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Source: Folia Zoologica, 67(2) : 69-77

Published By: Institute of Vertebrate Biology, Czech Academy of Sciences

URL: https://doi.org/10.25225/fozo.v67.i2.a1.2018

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Folia Zool. – 67 (2): 69–77 (2018) DOI: 10.25225/fozo.v67.i2.a1.2018

Clinical examination methods and investigation into the occurrence of endoparasites, Borna disease virus and genetic variability in the garden dormouse, *Eliomys quercinus*

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Received 31 December 2017; Accepted 11 June 2018

Abstract. Facing the ongoing decline in garden dormice (*Eliomys quercinus*) in large parts of Europe, data about genetic diversity, weight, size, and parasite burden were collected from live individuals (n = 156) from Hesse and Rhineland-Palatinate, Germany. Blood was collected under Isoflurane-oxygen-anaesthesia from the anterior vena cava or jugular vein, respectively. Sex, weight, tibiotarsal and tail length of all animals were recorded. Genetic analysis (n = 64) using hair samples, viral examinations of oral, ocular and anal swabs (n = 156) and parasitological examination of faecal samples (n = 57) were performed. Genetic analysis of mitochondrial DNA revealed three haplotypes in the study area (WE4, WE4-3, WE5). Microsatellite analysis demonstrated a comparably high genetic diversity with 66 % overlap of alleles, a low genetic distance (D_{Jost} 5.6 %) and a low index of differentiation (F_{ST} = 0.02) between individuals from Hesse and Rhineland-Palatinate. All swab samples were negative for Borna disease virus. In 47 % of the faecal samples *Entamoeba* sp. cysts, *Eimeria* sp. oocysts, and eggs of trematodes, cestodes (*Hymenolepis* spp.) and nematodes (*Capillaria* spp., Strongylida) were detected. To our knowledge, this is the first report of inhalation anaesthesia, clinical examination, blood collection and assessment of Borna disease virus in living garden dormice.

Key words: health assessment, dormice, anaesthesia, blood collection, mitochondrial sequence, microsatellite analysis

Introduction

Populations of the garden dormouse, *Eliomys quercinus* (Rodentia, Gliridae), are facing a significant decline over the last decades. Especially in the eastern range of its distribution, the species may have disappeared from as much as 50 % of its former range during the last 30 years (Meinig & Büchner 2012, Bertolino 2017). The species has declined more than almost any other rodent in Europe (Temple & Terry 2007). Thus, garden dormice are listed as "near threatened" on the International Union for the Conservation of Nature (IUCN) Red List and are protected by Appendix III of the Bern Convention (The European Community, decision 82/72/EEC, Official Journal of the European Communities L38/1 of 10.02.1982); however, there is little information available on the current rate of decline (Bertolino et al. 2008).

The reasons for this decline are still not understood. Decreases in insect food availability through increasing use of pesticides and increases in monoculture are suspected causes, besides competition with other rodent species (Macdonald & Barrett 1993, Perez et al. 2013). In some regions, the number of garden dormice is also reduced by direct human intervention such as hunting and consumption or pest control measures to remove individuals from orchards and households (Carpaneto & Cristaldi 1995). To counteract the population decline in Germany regional translocation

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and species conservation projects were initiated in the state of Hesse. These focus on the release of individuals in suitable habitats after they had been trapped in houses by private persons and brought to rehabilitation centres.

Infectious diseases might be an important factor for the species decline in garden dormice too, but the and correlations between health status and parasitic burden are lacking, the significance of parasites and other pathogens for the health of garden dormice is unknown. In this regard, the situation of animals in rehabilitation centres and the potential of pathogen dissemination upon translocation and release has not been studied. Moreover, the presence of potentially

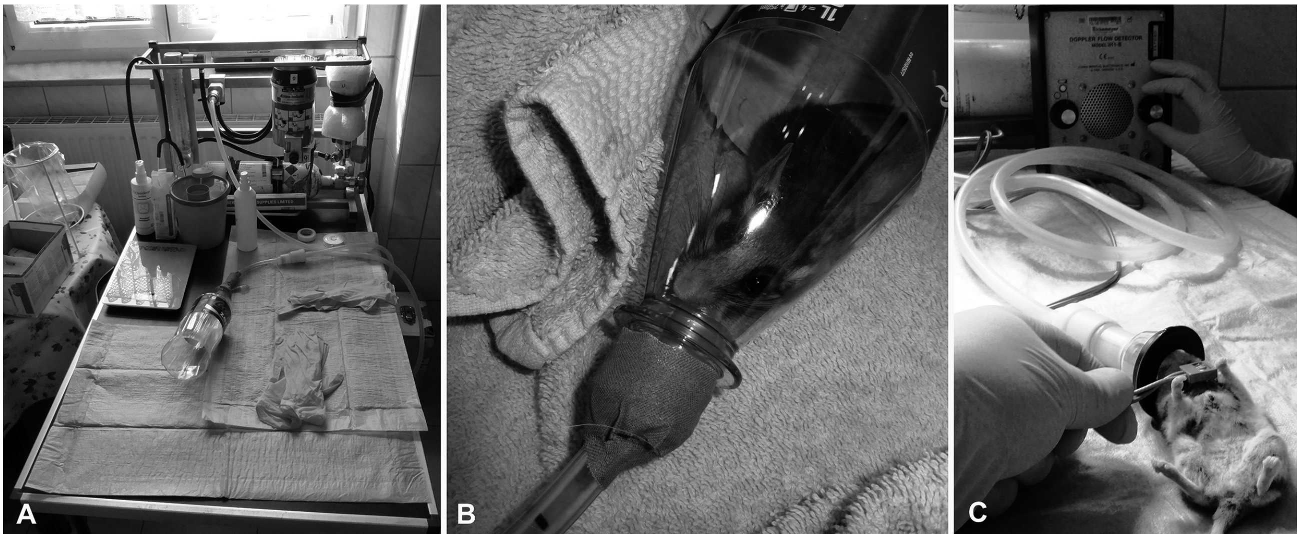

Fig. 1. Anaesthesia and narcosis monitoring. A) portable gas inhalation machine, B) bottle as tunnel-shaped induction mask, C) anaesthesia maintenance via face mask and monitoring via Doppler technique.

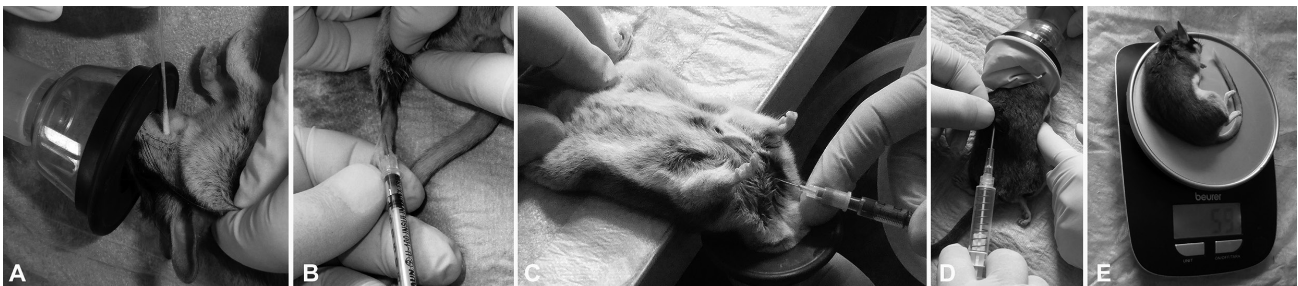

Fig. 2. Sampling, measuring and tagging of garden dormice. A) taking an oral swab, B) blood collection from saphenous vein, C) blood collection from anterior vena cava, D) subcutaneous PIT placement, E) weighing.

incidence of bacterial, viral or parasitic diseases in garden dormice is largely unknown. Moreover, the role of garden dormice as vectors or reservoirs for infectious and potentially zoonotic agents, as recently reported for shrews (Bourg et al. 2013, Weissenbock et al. 2017), is poorly understood. In previous studies, several parasites have been investigated in garden dormice (Chute 1960, Matuschka et al. 1999, Bertolino & Canestri-Trotti 2005, Kvičerová et al. 2011, Libois 2016, Makarikov 2017), but serological surveys failed to detect antibodies against viruses in single trapped garden dormice collected during euthanasia (Boulanger et al. 1996, Ledesma et al. 2009). As clinical examinations of living individuals zoonotic viruses such as Borna disease virus (Rott et al. 1991) has not yet been investigated in garden dormice and methods for swab sampling and blood collection have not been established for this species.

Genetic analyses have been performed in order to investigate phylogeographic patterns and to assess levels of genetic variability. Low levels of genetic diversity may result from population decline and constitute a potential future threat for the long-term survival of the species (Keller & Waller 2002, Charlesworth & Willis 2009). Former phylogeographic analysis of garden dormice across Europe revealed a high karyotypic diversity and a variable number of 48-54 chromosomes (Perez et al. 2013). Moreover,

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four evolutionarily significant units corresponding to Iberian, Italian, western European, and Alpine mitochondrial lineages have been identified, which were partially congruent with the chromosomal races (Perez et al. 2013). Even so the haplotypic variability has been demonstrated to be lower in northwestern European populations than on the Mediterranean region (Perez et al. 2013), data from distinct regions such as Hesse and Rhineland-Palatinate are missing. Alongside analysis of mitochondrial DNA, this study is the first analysing nuclear genetic diversity in the garden dormouse with microsatellites.

The aims of this study were firstly to establish humane methods and techniques to examine, anaesthetize and sample live garden dormice in order to enable studies on genetic structure of the population, biological scales and measures such as body weight and tibiotarsal length and to evaluate their accordance with animal welfare standards. Secondly, these methods should enable investigations of various potential pathogens in the future. In this regard, the presence of Borna disease virus genome and of endoparasite stages would be investigated, being two important examples for the examination of infectious agents in live garden dormice.

Material and Methods

Clinical examination, measuring of body weight, tibiotarsal and tail length and sampling of animals

Garden dormice (n = 156) originating from the German federal states Hesse and Rhineland-Palatinate were examined in a wildlife rescue centre prior to release. Using protective gloves, individuals were taken from artificial nest boxes, which were later used as release boxes, and transferred into a tunnel-shaped induction mask. To reduce stress and to prevent tail autotomy, inhalation anaesthesia was performed using an Isoflurane-oxygen-mixture (5 % induction via tunnel-shaped induction mask, 1.5-2.5 % maintenance via a face mask for laboratory rodents; Isofluran CP, CP-Pharma Handelsgesellschaft mbH, Burgdorf, Germany) via a portable gas inhalation machine (Marmed, Cölbe, Germany) (Fig. 1A, B). Anaesthetised animals were placed in dorsal recumbency and monitored constantly by visual inspection of respiration and cardiovascular doppler monitoring (Parks Medical Electronics, Aloha, OR, U.S.A.) (Fig. 1C). To this end, the sensor of the doppler was placed just ventral to the heart, median in the upper third of the thorax. Moreover, measuring peripheral oxygen saturation (SpO_2) was attempted at the animals' feet using tongue clips (Veterinary Handheld Pulse Oximeter UT100V, Utech Co. Ltd., Chongqing, China).

Dry swabs from mouth (Fig. 2A), eyes and anus were taken, faeces and urine was collected if released. Sex, weight, tibiotarsal and tail length of all animals was recorded. Body mass was measured with a digital scale to the nearest gram for all animals handled (Fig. 2E). Hind foot and tail length where measured with a ruler to the nearest millimeter. The measurements and weight of males and females were compared using a paired t-test (MS Excel 2013, Microsoft Corporation, Redmond, WA, U.S.A.).

In the absence of descriptions in dormice, blood collection was pre-assessed in 45 animals regarding accessibility of the vein, side effects of blood collection, such as haemorrhages or haematomas, and handling time needed to collect 0.2-0.4 ml of blood from each animal. Thereafter, in 45 animals blood was taken peripherally from the cephalic and/or the saphenous vein (Fig. 2B) and compared to central blood collection from the anterior vena cava or the jugular vein (Fig. 2C) in 45 other animals. Venipuncture was attempted four times in order to collect a minimal amount of 0.2 ml of blood, otherwise blood collection was rated as unsuccessful. After this pre-assessment in 90 animals, the anterior vena cava and the jugular vein were selected as preferred blood collection sites in all remaining animals. Blood smears and lithium heparin plasma were stored for further investigation.

Hair samples were taken by pulling out hairs including hair follicles from the back of the animals using a haemostat. Passive integrated micro-transponder (PIT) tags (AL-Vet Mini ISO transponder, Albrecht GmbH, Aulendorf, Germany) were placed subcutaneously between the shoulders (Fig. 2D).

All procedures on animals were in accordance with German animal welfare laws and permitted by the competent authority (RP Darmstadt Az: V54-19 c20/15).

Parasitological examination

Faecal samples were collected directly from individuals when the animals defecated during the procedure. They were immediately saved in SAF-medium and subsequently analysed microscopically using standard procedures (Marti & Escher 1990). Additionally, Copro-antigen ELISAs for *Giardia* (ProSpect® Giardia Microplate Assay, Oxoid Ltd., U.K.) and *Cryptosporidium* (ProSpect® Cryptosporidium Microplate Assay, Oxoid Ltd., U.K.) were performed according to manufacturer's instructions.

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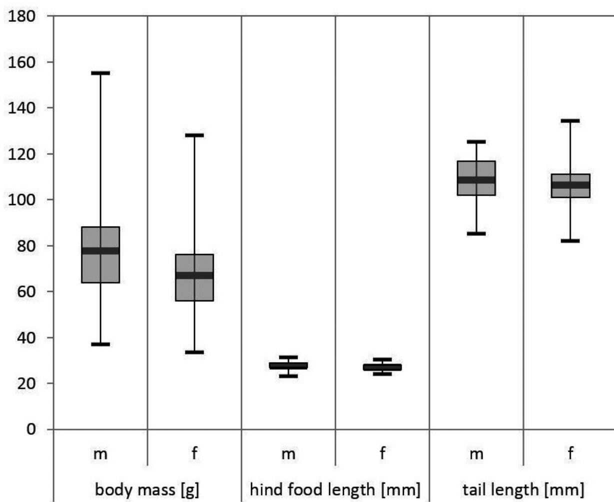

Fig. 3. Weight, tibiotarsal and tail length of garden dormice.

Table 1. Overview of the results of the parasitological examinations.

Parasite family and genus		Prevalence in samples examined (n = 57)
Protozoa	*Eimeria* sp.	2 %
	Entamoeba sp.	2 %
Cestoda	*Hymenolepis* spp.	25 %
Nematoda	*Capillaria* spp.	14 %
	Strongylida	2 %
Trematoda	not specified	2 %

Virological examination

The presence of Borna disease virus genome was examined via conventional reverse transcriptase (RT)-polymerase chain reaction (Thermocycler PTC-200, M. J. Research, St. Bruno, Canada) using primers (ABV_Lconsensus, ABV_Nconsensus) for the detection of L and N genes of Borna disease virus according to standard methods (Kistler et al. 2008). To this end, total RNA was extracted using the RNeasy Mini Kit (Qiagen, Hilden, Germany). Isolated RNA was transcribed by random hexamer primers using RevertAid H Minus Reverse Transcriptase according to manufacturer's information (Thermo Fisher Scientific, Dreieich, Germany). Resulting complementary DNA (cDNA) was used for PCR. PCR reactions carried out with High Fidelity PCR Enzyme Mix (Thermo Fisher Scientific, Dreieich, Germany) in 35 cycles of PCR: 94 °C, 30 seconds; 52 °C, 30 seconds; 72 °C, 30 seconds. PCR products were analyzed by agarose gel electrophoresis (1 % agarose gel).

Table 2. Results of the genetic analyses. Abbreviations: number of samples (n), number of alleles (A), number of private alleles (pA), effective number of alleles (A_E), detected heterozygosity (H_O) and expected heterozygosity (H_E).

Region	n	A	pA	A_E	H_O	H_E
Belgium	5			2.5	0.54	0.63
Netherlands	11			1.8	0.31	0.42
Hesse	32	33	6	3.0	0.52	0.67
Rhineland-Palatinate	32	35	8	3.6	0.59	0.73

Genetic analysis

DNA of hair from 64 of 134 hair samples was extracted using standard QIAGEN® kits. PCR conditions and analysis followed the protocols used in previous studies and was performed using Biometra®T1 Plus

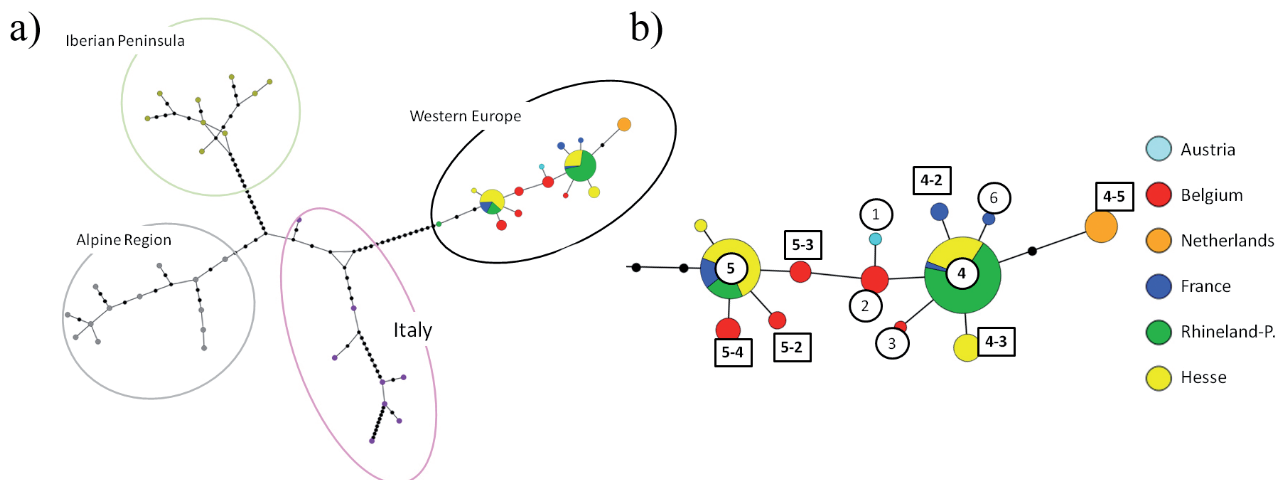

Fig. 4. a) Haplotype network using all known haplotypes of *Eliomys quercinus* in Europe (Perez et al. 2013) including haplotypes of this study. b) Haplotypes found in this study belong to the western European clade. Haplotype names in boxes are newly described all others were already described by Perez et al. 2013.

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thermocyclers (Reiners et al. 2014, Steyer et al. 2016). Mitochondrial sequence analyses of cytochrome-*b* (Cyt*b*) gene (655 base pairs) were done as previously described (Perez et al. 2013). Microsatellite analysis was performed using eight microsatellite markers (Václav Mikeš pers. comm., Hürner et al. 2009). Thirty-two samples were selected from dormice from Hesse and 32 from individuals from Rhineland-Palatinate, two German regions separated by the River Rhine. A minimum of 30 genotypes are necessary for a profound characterisation of genetic diversity and genetic distances. Results were compared to genetic data from garden dormice from the Netherlands (n = 11), Belgium (n = 14), France (n = 4) and Rhineland-Palatinate, Germany (n = 1). In order to determine the geographic signature of garden dormice in these regions basic population genetic parameters and principal coordinates analysis (PCA) were calculated using GENALEX6 (Peakall & Smouse 2006). Genetic distances were estimated by classic F_{ST} and standardized D_{Jost}, where the latter is more prone to unbalanced data (Jost 2008). For Bayesian interference of population structure, the program STRUCTURE was used (Pritchard et al. 2000). The haplotype network was drawn using popart (Leigh & Bryant 2015).

Results

Anaesthesia, clinical examination, scaling, measuring and sampling of animals

Isoflurane anaesthesia and clinical examinations were performed without complications. Animals ran unhesitatingly into the tunnel shaped induction mask, when it was darkened by placing towels around it, and induction time was always below 30 seconds using a 5 %-Isoflurane-oxygen-mixture. Thereafter, maintenance of anaesthesia was feasible at 1.5-2.5 %-Isoflurane-oxygen-mixture via a face mask. Rhythm and quality of heart beats could be monitored constantly by using the doppler technique placed at the upper third of the ventral thorax. In contrast, it was not possible to measure peripheral oxygen saturation (SpO^2) at the animals' feet, ears or tongue using feline tongue clips.

Females of garden dormice were significantly ($p < 0.005$) lighter than males but other body measurements did not differ significantly ($p > 0.05$). An overview of body weight, tibiotarsal and tail length of the animals handled is shown in Fig. 3.

Collection of 0.2-0.3 ml of blood was successful in 155 out of 156 animals, without the occurrence of subsequent complications, by puncture of peripheral veins (cephalic or saphenous vein) (n = 45 animals) or central veins (jugular vein or cranial vena cava) (n = 111 animals). Due to the small diameter of peripheral veins, blood collection at the distal parts of the fore legs (cephalic vein) and of the hind legs (saphenous vein) were only feasible using 30 Gauge sized insulin cannulas (needle diameter 0.3 mm) (OMNICAN Insulinspritze 0.5 ml U40 0.3 × 8 mm or Sterican Insulin 30 Gauge 0.3 × 12 mm, B. Braun Melsungen AG, Melsungen, Germany). To assist this, hairs at the puncture site were combed aside after application of alcoholic disinfectant spray, and an assistant compressed the proximal vein at the medial aspect of the upper legs. In general it was difficult to enter and to stay long enough inside the vein to collect sufficient blood volume. Moreover, peripheral veins were prone to collapse and mild to midgrade haematoma was seen in the majority of cases. In 10/45 animals the puncture of two, in 1/45 animals the puncture of three and in 1/45 animals the puncture of four peripheral veins was necessary in order to collect 0.2-0.3 ml of blood.

Blood collection from the anterior vena cava or jugular vein was performed using a 27 Gauge needle (Sterican 27 G 0.40 × 25 mm). The animal was moved to the edge of the table and the animal's head and neck were held downwards at a 45 degree angle. After disinfection with alcoholic disinfectant spray, the needle was inserted paramedian at the ventro-lateral throat region close to the cranial thoracic aperture. Subcutaneously, inside the syringe a vacuum of 0.05-0.1 ml was adjusted and the needle was moved carefully towards the opposing hip joint until blood was visible inside the conus of the cannula. In some cases, careful withdrawal of the needle was necessary to achieve blood collection. In some animals blood collection was successful after inserting the needle less than 5 mm, but in others insertion of more than 10 mm was necessary. The former were assigned to jugular venepuncture while the latter were assigned to puncture of the cranial vena cava. In 2/111 animals an increased respiratory rate (increased respiratory frequency and depth) was noticed during insertion of the needle towards the chest which may have been associated with penetration of respiratory tract organs (trachea, bronchi or lung). However, respiration normalized shortly after blood collection in these two animals.

Blood collection from the anterior vena cava or jugular vein was performed much faster compared to saphenous and cephalic veins. This led to a reduced mean anaesthesia time of 5:33 (2:37-13:00) minutes compared to 10:19 (6:30-21:15) minutes.

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All animals recovered from anaesthesia within 1-2 minutes and were placed individually in artificial nest boxes to ensure complete arousal. After 3-4 hours resting time, these nest boxes were transported to the release site and placed in a cage. Animals were monitored for seven days there and re-identified by individual PIT reading. After seven days acclimatisation, doors of the release cage were opened and the animals were allowed to leave "soft release" method).

Parasitological examination

The results of the parasitological examination of 57 faecal samples are summarized in Table 1. In total, 47 % of all samples contained stages of endoparasites from various parasite classes (Protozoa, Cestoda, Trematoda and Nematoda) and 12 % pseudoparasites such as Acari (e. g. *Gamasina* sp.). *Eimeria* sp. oocysts measured on average 13.7 × 12 µm. Trematode eggs were on average 30 × 16 µm sized, capped, and contained a miracidium. Copro-antigens of *Giardia* spp. and *Cryptosporidium* spp. were not detected in any sample (n = 57).

Virological examination

Swabs from eye, mouth and anus of all animals were negative for the Borna disease virus genome.

Genetic analysis

Mitochondrial sequence of the Cyt*b* gene identified three haplotypes WE4 (n = 11), WE4-3 (n = 5) and WE5 (n = 16) in Hesse and two of these haplotypes, WE4 (n = 27) and WE5 (n = 5), in Rhineland-Palatinate (Fig. 4). Haplotype WE4-3 was only demonstrated in the region around the city of Wiesbaden (Hesse).

Microsatellite analysis of genomic DNA revealed a similar distribution of allelic numbers in Hesse (n = 33) and Rhineland-Palatinate (n = 35) in comparison to the total number of 41 alleles. The common overlap of alleles is 66 % and the genetic distance between both regions besides the River Rhine is 5.6 % (D_{Jost}) with a low index of differentiation (F_{ST} = 0.02). However, unique alleles that were found in a specific region were present in Hesse (n = 6) and also in Rhineland-Palatinate (n = 8).

Genetic diversity, represented by the effective number of alleles (A_E), detected heterozygosity (H_O) and expected heterozygosity (H_E), was high (Hesse: A_E: 3, H_O: 0.52, H_E: 0.67; Rhineland-Palatinate: A_E: 3.6, H_O: 0.59, H_E: 0.73) compared to the Dutch and Belgian populations (Belgium: A_E: 2.5, H_O: 0.54, H_E: 0.63; Netherlands: A_E: 1.8, H_O: 0.31, H_E: 0.42) (Table 2).

Individual genetic profiles of garden dormice were investigated using PCA and STRUCTURE, but failed to depict a clear regional profile except for individuals from one region in Rhineland-Palatinate (not displayed). The six animals originating from Bingen-Ockenheim showed a group-specific genetic pattern.

Discussion

Isoflurane inhalation anaesthesia was safely and easily applicable via a portable gas inhalation machine enabling easy and less stressful sampling compared to conscious animals. This was in accordance with experience of isoflurane inhalation anaesthesia in African dormice, *Graphiurus* spp., used in laboratory conditions (Kastenmayer et al. 2010). The anaesthesia offered benefits in respect of animal welfare and working safety. Heart rate and breathing frequency were lowered, injury of the tail (tail autotomy, a well known phenomenon in dormice), was prevented and staff were not injured during examination and sampling (Long & West 2012). Compared to reported anaesthesia and recovery times of injectable anaesthesia in rodents (10-120 minutes) (Gargiulo et al. 2012), the application of isoflurane anaesthesia had flexible anaesthesia times and a very short-term recovery time of at most two minutes in garden dormice.

Female garden dormice weighed significantly less than males, but other body measurements did not differ significantly between the sexes. Body weights might have been influenced by feeding and increased food availability at the rehabilitation centre, where animals were kept for several days or weeks prior to examination and release. The average weight of 78 g in males and 67.3 g in females were in accordance with limited weight data in a previous study (Matuschka et al. 1999). Previously, a weight above 50 g has been associated with mature and healthy individuals and a weight below 50 g to juveniles and individuals which have not fully recovered from hibernation (Matuschka et al. 1999).

In this study, blood collection from a large number of live garden dormice was reported. In previous studies blood collection has been performed during euthanasia in one and in three trapped individuals, respectively (Boulanger et al. 1996, Ledesma et al. 2009). Blood collection was achieved from the cephalic vein, the saphenous vein, the anterior vena cava and the jugular vein. The collection from the latter two central veins were adapted from established techniques in laboratory animals (Parasuraman et al. 2010) and the main benefits were faster completion of blood collection rather than using the saphenous and

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cephalic veins. In peripheral veins, blood flow was slower, the likelihood of haematoma development and the need to puncture more than one vein in order to obtain sufficient blood volume increased. Besides haematoma at peripheral veins and temporarily changed respiration in two animals after the puncture of central veins, no side effects of blood collection were observed during the study.

Subcutaneous PIT placement at the upper back was in accordance with procedures in laboratory animals and other small mammals (Deutsch 2015) and feasible without complication. However, in some animals a short scratching movement was observed upon awakening. Tagging the animals before translocation and release at the release site was sufficient to enable the identification and monitoring of individuals in this species conservation project. Reading the PIT tags was easily done, e.g. when animals slept in nest boxes, even from outside of the boxes.

Endoparasites found in animals in the present study are mostly reported to cause subclinical infections in dormice, with *Eimeria* spp. having a potentially pathogenic character (Makarikov 2017). The prevalence of Hymenolepididae in the faecal samples examined was higher, but the prevalence of *Eimeria* spp. and Strongylidae was lower than in a study in the Bohemian Forest and Český Les Mountains, Czech Republic (Strongylidae 7.4 %, Hymenolepididae 3.7 %, *Eimeria* spp. 85.2 %) (Kvičerová et al. 2011). This may be explained by husbandry related re-infection cycles and oocyst accumulation inside cages of the rehabilitation centre. This does not apply to free-ranging animals. Interestingly, *Eimeria* sp. oocysts in the present study may belong to an *Eimeria* species which has not been reported in garden dormice before, as oocysts were smaller compared to all other reported *Eimeria* spp. from garden dormice (Bertolino & Canestri-Trotti 2005, Kvičerová et al. 2011). However, this hypothesis cannot be proven by parasitological examination of the faeces alone and further molecular biological and/or transmission studies are needed. Acari such as *Gamasina* sp. are regarded as pseudoparasites. They are usually ingested with the food and are only short-term transient in the animals' gastro-intestinal tract.

The genome of Borna virus was not detected in any samples from garden dormice and thus it does not seem to be present in the population examined. Therefore, there is no suggestion that garden dormice may be a reservoir or vector for Borna disease virus as described previously in shrews (Bourg et al. 2013, Weissenbock et al. 2017). As garden dormice are phylogenetically closely related to squirrels (Blanga-Kanfi et al. 2009), the presence of recently discovered variegated squirrel Borna virus should be investigated in future studies. The PCR used in the present study (Kistler et al. 2008) may have failed to detect variegated squirrel Borna virus and therefore samples should be screened again using a different, recently developed RT-PCR (Hoffmann et al. 2015).

Genetic investigations identified the haplotypes WE4, WE4-3 and WE5 in the present study of garden dormice. WE4 and WE5 have been identified previously in France and WE4 in Leiwein (Rhineland-Palatinate, Germany; (Perez et al. 2013), while Haplotype WE4-3 has not been identified elsewhere. However, WE4-3 differs by only one base pair from WE4 and thus seems to be closely related. In comparison to all available haplotypes from garden dormice in Eurasian countries, the three detected in this study seem to match to the geographical lineage of West Europe as defined previously (Perez et al. 2013).

Genetic diversity of the garden dormouse population examined in Hesse and Rhineland-Palatinate was high and equally distributed compared to isolated populations in Belgium and the Netherlands. Detected heterozygosity (H_O) was lower than expected heterozygosity (H_E) which points towards a small-scale population structure in the regions. However, further studies with a strategic sampling approach and a higher number of samples are needed to confirm this statement.

Surprisingly the River Rhine seems to be no genetic barrier for the garden dormice in Hesse and Rhineland-Palatinate, which contrasts with studies describing such barriers for other species (Würstlin et al. 2016). This finding may be explained by accidental or intentional transport of dormice by humans from one side of the river to the other or by migration over anthropogenic bridges. Therefore, with regard to the translocation project, it can be concluded that animals may be released at suitable release sites independent of their specific origin without the risk of falsifying genetic patterns.

The group-specific genetic pattern of animals originating from Bingen-Ockenheim may be a result of genetic separation, but it is also likely that the sampled individuals were more closely related i.e. representing a family structure. As samples of individuals were collected spatially, future studies should either sample dormice equally stratified on a broad scale, or if relatedness is of special interest, should be sampled intensively on a small scale.

The genetic analysis of garden dormice in this study was the first using microsatellites and covering so

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many samples. As in this study, genetic analysis should be an integral part of reintroduction projects to confirm the genetic origin of individuals (La Haye et al. 2017). To our knowledge, this is the first report of clinical examination, anaesthesia and sampling in living garden dormice. The methods applied for sampling and measuring were performed without any complications. In the future, other pathogens may be included in the examination as this study provides a solid basis for sample collection and processing in a wild dormouse species.

Acknowledgements

The authors thank the Lower Nature Conservation Authority (Untere Naturschutzbehörde) of Rheingau-Taunus-Kreis, Germany for funding and Dr. Dirk Enderlein from the Clinic for Birds, Reptiles, Amphibians and Fish of Justus Liebig University Giessen for critical revision of the manuscript and assistance with the virological examination. Moreover, the authors thank Nadine Vervoort from the rehabilitation centre for garden dormice in Wiesbaden and Wolfgang Jost and his team from the Nature and Biodiversity Conservation Union (NABU e.V.) group Niedernhausen for their assistance during handling, examination and release of the dormice and Dr. Dania Richter for critical revision of the manuscript.

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