20 Tree shrews

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Biological overview

General biology

The first report of tree shrews dates back to 1780. The description and an accompanying sketch were from William Ellis who maintained a naturalist's journal during Captain's Cook third Pacific voyage of the ship *Discovery*. Uncertainties concerning the taxonomic affinities of tree shrews originated with this description in which tree shrews were designated 'squirrels', a confusion that still occasionally persists today. About 80 years ago, a variety of reports described similarities between tree shrews and primates, and the conclusion that there was a direct phylogenetic relationship between modern tree shrews and primates was predominantly made by Le Gros Clark (1924), largely on the basis of brain anatomy. His view was endorsed in G.G. Simpson's classification of the mammals (Simpson 1945). In the following years, several authorities (see eg, Luckett 1980) had doubts about this phylogenetic link and, as a result, excluded tree shrews from primates. An intensive discussion of tree shrews and their phylogenetic relationships is provided in Luckett (1980), Martin (1990) and Emmons (2000). Today, tree shrews are placed in their own order, Scandentia, and according to very recent molecular phylogenetic studies they are placed together with primates and dermoptera within the clade Euarchonta (Kriegs *et al.* 2007). Currently the tree shrew genome is being cloned as part of the Mammalian Genome Project, funded by the National Institutes of Health (NIH).

Tree shrews (family Tupaiidae) are subdivided in two subfamilies: the diurnal subfamily Tupaiinae containing five genera (*Tupaia*, *Anathana*, *Dendrogale*, *Lyonogale*, *Urogale*) and the nocturnal subfamily Ptilocercinae, with a single genus, the pen-tailed tree shrew *Ptilocercus*. The geographic distribution of the Tupaiidae extends from India to the Philippines, and from Southern China to Java, Borneo, Sumatra and Bali. Natural habitats are tropical forests and plantation areas (Table 20.1).

In general, tree shrews are similar to squirrels in their external appearance and habits and the Malay word 'tupai' (from which the name *Tupaia* is derived) is used for both tree shrews and squirrels, whereas the Malay word 'tana' (found in the species *Lyonogale tana*) is used only for tree shrews. Despite their name, tree shrews have nothing to do with real shrews and most species of tree shrews are much more active on the ground than in trees. Although there are clear differences between tree shrew species, they share a basic common pattern that can be described with reference to the relatively well known Belanger's tree shrew *Tupaia belangeri* (Figure 20.1). All are relatively small, agile and in general omnivorous with a preference for small fruits and invertebrates, especially arthropods. Tree shrews range from the predominantly arboreal (*Dendrogale*, *Tupaia minor*, *Ptilocercus*) to the predominantly terrestrial (*Lyonogale*, *Urogale*), but most tree shrew species are semi-arboreal and usually forage on the ground. The terrestrial tree shrews have a long snout and sharp claws both of which are used to obtain food by rooting through the leaf litter on the forest floor. Species which are more arboreal are smaller than the terrestrial species. They have shorter snouts, smaller or poorly developed claws, long tails and more forward-facing eyes. When eating, all species will hold food between the front paws. In general, tree shrews have a well developed visual system and, for some species, colour vision has been documented. The vocal repertoire of *Tupaia belangeri* consists of eight distinct sounds. Within this repertoire, four basic acoustic structures can be distinguished which can be associated with functional categories such as alarm, attention contact and defence (Binz & Zimmermann 1989). No ultrasonic vocalisations could be found in *Tupaia belangeri* (Kirchhof *et al.* 2001). The same authors report that during agonistic encounters, adult males elicit five distinct call types, partially with graded variants. The calls show harmonic or noisy spectra ranging from 0.4–20 kHz. The call structure depends on the dominant status and the motivation of the individuals. Increasing pitch indicates increasing fear, while decreasing pitch and larger frequency range indicate increasing aggression (Kirchhof *et al.* 2001).

All tree shrews seem to use nests both for sleeping and rearing of offspring. Nests may be located in trees or on the ground level. Even tree shrews, which spend most of their time in trees, avoid climbing on fine branches and do not leap within or between trees. Typically, they use broad branches as support and use trees as a vertical extension of the terrestrial substrate (Martin 1990).

Size range and lifespan

Depending on the species, the body weight of tree shrews ranges between 45 and 350 g (see Table 20.1) with adult

1 **Table 20.1** Tree shrews, Scandentia, their biological data and distribution (with modifications from van Holst (1988)). Tree shrews, Scandentia, their biological data and distribution (with modifications from van Holst (1988)) (BW, body weight; HBL, head–body length; TL, tail length; NN, number of nipples; GP, gestation period; L, litter size; BIW, birth weight; W, weaning; P, puberty; L, longevity in captivity).

	Body	*Reproduction*	*Life history*	*Distribution*
Subfamily Tupaiinae *Tupaia* (tree shrews) *T. belangeri, T. glis, T. gracilis, T. javanica, T. longipes, T. minor, T. montana, T. nicobaria, T. palawanensis, T. picta, T. splendidula*	BW: 50–270 g HBL: 12–21 cm TL: 14–20 cm NN: 1–3 pairs	GP: 41–55 days L: 1–5 BIW: 6–10 g	W: around 30 days P: around 2 months L: 9–12 years	Tropical forests, semi-terrestrial
Lyonogale (Malaysian tree shrews) *L. tana, L. dorsalis*	BW: approx. 300 g HBL: approx. 22 cm TL: approx. 17 cm NN: 2 pairs	GT: 45–55 days L: 1–4 BIW: approx. 10 g	W: around 30 days P: around 2 months L: Unknown	Mainly terrestrial, primary and secondary forests
Urogale (Philippine tree shrew) *U. everetti*	BW: 220–350 g HBL: approx. 20 cm TL: approx. 15 cm NN: 2 pairs	GP: approx. 55 days L: 1–4 BIW: approx. 10 g	W: around 30 days P: probably 2 months L: 6 years	Terrestrial
Anathana (Indian tree shrew) *A. ellioti ellioti, A. ellioti pallida, A. ellioti wroughtoni*	BW: approx. 180 g HBL: approx. 19 cm TL: approx. 18 cm NN: 3 pairs	Unkown	Unkown	Tropical forests, semi-terrestrial
Dendrogale (smooth-tailed tree shrew) *D. melanura, D. murina*	BW: approx. 60 g HBL: approx. 13 cm TL: approx. 13 cm NN: 1 pair	Probably like *Tupaia*	Probably like *Tupaia*	Mainly arboreal
Subfamily Ptilocercinae *Ptilocercus lowii* (pen-tailed tree shrew)	BW: approx. 15 g HBL: approx. 14 cm TL: approx. 17 cm NN: 2 pairs	GP: unknown L: probably 1–4 BIW: unknown	Unknown	Nocturnal, arboreal, tropical forests

males being usually heavier than adult females (own observation). Their lifespan in the wild is still unknown but in captivity, *Tupaia glis* (Bever & Sprankel 1986) and *Tupaia belangeri* (own observations) can live 10 years or more.

Social organisation

Despite extensive morphological description and behavioural studies in the laboratory, remarkably little is known about the behaviour and the ecological roles of tree shrews in the wild (Emmons 1991, 2000; Emmons & Biun 1991). Based on observations of Kawamichi and Kawamichi (1979), males of the common tree shrew *Tupaia glis*, which are close relatives to the Belanger's tree shrew, have relatively stable home ranges of about 2 acres. The territory of an adult male overlaps to a certain extent with the home range of one adult female and also includes the ranges of a small number of juveniles. This suggests that common tree shrews are basically monogamous in the wild, which is in agreement with observations made in the laboratory where tree shrews can be effectively maintained in pairs. The same authors also reported territorial marking behaviour using the chest gland, and territorial fights between adults of the same sex. Chemical signals play an important role in territorial behaviour of male tree shrews. Scent substances are found in glandular secretions, urine, faeces and saliva, and contain information concerning the identity and physiological state of the individual. Laboratory experiments have shown that in males, both the production of the scent substances and the marking behaviour are controlled by androgens (von Holst & Buergel-Goodwin 1975; von Holst & Eichmann 1998; Eichmann & von Holst 1999).

Standard biological data

The dental formula of the Tupaiidae is: $I^2_3C^1_1Pm^3_3M^3_3$ (Butler 1980). Core body temperature and its circadian rhythm have been studied by telemetry in *Tupaia belangeri*. Minimal body temperature during the night was about 35 °C while during day time the core temperature increased to a maximum of about 40 °C (Refinetti & Menaker 1992 and own observations). This day/night difference of about 5 °C is much larger than that of most endotherms. Since body temperature rhythm is synchronised with the rhythm of locomotor activity, the diurnal temperature curve shows a bimodal shape which clearly differs from the cosine waveform that characterises the temperature rhythms of other species (Figure 20.2).

Systolic blood pressure recorded using the tail-cuff method similar to that often used in rats yielded a mean systolic blood pressure of 125 mmHg (Fuchs *et al.* 1993). When using this technique it is not necessary, as is the case with rats, to warm the tree shrew's tail before measurement.

Heart rates in tree shrews show a surprising pattern of variance. Telemetric analysis has revealed that heart rate is strictly correlated with the behaviour and the emotional status of the animals. A heart rate between 240 and 300 beats/min (own observations) is characteristic of resting periods, while during physical activity, heart rate is in the range of 250–350 beats/min and can increase up to 650 beats/min in emotionally exciting situations (Stohr 1988; Muller & Hub 1992). *Tupaia belangeri* shows a high locomotor activity revealing a clear bimodal pattern with a clear trough in the early afternoon (Kurre & Fuchs 1988) (Figure 20.2).

Figure 20.1 Adult male *Tupaia belangeri* from the German Primate Center.

Reliable data on serum constitutents are only available for *Tupaia belangeri*. Table 20.2 summarises serum values reported by Schwaier (1975). An overview of serum and endocrine data is given by von Holst (1977). Erythrocyte numbers are in the range of 8×10^6/mm^3, leucocytes in the range of 3×10^3/mm^3 and mean thrombocyte numbers in the range of 170×10^3/mm^3 (Zou *et al.* 1983). Basal concentrations for plasma norepinephrine (noradrenaline) range from 2.8–36.3 ng/ml and for epinephrine (adrenaline) from 1.3–19.1 ng/ml (Fuchs 1984). Corticosterone is the principal corticosteroid (mean 9 ng/ml) in the peripheral plasma and in unstressed animals the corticosterone : cortisol ratio is 4.5 : 1 (Collins *et al.* 1984). Values of urinary hormones for *Tupaia belangeri* are summarised in Table 20.2.

Breeds, strains and genetics

When selecting a breeding stock for tree shrews, it is important to know the exact origin of the animals. Sometimes, it can be extremely difficult to distinguish between closely related species since traditional classifications do not provide substantial help. Based on their external morphology alone, *Tupaia glis* and *Tupaia belangeri* are very difficult to distinguish from one another. The exact taxonomic classification could be ascertained by means of geographical origin, morphological criteria, cytogenetic analysis and analysis of acoustic signals (Toder *et al.* 1992).

Sources of supply

The 3Rs are a fundamental ethical requirement in laboratory animal science (see Chapter 2). To refine animal experiments and to reduce the number of animal subjects to a minimum, full control over the genetic background and lifespan of the subjects is required. Therefore, it is strongly recommended that animals should only be purchased from laboratory breeding colonies. Information on sources of supply can be

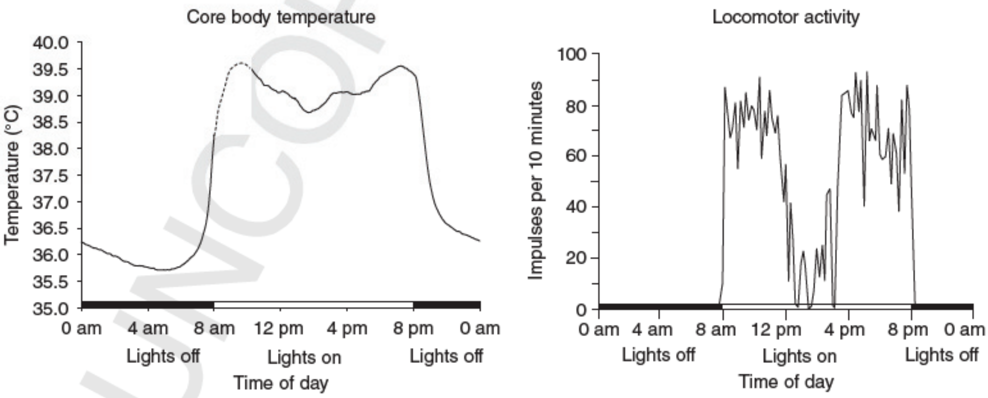


Figure 20.2 Circadian pattern of core body temperature and locomotor activity.

Table 20.2 Physiological data of adult Tupaia belangeri. Blood serum values from Schwaier (1975) and morning urine values from Fuchs (1988).

Parameter	*Serum/plasma*			*Urine*			
	Unit	*mean*	*SD*	*Gender*	*Unit*	*mean*	*SD*
Na^+	mmol/l	141	2.7	M	μmol/ml	95	34
				F	μmol/ml	120	48
K^+	mmol/l	5.2	0.75	M	μmol/ml	85	65
				F	μmol/ml	152	43
Na^+/K^+				M		1.05	0.5
				F		0.82	0.3
Mg^{2+}	mmol/l	2.12	0.24	M	μmol/ml	3.5	2
				F	μmol/ml	4.3	2.2
Ca^{2+}	mmol/l	5.58	0.88	M	μmol/ml	1.25	0.6
				F	μmol/ml	1.35	0.5
Fe^{2+}	μmol/l	95					
Cl^-	mmol/l	105	3.3				
Osmolarity	mOsmol/l	318	7.8	M	mOsmol/kg H_2O	2000	750
				F	mOsmol/kg H_2O	1950	350
pH				M		6.75	0.6
				F		6.75	0.4
Creatinine	μmol/l	62		M	μmol/ml	10.5	4.4
				F	μmol/ml	11.2	3.8
Urea		25.8	7.8	M	μmol/μmol Crea	0.9	0.8
				F	μmol/μmol Crea	0.9	0.2
Uric acid	mol/l	48		M	μg/μmol Crea	16	14
				F	μg/μmol Crea	15	8
Cholesterol	mmol/l	2					
Triglycerides	mol/l	1					
Glucose	mg/100 ml	115.9	16.3	M	mg/μmol Crea	0.1	0
				F	mg/μmol Crea	0.08	0
Protein	g/100 ml	6.5		M	mg/ml	2.65	1.1
				F	mg/ml	0.3	0.2
Protein/Crea				M	mg/μmol Crea	0.25	0.1
				F	mg/μmol Crea	0.27	0.1
Prolactin	ng/ml	12					
Cortisol	μg/ml	8.8					
Corticosterone				M	pg/μmol Crea	335	130
Gastrin	pg/ml	55					
GPT	U/l	10.9	6.2				
GOT	U/l	58	16.8				
LDH	U/l	1872	802				
AP (age 14–27 months)	U/l	90.8	48.2				
GH	ng Eq/ml	>50					
Epinephrine (adrenaline)	ng/l	7.5–11		M	pg/μmol Crea	47	39
Norepinephrine (noradrenaline)	ng/l	5–6.9		M	pg/μmol Crea	103	75
ACTH	pg/ml	65					
TSH	pg/ml	3.5					
FSH	pg/mg	89					
LH	ng/ml	24					

obtained from the German Primate Center, Göttingen, Germany[1].

Uses in the laboratory

Evidence derived from studies on the brain of *Tupaia* by Sir Wilfred Le Gros Clark played a major role in the acceptance of the classification of Tupaiids as primates. Their popularity as experimental subjects in neurobiology, in particular neuroanatomy, has been a direct consequence of their former phylogenetic status in which they were classified as primitive primates (Campbell 1980). The vast majority of experimental work with *Tupaia* has been on the visual system since they were considered ideal subjects to gain insight into the organisation of the early primate visual system. However, it became clear from comparative studies that *Tupaia* possesses none of the features characteristic of primate visual systems (Campbell 1980).

[1] http://www.dpz.eu

Tree shrews have proved to be useful animal models in many instances where a small omnivorous non-rodent species is required (Cao *et al.* 2003). Of course, they should only be used where it is appropriate and necessary for the study. They can be used in many fields of preclinical research such as toxicology and virology, in particular in studies investigating herpes and hepatitis viruses (Hunt 1993; Xu *et al.* 2007). Further, various aspects of behaviour, infant development, communication and social structures can be studied in tree shrews (Martin 1968a, 1968b; Hertenstein *et al.* 1987). Based on a study by von Holst (1972), psychosocially stressed male tree shrews were thought to be a suitable model to study the mechanisms of acute renal failure. However, the authors and others (Steinhausen *et al.* 1978) were unable to replicate these results.

The pronounced territoriality, especially in male tree shrews, can be used to establish natural challenging situations under experimental control in the laboratory. When living in visual and olfactory contact with a male conspecific by which it has been defeated, the subordinate Belanger's tree shrew shows dramatic behavioural, physiological and neuroendocrine changes. As we know today, these stress-induced alterations result entirely from the continuous visual presence of the dominant conspecific. In contrast, dominant tree shrews show no noticeable biobehavioural alterations. It is an interesting aspect of preclinical research that many of the alterations in subordinate tree shrews are similar to the symptoms observed in depressed patients and can be counteracted by several classes of antidepressant drugs (Fuchs 2005).

There is a high degree of genetic homology between tree shrews and primates for several receptor proteins of neuromodulators (Fuchs & Flugge 2002) and the amyloid-beta precursor protein (Pawlik *et al.* 1999); this and the three to four times longer lifespan of tree shrews than that of rodents (Keuker *et al.* 2005), suggest that this species may possibly come to be used in future studies focusing on aging-related brain changes in socially homogeneous and stable cohorts.

Laboratory management and breeding

General husbandry

Housing

Tree shrews have been housed in enclosures of various sizes. Cages and cage equipment should be adapted to the natural behaviour patterns of the animals, providing enough space for their locomotor activities. The cage equipment thus should include substrates for climbing, such as suitable branches and wire mesh. A broad branch (diameter approximately 7 cm), board or tube should be fixed near the top of the cage where the animals can rest during their siestas. Objects for scent marking such as branches, and pasteboard tubes for hiding and marking should also be offered. Outside the cages, wooden sleeping boxes and, for breeding pairs, a separate nest box and nesting material should be provided. Schwaier (1973) recommended the installation of tunnels made out of flexible plastic tubing, of suitable diameter, which can be fixed outside the cages allowing opportunities for greater travel. When cages are side by side they must be separated by opaque screens, which prevent interactions other than the exchange of calls and dispersed odours. Cages face to face separated by a corridor are quite satisfactory because they allow visual interactions without the threat of an immediate attack. Below the cages are waste trays with sawdust or paper to catch excrement and food. In general, the caging conditions should allow control and observation of the animals during the active period by the animal care staff. Construction of the animal facilities should allow each room to be emptied of animals from time to time to allow for cleaning, disinfection and repairs. In the rooms all possible routes of escape must be screened with small diameter wire mesh. To avoid startling the animals, it is recommended that staff should give a sign to the animals before entering an animal room, for example, by knocking on the door.

Tree shrews are best housed at a temperature of approximately 25 °C. Temperatures less than 20 °C can be dangerous for the offspring. Humidity is also critical. Experience suggests that minimal levels required are in the range of 45–50%.

Little systematic research has been carried out on housing of tree shrews; a description of a successful facility is provided here. In the German Primate Center, Göttingen, *Tupaia belangeri* are housed in steel cages (size 50 cm × 80 cm × 130 cm (w × d × h) (Figure 20.3), or 65 cm × 85 cm × 85 cm (w × d × h)). Outside the cages are wooden nest boxes with removable covers (18 cm × 15 cm × 15 cm (w × d × h); entrance 6 cm diameter). These boxes are made from waterproof plywood, which is highly resistant to water and heat and can be effectively cleaned. As the animals stay in the box all night, moisture arises and the animals become damp unless open-pored material is used for the nest box. Therefore, boxes made of plastic or metal are inappropriate. The animals can be locked in the box by a shutter and the box can be removed from the cage. Breeding pairs are housed in modular units (two units with nest boxes) which can be separated by a wire mesh frame.

The animal quarters are air-conditioned with a relative humidity of 60 ± 7%, a temperature of 27 ± 1 °C, and a 10-fold air-exchange per hour. The animal rooms are illuminated (L : D = 12 : 12) from 8:00 am to 8:00 pm with six neon lamps (58 W each, light intensity about 900 lux). After lights are on and before lights are shut down a 30 minute 'sun rise' and a 30 minute 'sun set' with reduced light intensity are programmed. In addition to the neon lamps, each room is equipped with two ultraviolet lamps, which are regulated by a separate timer. The total UV exposure time per day is 2 h (four intervals of 30 minutes each). During the night there is no natural or artificial illumination of the rooms. Each animal room is equipped with one loudspeaker, which is active from 8:30 am to 7:30 pm broadcasting news, reports and some music at low volume. Cages are cleaned once a week with water, the paper under the cages is changed daily, and the waste trays with sawdust are cleaned once a week. No detergents are used in the animal rooms.

Presentation of food and water

Food is supplied in glazed stoneware or stainless steel dishes (diameter about 8 cm, height about 3 cm) which are

Figure 20.3 Housing and rearing *Tupaia belangeri* in the German Primate Center. Upper row: Animal room, a single cage and a nest box closed with a slider. Middle row: Catching a tree shrew from its nest box with a cloth. Lower row: Housing for newborn tree shrews.

changed and cleaned daily. Tap water is provided in bottles or in dishes.

Identification and sexing

Animals should be individually marked, which can be achieved by subcutaneous implantation of transponders or by cutting patterns into the tail hair. Tattooing is hard to perform and the marks often do not last long. Other methods such as notching the inside of the ear or even amputation of claws are prohibited by law in most countries.

The external genitalia of adult male tree shrews consist of a slender and elongate penis, which is posterior to scrotal testes. Retraction of the testes into the abdominal cavity can occur under experimental conditions of stress. In female *Tupaia*, *Lyonogale*, *Urogale* and *Dendrogale*, the clitoris is greatly elongated and grooved on its ventral surface. In neonatal *Tupaia*, the urethra enters the clitoris and extends throughout its length as the clitoral urethra, whereas in the adult the urethra opens together with the vagina as a urogenital sinus at the base of the clitoris. Therefore, infant and juvenile females and males might sometimes be mixed up because the vaginal orifice at the base of the clitoris is sealed. In contrast to the penis, the clitoris does not have a tubular sheath (Figure 20.4).

Health monitoring, quarantine and barrier systems

From the information available to date, the maintenance of tree shrews involves very little risk to human health but, nevertheless, they should be handled with caution. Routine colony health-screening procedures should be carried out and veterinary assistance must be available. Newly acquired animals must be kept in quarantine and require veterinary treatment for external and internal parasites. As pointed out earlier, environmental and social stressors may be a cause of health problems in tree shrews. They may induce sudden and dramatic weight loss or even wounds. Therefore, animal care staff should routinely monitor the animals for signs of illness, such as no food and water intake,

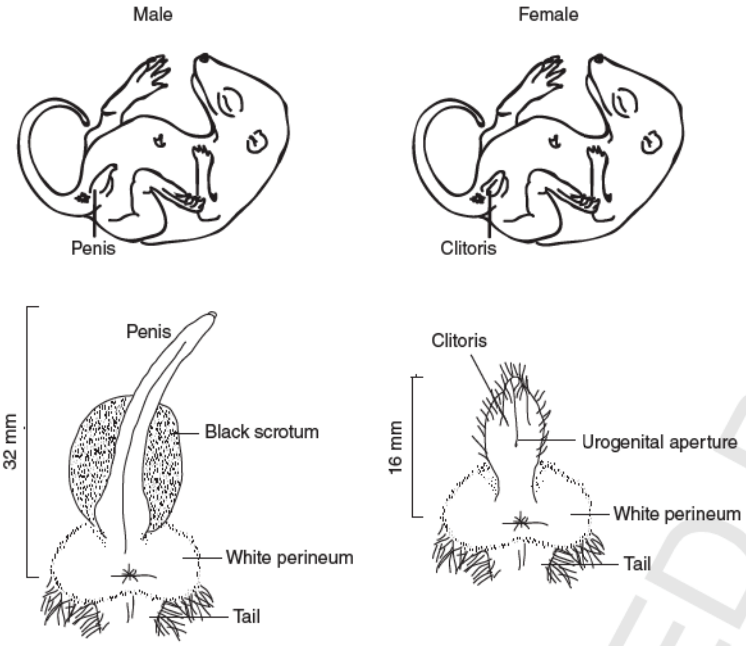


Figure 20.4 Ventral view of the external genitalia of infant and adult tree shrews (*Tupaia belangeri*). Note the penis of the infant tree shrew is twice as long as the clitoris and the anterior position of the scrotum in the adult male tree shrew (Bearder & Pitts 1987, adapted from Martin 1968a).

diarrhoea, weight loss, aberrant body posture or movements, rough fur and lethargy or apathy. When the animals are handled for the routine weighing procedure (at least once a month), they should also be checked for signs of cataracts or tumours.

Transport

For shipment, each individual should be confined to a small dark wooden compartment or its nest box. The size of the compartment is determined by the body weight of the animal and both national and international regulations. It must have openings for sufficient ventilation and should be lined with nesting material. Moist fruits, cooked moist rice and water in a gel form (available commercially) should be provided. Before shipping, animals should be habituated to water provided in gel form. See also Chapter 13.

Due to the high metabolic rate and the sensitivity of tree shrews to disturbances, newly arrived animals are often quite exhausted. For recovery, they should be supplied with sugar water or apple juice and a high-calorie diet. The sleeping boxes should be warmed with an electric cushion (30 °C), lights in the room should be dimmed and disturbances should be avoided.

Breeding

Adults

Females can give birth to their first litter at an age of about 4 months. Males become fertile between 4 and 5 months. The best age for first pregnancy seems to be between 6 and 9 months. If the animal is older, problems such as infertility, stillbirth, cannibalism or abortion occur more often. In males, stress can result in testicular inactivity (Fischer *et al.* 1985; Brack & Fuchs 2000).

Identifying fertile state

Based on the length of the intervals between copulation periods observed in captivity, several authors have suggested an 8–12 day (anovulatory) oestrous cycle in various species of *Tupaia* and ovulation is supposed to be induced by copulation (Martin 1990). However, it is still a matter of discussion whether ovulation is triggered by copulation (induced ovulation) and/or whether an oestrus cycle exists. No cyclic changes in vaginal smears have been detected by the authors or others. In addition, the authors could not find any cyclic alterations in urinary excretion of sex hormones. According to Cao *et al.* (2001) ovulation can be induced by combined injections of pregnant mare serum gonadotrophin and human chorionic gonadotrophin.

Mating systems

In tree shrews, mating is one of the critical and in many cases most difficult part of breeding. If a female accepts a male, copulation may be observed within a few hours. In many cases, however, placing an adult male and an adult female together in one cage will result in aggressive interactions. There are two main reasons for these fights. One is that the individuals just do not like each other; 'love at first sight' and its cardiovascular consequences have been described by von Holst (1987). Another reason is territoriality. If the mating cage is the territory of one partner, this animal –

male or female – defends its area against the intruder. In many cases the animals become gradually familiar with another and amicable physical attractions can be observed at the 'border' between the cages so that the partition can be removed after some days. However, if aggressive interactions or fights do not cease, other partners have to be tested. When a well matched couple is found and stable pair-bonding is established, constant reproductive success is guaranteed. Under natural and laboratory conditions, breeding may occur at any time of the year and no seasonal breeding peaks have been described.

When the female leaves the nest after giving birth and having suckled the young, copulation with the male usually occurs within a few hours. Therefore, leaving a couple together is convenient and ensures regular births. Repeated pregnancy cycles are typical of a highly successful breeding colony of *Tupaia belangeri*, and female receptivity and copulation are often confined to the post-partum oestrus (Martin 1968a, 1968b).

Conception and pregnancy

In *Tupaia belangeri*, pregnancy can be detected by palpation from the second week of gestation onwards. Significant weight gain (30–50 g) and marked swelling of the abdomen are observed within 2 weeks before term. Duration of pregnancy in regular breeding pairs is in the range of 41–45 days. Breeding success is a good indicator of the general condition of the colony. Even with harmonious, healthy and well nourished breeders, successful breeding can be disrupted a variety of disturbances. Tree shrews are highly susceptible to stress and many of the problems in housing them are related to this. Loud noises, strange persons and unfamiliar care staff, overcleaning, inadequate furnishing of the cages and crowding have all been shown to be the reasons for abortion, shortened pregnancies, cannibalism or reduced amounts of milk resulting in starvation of the offspring.

Nesting

About 1 week before term, the female starts to carry nesting material into one of the two nesting boxes. For nesting material, we offer shredded paper, wood-wool or dry leaves. If no nesting material is available, the females will use pellets.

Parturition

After a relatively short gestation period, tree shrews give birth to naked and altricial pups. In most cases births occur in the morning hours, but sometimes they also occur in the afternoon.

Early development

The infants are born without fur; their ears open at around 10 days and their eyes after 20 days. The development strongly depends on the milk supply and health condition. The nipple count for all species of tupaiids falls within the range of one to three pairs. Field and laboratory studies indicate that the number of pups per litter is one to four (in some cases in the authors' colony there were five young per litter which were successfully raised). The birth weight is about 10 g.

Immediately after birth, the young are nursed and the weight of optimally fed babies is in the range of 14–20 g. Females tend to be a little heavier than males. Schwaier (1973) reports a litter size of 2.23 and sex ratio of 0.82 (males: females). For another colony of *Tupaia belangeri*, a sex ratio of 1.8 and a litter size of 2.4 was reported (Hertenstein *et al.* 1987). A survey of the *Tupaia belangeri* colony at the German Primate Center, Göttingen (1984–2007, total 2962 animals) revealed a sex ratio of 0.95 (m : f) and the following litter sizes: singletons: 158; twins: 462; triplets: 461; quadruplets: 113; quintuplets: 9.

A detailed description of growth and reproductive development in *Tupaia belangeri* from birth to sexual maturity is given by Collins and Tsang (1987) and Hertenstein *et al.* (1987).

The maternal behaviour in *Tupaia* is unusual among mammals and has been described in detail by Martin (1968a, 1968b). The tree shrew species that have been investigated (*Tupaia belangeri*, *Tupaia minor*, *Lyonogale tana*) all show an unusual nursing schedule with the infants kept in separate nests and visited by the mother for suckling only once every 48 h.

The pups receive about 5–10 ml milk. Due to the thin skin the dilated stomach appears as a light patch in the abdomen (Figure 20.5). The fat content in tree shrew milk is very high (about 25%) while the sugar concentration is low. The energy content of the tree shrew milk lies within the range of other mammals which is in general related to body size (Martin 1990). Since any suckling visit takes only 5–10 minutes, a tree shrew infant will be in contact with its mother less than 2 h during the 30 day nest phase, following which the infant is independent of its mother for milk supply. Thus, tree shrews have the lowest mother–infant contact and parental investment yet described for viviparous mammals (Martin 1990). The pattern of minimal mother–infant contact is strikingly different from the characteristic primate pattern of elaborated maternal care, juvenile dependence and enhanced social organisation (Martin 1990). In a recent field study, Emmons and Biun (1991) investigated the maternal behaviour of the Malaysian tree shrew *Tupaia* (*Lyonogale*) *tana*. Their observations confirmed the peculiar 'absentee' system previously demonstrated only in the laboratory. For the first month of life, the pups stay in a nest apart from the mother, who visits them every other day to nurse them for about 2 minutes. After they leave the nest, the mother spends a lot of time with them daily for at least 3 weeks. The male that shared the mother's territory frequently interacted with the mother during the nestling phase of the pups, but had no contact with them.

Death of young is due to premature birth (non-inflated lungs, interstitial pneumonia), cannibalism or starvation. Cannibalism of the new-born young by the mother or other adults occurs under stressful laboratory conditions which contribute to high mortality and also leads to modifications of maternal suckling behaviour. It has been observed by von Holst (1969) that increasing stress in the group modifies the suckling intervals and led to increased cannibalism of young *Tupaia belangeri* under experimental conditions.

Figure 20.5 Newborn *Tupaia belangeri* before (left) and after suckling (right). Due to the thin skin the dilated stomachs appear as light patches (*) in the abdomen.

According to reports in the literature, newborn *Tupaia belangeri* are marked by a maternal scent substance which protects against cannibalism (von Stralendorff 1982). In contrast to these observations, the authors were successful with cross-fostering strategies in cases where the mothers were unable to suckle and raise their offspring.

In order to avoid cannibalism and to control suckling success the authors separate the neonates from the parents immediately after birth. For the next 3 weeks they are kept in a nest box elsewhere in the animal facility (Figure 20.3). Since temperature regulation is immature in newborn tree shrews the floor of the nest box is warmed by a temperature-controlled heating cushion (eye heating cushion, temperature about 27°C) during the first 10 days (Figure 20.3). If they are kept too warm, they will develop a lighter fur colour; as they get older, the animals turn to their normal colour. Mothers are transferred to their litter every day for a maximum of 30 minutes. It is important to note that some females suckle their young only every second day; others have very clear daily time windows within which suckling will take place. The best suckling rhythm for each breeder can only be found by careful observation of individual animals.

Hand rearing

The extremely high fat content of the tree shrew milk is probably one reason why hand rearing is regarded as being very difficult. For successful hand rearing Tsang and Collins (1985) developed a liquid formula and a protocol which conforms to the natural weaning pattern of *Tupaia belangeri.*

Weaning and rearing

Tupaia belangeri can be weaned around day 35. In the authors' colony the young are separated from the parents at 50–60 days of age. Weaned *Tupaia* of the same sex and about equal age can be housed in peer groups (up to 10 animals depending on the size of the cage and the number of nest boxes). At 8–10 weeks of age, females and males gradually become fertile and consequently aggressive interactions increase. It is then time to separate the animals. In many cases smaller all-female groups (three animals) are stable. Males are housed singly or together with a female.

Feeding

Natural and laboratory diets

Tree shrews of the genus *Tupaia* are predominantly insectivorous. Besides insects they use fruit to add extra calories or nutrients such as calcium to a high-protein diet.

At the authors' colony, as basic food *Tupaia belangeri* are provided with a specially developed pelleted *Tupaia* diet. In addition, the animals get small pieces of fruit (such as apples, oranges, bananas, grapes, kiwi) and vegetables twice a week. Once a week, they get fruit juice and vitamins, cooked eggs or baby food and on weekends, small pieces of crisp bread. As rewards they get meal-worms, raisins, pieces of banana, dates and figs. Breeding pairs or recovering animals get, in addition to the standard food, cat chow or mashed bananas.

In the laboratory, tree shrews are reported to eat almost anything. Therefore, when pellets are not available they can be fed with steamed rice and chopped beef heart; they especially prefer soft, fat and sweet food, and all kinds of fruits and vegetables. There are, however, indications for allergic mechanisms against soybean products and oat flakes (Brack *et al.* 1990).

Water

As judged from the water content of their faeces, tree shrews absorb little water from their ingesta and they cannot stay without water for more than 1 day without serious problems. Consequently, water bottles must be controlled daily and water must always be present *ad libitum*; the mean water intake is 350 ml/kg. Tap water should be changed at least once a week. Bottles and nipples should be washed with hot water or sterilised between changes. No detergents should be used.

Laboratory procedures

Handling and training

For quick and easy catching tree shrews can be locked in their nesting boxes as the animals usually slip into the boxes as soon as somebody enters the room. When removing the animals and to protect the experimenter's hands, the animal is gently wrapped in a cloth (40 cm × 40 cm) (Figure 20.3). Since their teeth are small, bites are not dangerous but can be painful. Using this technique, no difficulties occur with the usual procedures such as daily external body inspection, weighing, urine and blood collection, temperature and blood pressure recording, and application of substances by different routes. Tree shrews can be easily trained for memory tests, for example, by positive reinforcement techniques (Ohl *et al*. 1998; Bartolomucci *et al.* 2001; see also Chapter 16).

Physiological monitoring

Rectal temperature can easily be measured by thermometers of the type used in humans. Transmitters can be implanted for long-term studies.

Collection of specimens – blood, urine

Blood withdrawal (about 500 µl) by puncturing the venous plexus at the lower side of the tail with a small scalpel is recommended by some authors. Prior to puncture, the tail should be shaved and rubbed with a silicon paste which improves the blood collection. Blood flow can be enhanced by holding the tail under a heating lamp. This technique, however, requires experience and is therefore often unsatisfactory. Another approach is described by Schwaier (1974), taking blood from the saphenous vein. Following the recommendations of GV-SOLAS for small laboratory animals not more than 0.7 ml blood per 100 g body weight at a time should be collected. In cases of repeated blood sampling (eg, over 2 weeks) not more than 0.07 ml/100 g body weight should be collected within 24 h. Despite being completely relaxed during blood sampling, the procedure *per se* seems to be stressful for the animals as documented by increased basal heart rate over several days after one blood sampling procedure (Stohr 1988).

Morning urine can easily be collected from animals that have been confined to their nest box shortly before the lights turn on in the animal rooms. A slight massage of the hypogastrium gives between 1 and 7 ml of urine. Another approach is to place plastic mats with wells under the cages and to collect the urine later with a pipette out of the wells.

Urine analysis has proved to be a stress-free and reliable procedure for long-term monitoring of the physiological status of *Tupaia belangeri*. Among these are parameters for metabolic activity (Johren *et al.* 1991), various bioactive compounds (Collins *et al*. 1989; Fuchs & Schumacher 1990; Fuchs *et al.* 1992) and urinary proteins which play a crucial role in olfactory communication (Weber & Fuchs 1988). Age and time of the day may have an impact on the values (Fuchs 1988; van Kampen & Fuchs 1998). For urinary data see Table 20.2.

Administration of medicines

Most routes of applications, such as subcutaneous, intramuscular, intraperitoneal or oral, are easy to perform. For intravenous injections it is recommended to use the saphenous vein (Schwaier 1974).

Anaesthesia

Adequate anaesthesia is a prerequisite for using an animal species in the laboratory for a wide range of experiments. Early studies used pentobarbital in a comparatively high dose of 75 mg/kg.

A quick and safe injection anaesthetic is the so called Goettinger Mixture II (GM II) consisting of ketamine (50 mg/ml), xylazine (10 mg/ml) and atropine (0.1 mg/ml). The dosage is 0.1 ml/100 g body weight. Anaesthesia usually occurs within 5 minutes of the intramuscular injection, and general anaesthesia lasts about 20–45 minutes. For longer general anaesthesia, inhalation anaesthesia is recommended. For inhalation anaesthesia, animals had to be artificially ventilated through an endotracheal tube (home-made from high-med-PE-micro-tube, inner diameter 1.75 mm, outer diameter 2.08 mm). Our experiences with a respirator for small animals show that 0.5–2% isoflurane in a mixture of 30% oxygen and 70% N_2O, with a respiration rate of 35 per minute with an inspiratory phase of 35% and plateau phase of 5% work fine for *Tupaia belangeri.*

Inhalation anaesthesia is used following induction by injection anaesthesia (eg, GMII). The jaw bone is placed on the fingers and the head is fixed by placing the thumbs behind the skull. The mouth is opened by introducing the laryngoscope. Sometimes the epiglottis can be held down with the tip. After inserting the endotracheal tube, correct placement must be checked by auscultation; once corrrect placement is confirmed, the tube can be held in place with a strip of adhesive tape (Figure 20.6).

Euthanasia

The use of animals in research, including euthanasia, is a sensitive issue. Because of differences in national regulations, each researcher will be required to obtain clearance from the local or national ethical committee for research prior to conducting euthanasia. In our opinion, cervical dislocation is not an appropriate method of euthanasia for tree shrews. We recommend either an overdose of sodium

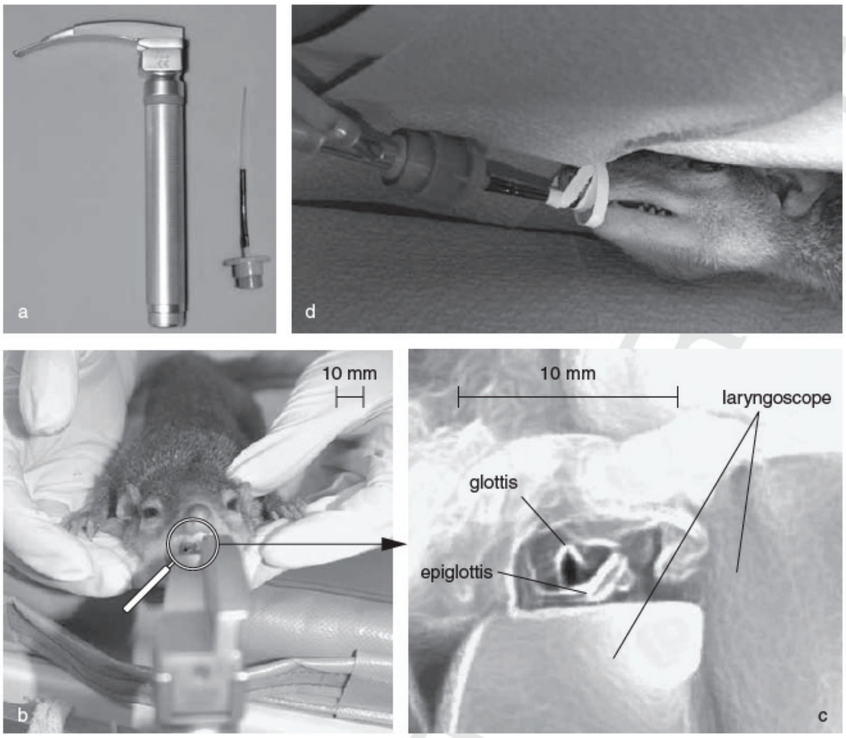


Figure 20.6 Intubation of a tree shrew (*Tupaia belangeri*) for inhalation anaesthesia. (a) Laryngoscope and homemade micro-tube (for details see text). (b) Fixation of the head and position of the laryngoscope. (c) Enlarged view of (b) showing glottis and epiglottis. (d) After successful intubation, the tube is held in place with a strip of adhesive tape.

pentobarbital, for example, or decapitation. Carbon dioxide has been used on tree shrews and has proven to be an appropriate means of euthanasia. In any case, after exposing animals to an overdose of anaesthetics it is always mandatory to confirm death by, for example, cervical dislocation, as an appropriate secondary means of euthanasia.

Common welfare problems

Health problems

Tree shrews experience relatively few health problems. However, our experience shows that gastritis can be a frequent problem. In this case, animals stop eating and a lack of faeces can also be observed. Gastritis can be effectively treated with cimetidine (10 mg/kg body weight, twice daily orally).

Diarrhoea is another symptom often seen in a colony. Mostly *Escherichia coli variatio haemolytica*, *Klebsiella pneumonia* or protozoa (*Giardia*, *Trichomonas*, *Entamoeba*) are the cause of this symptom. Before antibiotic treatment of diarrhoea, appropriate diagnostic and resistance tests are strongly recommended.

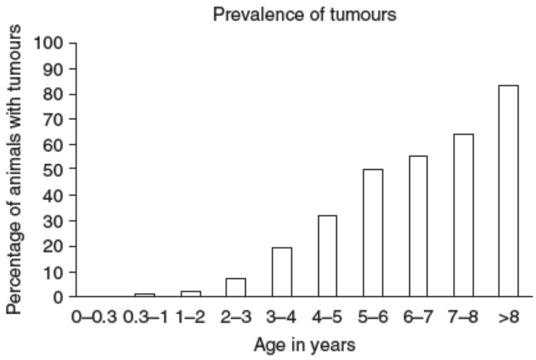
Infection with the cestode *Tupaiataenia quentini* was successfully treated with praziquantel (Brack *et al.* 1987). Intestinal trichomoniasis with *Tiritrichomonas mobilensis* (mostly in the caecum) has also been described as well (Brack *et al.* 1995). Enteropathy of the upper digestive tract was due to a foodstuff allergy against oat flakes and soybean products (Brack *et al.* 1990).

Penis prolapse is another possible health problem. We found this in several males of different ages with unknown cause. The prolapse is not lethal by itself, but since there is no effective treatment and the penis becomes irritated and swollen, in most cases, the animal has to be euthanased.

Automutilation can often be found if the cages are too small, overcrowed or the animals are disturbed too much. Moving the animal to a bigger or quieter area in the facility is the first step in treatment. In some cases, standard antibiotic treatment and amputation of the affected body part (eg, a toe or the distal part of the tail) is necessary.

Tupaia seem to be prone to the spontaneous development of gallstones with fatty and cholesterol-rich diets (Schwaier 1979). In two reports mite infestations were described (Bever 1985; Brack *et al.* 1989).

Most tumours of the genital system of male animals have a Leydig cell origin and occur unilaterally. Tumours of the female genital system are predominantly mammary tumours or sometimes ovarian tumours. Tumours of the haematopoietic system are malignant lymphomas; tumours of the integument occur mostly in the jugulo-sternal gland. Similar to humans, the incidence of tumours increases with age (Brack



Types of tumours	
Genital system	34%
Haematopoietic system	24%
Respiratory system	22%
Integument	8%
Gastrointestinal system	5%
Urinary bladder	2%

Figure 20.7 Common spontaneous tumours and incidence of tumours, which rises with age (with modifications from Brack 1998).

1998). The most common spontaneous tumours are summarised in Figure 20.7.

Two tree shrew specific viruses are known and reported in the literature so far: the Tupaia herpes virus (THV) (five different types), which might be a cause for tumours (Darai *et al.* 1982), and the potentially non-pathogenic Tupaia paramyxovirus (TPMV) (Tidona *et al.* 1999).

Concluding remarks

Despite their obvious attractiveness there are limitations to the use of tree shrews in research. Major limitations are housing and breeding, both of which are time consuming and expensive. Obviously, this constraint explains why many laboratories are not capable of using these animals for their study programmes. Further research is required to collect basic data on tree shrews to inform their housing and care.

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