52.6%) with median ages of 432 d (187 to 744 d) and 390 d (158 to 715 d), respectively. The average weight for both males and females was 3.0 g (2.1 to 4.2 g and 2.3 to 4.3 g, respectively). The torso length of the females was 4.8 cm (4.0 to 5.4 cm), while the males had a length of 4.6 cm (4.2 to 5.0 cm). In addition, the total body length including tail was 7.2 cm (6.0 to 7.6 cm) for females and males (Figure 1 D).

Socialization and breeding. For breeding, shrews build nests and use special stones with labyrinth-like passages (Figure 4). The gestation period is about 27 to 28 d, and the offspring are born naked and blind. Males have a gland on their flanks that produces a musky smelling secretion.[14] Females have 3 mammary glands on each side. External sex differences are well developed at 23 d after birth when weaning occurs and are easy to use for sex identification (Figure 1 B, C and E). Females are ready to mate again immediately after they give birth and, thus, may give birth as early as 4 wk after the previous litter. After 7 but no later than 8 wk after birth, the sexes in the litter should be separated. Males and females can be kept together as sibling groups separated by sex. In our experience, new pairings are best formed at 12 wk of age for males and 16 wk of age for females. In our experience, fertility decreases significantly after a breeding period of one year.

Since 2006, a total of 279 pairings have been established to date. Of these breeding pairs, 217 pairs (77.7%) successfully produced a first litter while 62 breeding pairs were unsuccessful, of which some were remated. Only weaned animals (that is, shrews that had reached the age of 23 d) were included in the evaluation of progeny. From 217 successful matings, a total of 1,129 pups were produced in up to 15 litters per breeding pair. Generally, the first litter produced the most progeny, which was a median of 3 (1 to 7) pups (Figure 6). The maximum number

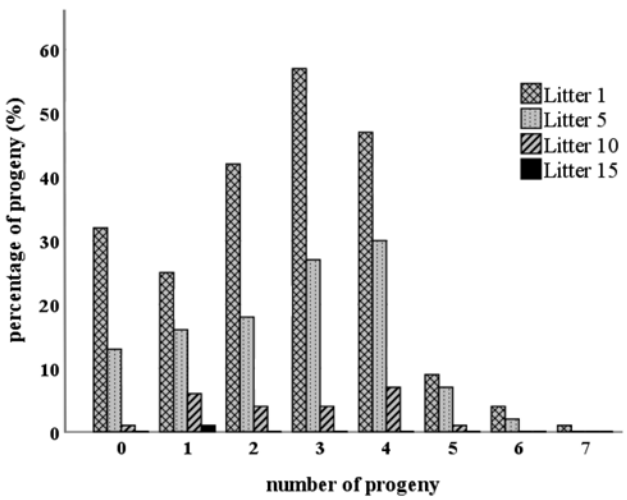

Figure 6. Comparison of the number of progeny per breeding pair (n = 217) in selected consecutive litters of the same breeding pair. The graph illustrates a distinct decline in the number of progeny per litter with increasing numbers of litters per breeding pair, each from litter one to litter 5 and litter 5 to litter 10. Only one 15th litter was recorded with a single pup during the entire period of investigation. The most frequent are 3 and 4 offspring (58 and 48%) born in the first litter.

Table 1. Tabular presentation the number of individual litters with the corresponding litter size. The occurrence of a certain number of pups (0 to a maximum of 7) is given in absolute numbers and in percentages for each litter, grouped per column into the consecutive litter number (1 to a maximum of 15 litters per breeding pair). The percentages show the distribution of pups in litters 1 to 7. A decrease in litter size occurred from the tenth litter and afterwards. A 14th and 15th litter was achieved by one single breeding pair, in which 3 and one offspring were determined, respectively.

	litter 1		**litter 2**		**litter 3**		**litter 4**		**litter 5**		**litter 6**		**litter 7**		**litter 8**	
Total number of pubs (n = 1129)	217		201		173		145		113		86		69		46	
Number of pups	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
0	32	14.7	22	10.9	19	11.0	18	12.4	13	11.5	9	10.5	8	11.6	2	4.4
1	25	11.5	24	11.9	18	10.4	17	11.7	16	14.2	12	14.0	7	10.1	8	17.4
2	42	19.4	30	14.9	28	16.2	24	16.6	18	15.9	14	16.3	11	15.9	15	32.6
3	57	26.3	41	20.4	37	21.4	34	23.4	27	23.9	23	26.7	18	26.1	11	23.9
4	47	21.7	58	28.9	49	28.3	28	19.3	30	26.5	16	18.6	12	17.4	5	10.9
5	9	4.1	22	10.9	18	10.4	23	15.9	7	6.2	9	10.5	10	14.5	5	10.9
6	4	1.8	4	2.0	4	2.3	1	0.7	2	1.8	2	2.3	2	2.9	0	0
7	1	0.5	0	0	0	0	0	0	0	0	1	1.2	1	1.4	0	0

	litter 9		**litter 10**		**litter 11**		**litter 12**		**litter 13**		**litter 14**		**litter 15**	
Total number of pubs (n = 1129)	34		23		12		4		4		1		1	
Number of pups	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
0	3	8.8	1	4.3	2	16.7	1	25.0	2	50.0	0	0	0	0
1	7	20.6	6	26.1	4	33.3	3	75.0	1	25.0	0	0	1	100
2	7	20.6	4	17.4	5	41.7	0	0	1	25.0	1	100	0	0
3	9	26.7	4	17.4	1	8.3	0	0	0	0	0	0	0	0
4	7	20.6	7	30.4	0	0	0	0	0	0	0	0	0	0
5	1	2.9	1	4.3	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0

of 7 pups was achieved only three times within the given time frame. Litter size fell significantly from litter 9 onwards (Table 1).

Discussion

Maintaining appropriate husbandry conditions for Etruscan shrews has been described as demanding,[14,15,21,31,34] with daily need for live insects as most crucial and irreplaceable[15] aspect. Breeding may be even more difficult. However, our experience shows that maintenance and breeding may be successful long-term, if certain prerequisites are met.

Etruscan shrews may be less sensitive to the space available than other species[31] and the cage sizes used seem to be well accepted,[14] as was the natural and varied enrichment. Etruscan shrews are strongly thigmotactic,[14] which requires sufficient high-quality bedding.

The shrews in our facility show a life expectancy of more than 3 y, perhaps due to a supportive environment and the absence of natural enemies. Life expectancy under natural conditions is uncertain, but a life expectancy in captivity of up to 2.8 y has been described[31] and was 3.3 y for some animals in our colony.

Due to the constant light, temperature, and food available in captivity, seasonal behaviors observed in the wild may not occur. However, this lack of seasonality does not appear to have any observable adverse impact on breeding or hunting behavior.[19] Even more so, we achieved year-round mating regardless of seasons, whereas free-living shrews reproduce only from April to October.[14,27]

To the authors' knowledge, the maximum litter number per breeding pair in the wild is unknown but was determined to be as high as 15 in our facility. Furthermore, litter sizes of 2 to 6 pups have been described[4,27] whereas animals maintained in our care had an average of 3 and a maximum of 7 pups per litter, despite the females having only 6 teats.

In conclusion, eco-ethological observations and other research-driven interventions can be adequately performed only after a colony has been acclimatized and successfully established. For exotic species such as the Etruscan shrew, specific conditions are necessary to maintain the highest possible standard of animal welfare and wellbeing, which is a key prerequisite for obtaining reliable research results.

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