Ann. Zool. Fennici 50: 256–261 ISSN 0003-455X (print), ISSN 1797-2450 (online)
Helsinki 31 October 2013 © Finnish Zoological and Botanical Publishing Board 2013

The use of silicone casts in collection of morphological data from free-ranging wildlife — the case of tracheobronchial anatomy of the Eurasian lynx (*Lynx lynx*)

Juha Laakkonen* & Matti Kivalo

*Division of Veterinary Anatomy and Embryology, Department of Veterinary Biosciences, Faculty of Veterinary Medicine, B.O. Box 66, FI-00014 University of Helsinki, Finland (*corresponding author: juha.laakkonen@helsinki.fi)*

Received 3 Jan. 2013, final version received 21 Mar. 2013, accepted 28 Mar. 2013

Laakkonen, J. & Kivalo, M. 2013: The use of silicone casts in collection of morphological data from free-ranging wildlife — the case of tracheobronchial anatomy of the Eurasian lynx (*Lynx lynx*). — *Ann. Zool. Fennici* 50: 256–261.

We tested the usefulness of silicone casts for gathering morphological data from free-ranging wildlife by documenting the tracheobronchial anatomy of the Eurasian lynx (*Lynx lynx*). The silicone compound accurately reproduced the main aspects of the tracheobronchial branching but the demonstration of smaller airways was less accurate. Evaluation of air-inflated specimens and tracheobronchial casts showed that the right lung consisted of cranial, middle, caudal and accessory lobes, whereas the left lung was divided into cranial and caudal lobes. The left cranial lobe was further divided into cranial and caudal parts. The right cranial lobar bronchus was almost tracheal in location. The trachea had an average of 37 cartilages that showed a pattern of random anastomoses between adjacent cartilages. The silicone compound tested in this study holds promise for its use also under field conditions to gather quantitative morphological data.

Introduction

Gathering of morphological data from wildlife is important for the documentation of evolutionary processes. Many such studies focus on craniodental characters because these correlate with biological traits (size, diet, bite force), which are important for understanding the biology of the species (Viranta & Kauhala 2011). Skeletal specimens are also considerably easier to gather and store than high quality soft tissue specimens from free-ranging individuals.

The recent rapid growth of the Eurasian lynx (*Lynx lynx*) population in Finland to ~2400 individuals provided opportunities for the acquisition of research material of this elusive species for various biological studies through increased hunting effort (Deksne *et al*. 2013). Because previous references to the gross anatomy of the Eurasian lynx are scarce (Görinz *et al*. 2006, Axnér *et al*. 2009) with the exception of studies on skull morphology (Červený & Koubek 2000, Gomerčić *et al*. 2009), we seized the opportunity to gather data on various internal

organs of the lynx. One of the targeted organ systems was the respiratory apparatus that is an important morphological entity for studies on the comparative anatomy of vertebrates (Sanders & Farmer 2012). Besides documenting the basic morphological features of the lynx lungs, we tested whether a silicone compound originally developed for dentistry, and previously used for research on teeth (Evans *et al*. 2007), is useful for documenting and preserving the tracheobronchial tree morphology of the lynx.

Various techniques for obtaining accurate casts of the bronchial tree of vertebrates have been used since the 1950s (Tucker & Krementz 1957, Frank & Yoder 1966, Phalen *et al*. 1973, Perry *et al*. 2000), but many require laboratory facilities for processing. Because of the ease of use and the high hydrophilicity of the compound used in this study, we predicted that high quality casts could be produced also without extensive washing and drying of the specimens prior to casting. This would also allow for the preparation of multiple specimens under field conditions.

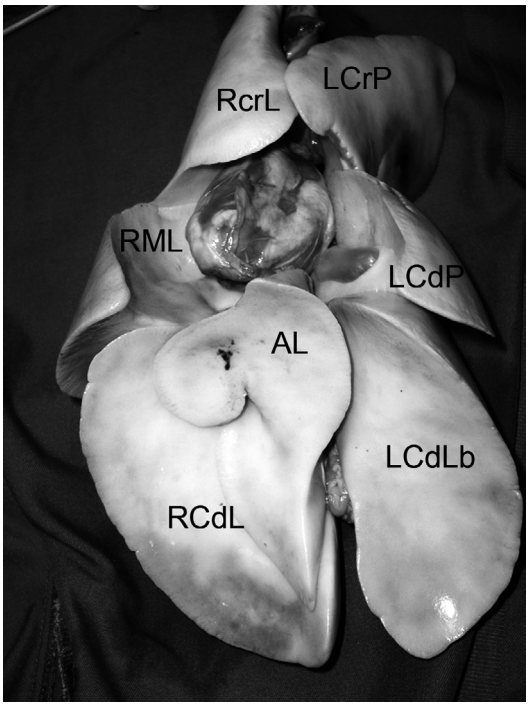

Fig. 1. Inflated lynx lungs and heart, ventral view. Right cranial (RCrL), middle (RML), caudal (RCdL), and accessory (AL) lobes. Cranial (LCrP) and caudal (LCdP) parts of the left cranial lobe, and caudal lobe (LCdLb).

Material and methods

Lynx were legally hunted during the 2011/2012 hunting season throughout its distribution area in Finland. Frozen carcasses obtained from hunters were shipped to the field station of the Finnish Game and Fisheries Research Institute where various samples for morphological and parasitological studies were taken. On necropsy, the sex and body weight of lynx was determined, and according to the method of Schmidt *et al*. (1997) the animals were classified as young individuals (body weight < 15 kg and age < 1.5 years) and adults (body weight > 15 kg and age > 1.5 years). Samples of intact lower respiratory tracts (n = 12) from adults were frozen (–20 °C) until later examination at the Faculty of Veterinary Medicine in Helsinki, where lungs were thawed and examined macroscopically for basic morphology. The division into lobes was evaluated and visualized with the use of air-inflated specimens (Fig. 1).

For the visualization of the tracheobronchial tree, silicone casts were prepared from three specimens. For casting, the airways were washed twice with tap water to remove blood and debris. The lungs were drip-dried. For casting, a two-component silicone (3M Express© 2 Light Body Standard Quick) was injected into the trachea using a gun-like silicone dispenser (Garant, with a 3M mixing tip), which mixed the two components during injection. This compound was chosen for its qualities of low viscosity and very high hydrophilicity. For the injection, the lungs were positioned horizontally (to allow adequate flow into all lobes) on a table, and silicone mix was applied separately into each bronchus (the correct position of the mixing tip was checked by palpation). The dispenser was hooked tightly into bronchus by a surgical clamp. The low viscosity mix flowed distally freely through the wet tracheobronchial lumina via digital pressure provided by the dispenser. When silicone material started to flow out around the applicator tip, the injection was deemed finished and the bronchi were closed with surgical clamps. At this point,

the surface of the lungs was examined for the pink silicone-mix to be seen through the serosa and parenchyma of the lung, which confirmed adequate airway flow of silicone. The silicone hardened in 2–3 minutes at room temperature (as indicated by the manufacturer's instruction manual) but all specimens were allowed to harden for 5–10 minutes to make sure that all parts of the cast hardened. After hardening, the specimens were placed in a bucket of sodium hypochlorite solution (14%, Sigma-Aldrich, Switzerland) in a horizontal position for the removal of lung tissue. During maceration, which took 7–10 days, the specimens were rotated daily (after hardening the silicone did not flow out of the trachea even when held upside down). For comparison, the bronchial tree of a mixed-breed domestic cat was prepared in a similar manner. The cat had been donated by the owner to the Faculty of Veterinary Medicine for research and teaching purposes. The cat had been stored frozen similarly to the lynx prior to the preparation of casts. In an effort to simulate field conditions during the preparation of specimens, one additional lynx specimen had been allowed to harden in a cold (+6 °C) storage room for an hour, after which it was boiled in tap water for four hours (for the removal of lung tissue). The finished casts were stored at room temperature and examined for branching of the bronchi and smaller airways. All diameter measurements of the bronchi were from an adult male weighing 21 kg. Anatomical terminology is in accordance with the International Committee on Veterinary Gross Anatomical Nomenclature (2012).

Additional samples of adult lynx lungs (n = 56) were obtained from the same source during the 2010/2011 hunting season for lung weight analysis. These lungs were cut off at the level of tracheal bifurcation on necropsy.

Results

The body weight of an adult lynx ranged from 15 to 29 kg (mean ± SD = 19.9 ± 3.07 kg). The mean ± SD weight of the lungs of an adult lynx was 124 ± 34.3 g (range = 75–211 g). The mean ± SD lung weight/body weight ratio was 0.70% ± 0.19%.

Silicone casts

The silicone compound accurately reproduced the branching and size of the principal and segmental bronchi but the demonstration of smaller airways was less accurate. Casts had no visible marks indicative of problems with air bubbles or water droplets. The material costs for one prepared tracheobronchial cast of the lynx was about 20 euros.

Trachea

The trachea began immediately caudal to the cricoid cartilage of the larynx and extended caudally to its bifurcation. The length of the trachea, from the first cartilage to the tracheal bifurcation, ranged from 11–15 cm (mean = 12.9). The lynx trachea contained between 36 and 39 (mean = 37) cartilages. The adjacent cartilages exhibited a pattern of random anastomoses. The tracheal cartilages were all open dorsally and confirmation did not vary throughout the organ. The cartilages were also visible in the silicone casts. The lumen of the trachea was round on cross section throughout the trachea. In a specimen weighing 21 kg, the diameter of the cervical trachea was 20 mm (averaged from five adjacent cartilages).

Lobulation and bronchial tree

The right lung of the lynx was divided by interlobar fissures into cranial, middle, caudal and accessory lobes. The apex of the right cranial lobe was pointed. The left lung was divided by inter-lobar fissures into cranial and caudal lobes (Fig. 1). The cranial lobe was subdivided into cranial and caudal parts by interlobar fissure that extended from the dorsal border of the lung to the ventral margin.

The bifurcation of the trachea gave rise to two principal bronchi (10–11 mm in diam.) which were directed caudodorsally and divided into lobar bronchi upon entering the lung parenchyma (Fig. 2). The right cranial lobar bronchus (10 mm in diam.) arose just cranial to or at the level of the tracheal bifurcation (Fig. 2). The right cranial lobar bronchus divided into 10–12 segmental bronchi, which varied in diameter

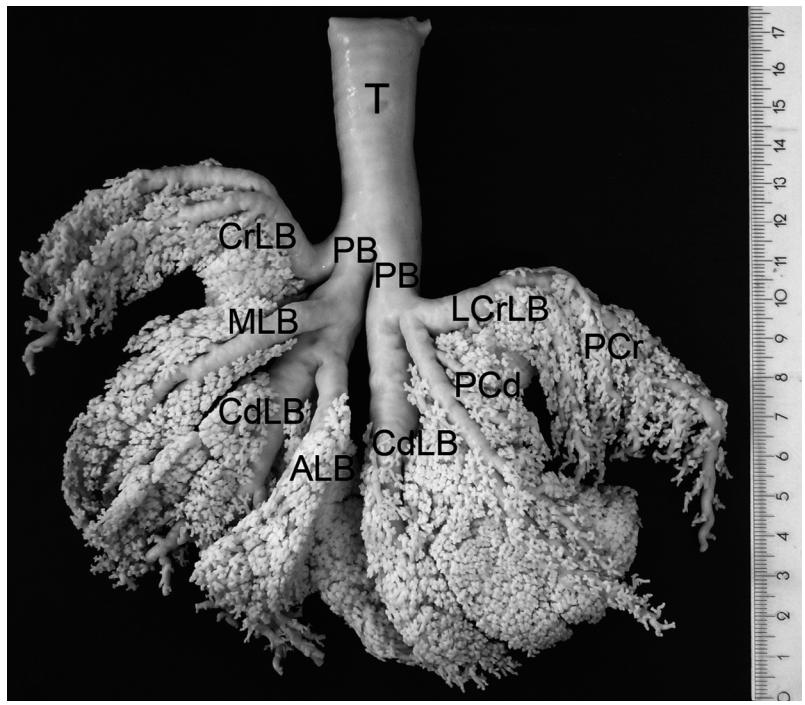

Fig. 2. Silicone tracheobronchial cast of the lynx, ventral view. Trachea (T), principal bronchi (PB, right and left), right cranial (CrLB), middle (MLB), right caudal (CdLB) and accessory lobar (ALB) bronchi. Pars cranialis (PCr) and pars caudalis (PCd) of the left cranial lobar bronchus (LCrLB), and left caudal lobar bronchus (CdLB).

from 4 to 6 mm. The subsequent divisions were irregular. The right principal bronchus gave rise to a ventrolaterally directed middle lobar bronchus (7 mm in diam.) approximately 1.5 cm from the tracheal bifurcation (Fig. 2). This middle lobar bronchus divided into six segmental bronchi (3–4 mm in diam.) of which two radiated craniolaterally, two caudolaterally and two laterally. The two caudolateral segmental bronchi run almost parallel after the bifurcation. All segmental bronchi divided further into multiple smaller airways (1.5–2 mm in diam.).

Caudal to the origin of the middle lobar bronchus, the right principal bronchus gave rise to the accessory lobar bronchus (7 mm in diam.) that emerged from the ventrocaudal aspect (Fig. 2). The right accessory lobar bronchus bifurcated into dorsal and ventral segmental bronchi (4 mm in diam.) each of which divided into multiple smaller airways (2 mm in diam.). From the point of origin of the accessory lobar bronchus, the principal bronchus continued caudally as the caudal lobar bronchus (10 mm in diam.). The first segmental bronchus (5 mm in diam.) to the right caudal lobe emerged from the dorsal wall of the caudal lobar bronchus near the origin of the accessory lobar bronchus. Caudal to this, a caudoventral (3 mm in diam.) and a lateral (5 mm in diam.) segmental bronchi arose. The caudal lobar bronchus (6 mm in diam. at this point) continued caudoventrally with subsequent irregular divisions into multiple smaller airways which ranged in diameter from 1.5 to 2 mm.

The left principal bronchi gave rise to a cranial lobar bronchus approximately 1.5 cm from the tracheal bifurcation (Fig. 2). The cranial lobar bronchus divided immediately into a larger (8 mm in diam.) laterally oriented segmental bronchus and into a smaller (5 mm in diam.) caudally directed segmental bronchus. Both of these bronchi divided into multiple smaller airways (1–2 mm in diam.; Fig. 2).

After the branching of the cranial lobar bronchus, the principal bronchus continued as the caudal lobar bronchus (10 mm in diam.) for approximately 1.5 cm before giving rise to 10–12 segmental bronchi which ranged in diameter from 3 to 5 mm. All divisions into bronchi and segmental bronchi were readily seen in the tracheobronchial casts but further divisions into smaller airways were difficult to distinguish in some cases.

Discussion

The silicone compound accurately reproduced the main aspects of tracheobronchial branching. This was confirmed by comparing the lung casts of the lynx to that of the domestic cat that in turn corresponded with the known tracheobronchial branching of cat lungs (Nickel *et al*. 1979). The surface of the casts showed no visible problems with air bubbles or water droplets. The demonstration of smaller airways (diameter 1–2 mm) in the lynx casts, however, was less accurate. This is not surprising considering that the specimens were frozen twice and also transported for long distances. Despite this, the casts are useful for comparative studies because histological sections are needed to identify the distal airways (branches of segmental bronchi, bronchioles) that differ in microanatomy. The visualization of the smaller airways could also be significantly improved by preparing the casts in the field from fresh specimens, and by injecting the lungs while still *in situ*. From fresh specimens, the analyses of the bronchial tree morphology could be further improved by filling them and comparing how the mold reflects the anatomy observed by dissection. Because of the hydrophilic properties of the compound used in this study, extensive drying of the specimens in laboratory conditions is not necessary before casting. Removal of the lung tissue can be done by boiling the specimens in the field and if needed, completing the removal process (with chemicals) in the laboratory. This allows the casted specimens to be stored and transported without cold storage facilities. The easy casting process makes it possible to prepare multiple specimens in a relatively short time for comparative anatomical studies.

The largest, most proximal intrapulmonary bronchi show considerable organizational variation between animal species (Nakakuki 1993, Hyde 2009). Most interspecific variation in airway branching between cats and lynx, however, was seen in the segmental bronchi. The branching pattern of the cat segmental bronchi is dichotomous and regular compared to that of the lynx (Nickel *et al*. 1979, Dyce *et al*. 2010, this study). As compared with those of the lynx, the segmental bronchi of the cat are relatively similar in diameter to each other. In the lynx, the diameter of adjacent segmental bronchi may differ considerably. This results in elongated bronchopulmonary segments that may reflect the generally elongated body shape of the lynx. Divisions into smaller airways were less regular and less predictable both in cat (Nickel *et al*. 1979, Dyce *et al*. 2010, this study) and the lynx (*see* above).

The mean ± SD lung weight/body weight ratio of the lynx was 0.70% which is similar to that reported by Colebatch and Mitchell (1971) for the domestic cat (mean ± SD = 0.76% ± 0.11%, n = 10). When comparing the absolute weight of lungs with the body weight, this value differs among animal species. However, Nickel *et al*. (1979) reported the average lung weight to be 1%–1.5% of the body weight. Similarly to cats, the apex of the right cranial lobe of the lynx was pointed differing from the rounded shape typical for dogs (Nickel *et al*. 1979). The location of the right cranial lobar bronchus of the lynx was almost tracheal. In some domestic (e.g. ruminants and pigs; Dyce *et al*. 2010) and wild animals (Nakakuki 1993, Gonlugur *et al*. 2005) the cranial lobe is independently ventilated by a bronchus detached from the trachea shortly before the bifurcation.

In conclusion, the silicone compound accurately reproduced the main aspects of the tracheobronchial branching of the lynx lung specimens and holds promise for use in the collection of quantitative morphological data also in field conditions. Casts can be stored virtually without change in room temperature for later use in research and teaching as well as in museum collections or displays.

Acknowledgements

Hunters are gratefully acknowledged for providing carcasses, and Anita Kenttälä, Jorma Korhonen and Sanna Kokko of the Taivalkoski field station for processing the carcasses. We thank Katja Holmala for coordinating the lynx research.

References

Axnér, E., Uhlhorn, H., Ågren, E. & Mörner, T. 2009: Reproductive maturation in the male Eurasian lynx (*Lynx lynx*): a study on 55 reproductive organs collected from carcasses during 2002–2005. — *Reproduction in Domestic Animals* 44: 467–473.

Červený, J. & Koubek, P. 2000: Variability of body size and skull dimensions of the lynx (*Lynx lynx*) in the Czech Republic. — *Lynx* 31: 5–12.

Colebatch, H. J. H. & Mitchell, C. A. 1971: Constriction of isolated living liquid-filled dog and cat lungs with histamine. — *Journal of Applied Physiology* 30: 691–702.

Deksne, G., Laakkonen, J., Näreaho, A., Jokelainen, P., Holmala, K., Kojola, I. & Sukura, A. 2013: Endoparasites of the Eurasian lynx (*Lynx lynx*) in Finland. — *Journal of Parasitology*. [In press].

Dyce, K. M., Sack, W. O. & Wensing, C. J. G. 2010: *Textbook of veterinary anatomy*, 4th ed. — Saunders, Elsevier, St. Louis, USA.

Evans, A. R., Wilson, G. P., Fortelius, M. & Jernvall, J. 2007: High-level similarity of dentitions in carnivorans and rodents. — *Nature* 445: 78–81.

Frank, N. R. & Yoder, R. E. 1966: A method of making a flexible cast of the lung. — *Journal of Applied Physiology* 21: 1925–1926.

Gomerčić, T., Gužvica, G., Duras Gomerčić, M., Frković, A., Pavlović, D., Kusak, J., Sindičić, M. & Huber, D. 2009: Variation in teeth number, teeth and skull disorders in Eurasian lynx, *Lynx lynx* from Croatia. — *Folia Zoologica* 58: 57–65.

Gonlugur, U., Efeoglu, T., Kaptanoglu, M. & Akkurt, I. 2005: Major anatomical variations of the tracheobronchial tree: Bronchoscopic observation. — *Anatomical Science International* 80: 111–115.

Görinz, F., Neubauer, K., Naidenko, S. V., Fickel, J. & Jewgenov, K. 2006: Investigations on reproductive physiology in the male Eurasian lynx (*Lynx lynx*). — *Theriogenology* 66: 1751–1754.

Hyde, D. M., Hamid, Q. & Irvin, C. G. 2009: Anatomy, pathology, and physiology of the tracheobronchial tree: Emphasis on the distal airways. — *Journal of Allergy and Clinical Immunology* 124: S72–S77.

International Committee on Veterinary Gross Anatomical Nomenclature 2012: *Nomina anatomica veterinaria*, 5th ed. (revised version). — Editorial Committee Hannover, Columbia, Gent, Sapporo.

Nakakuki, S. 1993: The bronchial tree, lobular division and blood vessels of the masked palm civet (*Paguma larvata*) lung. — *Journal of Veterinary Medical Science* 55: 425–429.

Nickel, R., Schummer, A. & Seiferle, E. 1979: *The viscera of the domestic mammals*. — Verlag Paul Parey, New York, USA.

Perry, S. F., Purohit, A. M., Boser, S., Mitchell, I. & Green, F. H. 2000: Bronchial casts of human lungs using negative pressure injection. — *Experimental Lung Research* 26: 27–39.

Phalen, R. F., Yeh, H.-C., Raabe, O. G. & Velasquez, D. J. 1973: Casting the lungs *in-situ*. — *Anatomical Record* 177: 255–263.

Sanders, R. K. & Farmer, C. G. 2012: The pulmonary anatomy of *Alligator mississippiensis* and its similarity to the avian respiratory system. — *Anatomical Record* 295: 699–714.

Schmidt, K., Jedrzejewski, W. & Okarma, H. 1997: Spatial organization and social relations in the Eurasian lynx population in Bialowieża Primeval Forest, Poland. — *Acta Theriologica* 42: 289–312.

Tucker, J. L. Jr. & Krementz, E. T. 1957: Anatomical corrosion specimens. — *Anatomical Record* 127: 655–676.

Viranta, S. & Kauhala, K. 2011: Increased carnivory in Finnish red fox females — adaptation to a new competitor? — *Annales Zoologici Fennici* 48: 17–28.