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COMPARATIVE ASPECTS OF THE MORPHOGENESIS AND MORPHOLOGY OF THE WING MEMBRANES OF BATS (CHIROPTERA) AND FLYING LEMURS (DERMOPTERA)

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Comparative Aspects of the Morphogenesis and Morphology of the Wing Membranes of Bats (Chiroptera) and Flying Lemurs (Dermoptera). Kovalyova, I. M. — The heterogeneity of formation of different areas in the wing membrane of Chiroptera and Dermoptera was established. The web between metacarpals and digits (chiropatagium) was formed by the mesenchyme which initially formed the forelimb rudiment. The plagiopatagium and propatagium were formed by proliferation of the trunk mesenchymal cells.

Key words: morphogenesis, forelimb, wing membrane, Chiroptera, Dermoptera.

Морфогенез и морфология летательной перепонки рукокрылых (Chiroptera) и шерстокрылов (Dermoptera) в сравнительном аспекте. Ковалёва И. М. — Установлена неоднородность формирования различных областей летательной перепонки рукокрылых и шерстокрыла. Летательная перепонка между пальцами грудной конечности рукокрылых и шерстокрыла (chiropatagium) сформирована мезенхимой, изначально формирующей почки конечностей. Боковая и плечеголовая области летательной перепонки (plagiopatagium и propatagium) этих животных формируются путем пролиферации мезенхимных клеток боковой стенки туловища.

Ключевые слова: морфогенез, грудная конечность, летательная перепонка, Chiroptera, Dermoptera.

Volant mammals use either gliding or flapping (active, powered) flight. There are many representatives of the extinct and recent orders (Volaticotherium, Marsupialia, Dermoptera, Rodentia) among mammals that have mastered gliding flight. But representatives of only one mammalian order, Chiroptera, have realized powered flight. However, the evolution of wings in mammals required specific developmental shifts from ancestral limb morphology to one for flight in both cases.

The wing membrane or patagium of bats can be subdivided into several regions: the propatagium, between the humerus, radius, and the wing's leading edge; the cheiro- or dactylopatagium between digits II–V, the plagiopatagium, interconnecting the lateral edge of the body, the hindlimb, the humerus and radius, and digit V; and the uropatagium, between the hindlimbs and including the tail, when present. Skeletal elements of the manus, metacarpals and phalanges, are greatly elongated in bats. The area of the interdigital membrane in bats provides bats with fine control of aerodynamic forces during flight (Panyutina Korzun, 2009).

The mammals that use exclusively gliding flight have wing membranes that include primarily the proximal elements of forelimbs, the humerus and the forearm, as well as those of the hindlimb, the femur and leg. Representatives of the order Dermoptera, the flying lemurs, have the most extensive wing membrane. This structure attaches to the head, neck, and all elements of the pelvic and thoracic limbs, on each side of the body, and the tail. A significant part of the wing membrane in flying lemurs attaches to the lateral part of the body. Skeletal elements of the flying lemur's hand are somewhat elongated in comparison with the basal mammalian condition, as observed, for example, in tree shrews. Metacarpals and distal phalanges make the main contribution to the length of the hand (Panyutina et al., 2012). Despite this, the interdigital membrane is a very small part of the total wing membrane (Panyutina, Korzun, 2009).

Comparative studies of prenatal development of the wing, particularly the wing membrane, of volant mammals are lacking.

We have described differences in the morphology and morphogenesis of different parts of the wing membrane in bats (Kovalyova, 2013). The problem of the morphogenesis of the flight membrane in gliding mammals, particularly in flying lemurs, has not been elucidated in the scientific literature. This work addresses that gap and also provides a comparative analysis of the patagium in representatives of two groups of volant mammals, *Cynocephalus variegatus*, a glider, and four bat species, *Myotis blythii*, *Nyctalus noctula*, *Rhinolophus bocharicus*, and *Rhinolophus hipposideros*.

Material and methods

The study material came from several species. First, we obtained the right forelimb and portions of the wing membrane of a stage 20, 19 mm GL (greatest length) embryo of a flying lemur *Cynocephalus variegatus* (family *Cynocephalidae*, Audebert). The material was provided courtesy of the Hubrecht Laboratory of the Netherlands Institute for Developmental Biology (Utrecht, The Netherlands). In addition, we obtained stage 14–25 embryos from two species of two bat families: *Myotis blythii* and *Nyctalus noctula* (Vespertilionidae) and *Rhinolophus bocharicus* and *Rhinolophus hipposideros* (Rhinolophidae) (table 1). All specimens examined were from material deposited in the collections of the Evolutionary Morphology Department of the Schmalhausen Institute of Zoology NAS of Ukraine.

Serial sections of flying lemur embryo (up to 5 µm thick) were stained with Mallory's trichrome; serial sections of bats embryos (up to 5 µm thick) were stained with Ehrlich's hematoxylin and eosin and with Mallory's trichrome. The slides were studied and photographed using an Axio Imager M1 light microscope (Karl Zeiss, Germany).

Table 1. Material examined

Species (number of specimens)	N specimens	Stage of embryos development	CRL, mm	Body mass, mg
Chiroptera				
RHINOLOPHIDAE				
<i>Rhinolophus bocharicus</i>	16	15	5.0	10.0
Kastschenko et Akimov, 1917 (9)	19	15	4.8	9.0
	4	16	5.0	13.0
	14	17	6.0	20.0
	5	17	6.9	43.0
	18	17–18	7.3	30.0
	21	17–18	7.2	32.0
	3	18	8.5	40.0
		20	–	–
<i>Rhinolophus hipposideros</i>	25	18–19	8.8	115.0
(Bechstein, 1800) (3)	51	20	12.0	363.0
	16	22	15.3	500.0
VESPERTILIONIDAE				
<i>Myotis blythii</i>	328	15	4.2	8.0
(Tomes, 1857) (11)	331	15	4.0	13.0
	343	18	9.0	100.0
	349	19	10.0	134.0
	364	22	15.5	300.0
	359	22	24.5	325.0
	162	23	24.5	3420.0
	164	23	28.0	5150.0
	358	23	–	5600.0
	357	23	–	6250.0
	167	24	–	10610.0
<i>Nyctalus noctula</i>	2	14	4.0	8.0
(Schreber, 1774) (8)	6	16	5.9	30.0
	9	17	6.2	39.5
	13	18	8.0	72.0
	16	19	9.1	123.5
	17	19	9.5	112.0
	18	20	11.8	232.0
	20	22	14.4	393.0
Dermoptera				
CYNOCEPHALIDAE				
<i>Cynocephalus variegatus</i> (Audebert) (1)	–	20	19.0	–

Results

Bats

The study of bat embryos established that by the 15th embryonic stage the rudiments of both limbs were elongated. The distal section of the forelimb rudiment was somewhat more elongated compared with the proximal, and formed the carpal plate. Mesenchymal cells condensed and formed precursors of various segments of the limb skeleton. It was noted that as mesenchymal cells of forelimb buds proliferated and differentiated, extensive mesenchymal cell fields remained between the fingers, forming the interdigital membrane or web of the chiroptagium. Thus, the portion of the bat wing membrane between metacarpals and phalanges at the 18th stage of prenatal development was formed by the mesenchyme that arose originally from the forelimb bud (fig. 1, A).

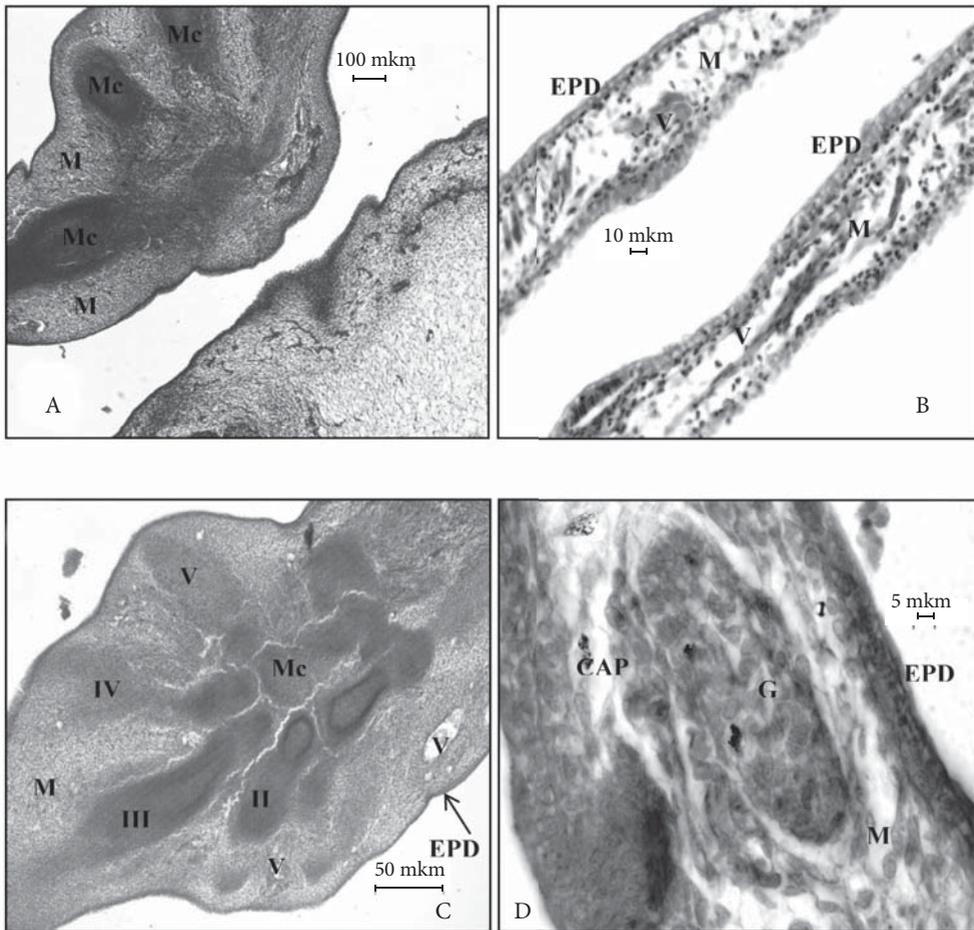


Fig. 1. Hand and wing membrane of bats embryos. A, B, C — x400; D — x1000: A — embryo *Myotis blythii* stage 18. Longitudinal section. The left forelimb bud with metacarpals rudiments: mesenchymal condensations of metacarpal rudiments (Mc), undifferentiated mesenchyme (M), epidermis (EPD). Stained with Ehrlich's hematoxylin and eosin; B — embryo *Rhinolophus hipposideros* stage 20. Cross-section. The wing membrane (uropatagium). The centre of hemopoiesis (G), epidermis (EPD), undifferentiated mesenchyme (M), blood vessels (V). Stained with Mallory's trichrome; C — embryo *Myotis blythii* stage 19. Longitudinal section. The right forearm. Metacarpal rudiments (Mc), digits rudiments (II III, IV, V), epidermis (EPD), undifferentiated mesenchyme (M), blood vessels (V). Stained with Ehrlich's hematoxylin and eosin; D — embryo *Myotis blythii* stage 22. Cross-section of the plagiopatagium skin. The centre of hemopoiesis (G), mesenchyme (M), epidermis (EPD). Stained with Ehrlich's hematoxylin and eosin.

The formation of the bat plagiopatagium, the region of the wing membrane between the body wall and the handwing, began with the 17th stage of prenatal development as a cranial primordium developed as a protrusion of the mesenchyme of the lateral body wall in the axilla. At the 18th embryonic stage, the thoracic and pelvic limbs of bats embryos had wide carpal plates. The caudal rudiment of the lateral membrane formed in the groin region, also by mesenchymal protrusion.

The cranial and caudal mesenchymal primordia of the plagiopatagium expanded and merged. Simultaneously, cell proliferation was observed at the surface epithelium of the lateral regions both limbs, contributing further to the formation of the plagiopatagium. As a consequence, epithelial tissues of the body and both the thoracic and pelvic limbs contribute to the formation of the plagiopatagium.

At the 19th embryonic stage, the mesenchymal primordia of the bat propatagium and uropatagium appeared. The epidermis of the membranes was composed predominantly of two layers of epithelial cells. During growth and extension from the body, hypodermal layers were not formed in the marginal areas of the skin in the plagiopatagium or uropatagium regions of the growing wing membranes. At the 20th embryonic stage of development, a single dermal layer between the ventral and dorsal layers of the epidermis was formed (fig. 1, B).

At the 20th stage of embryonic development in bats cartilaginous anlage of all skeletal elements of the thoracic limb were observed (fig. 1, C).

Thus, a large area of skin comprising wing membrane attached to the forelimb skeleton, body wall, and tail is composed of two layers of the epidermis (the ventral and dorsal surface of the membrane) and a differentiating mesenchyme layer disposed between them. Spherical formations or glomeruli, clusters of mesenchymal cells, are observed under the layer of epidermal cells (fig. 1, D). Subsequently, they form nests of hematopoiesis. Single capillaries were observed in the mesenchymal layer.

In bats, formation of the dense capillary net in the skin of the body and wing membrane was observed up to the 24th stage. The structure in the propatagium, plagiopatagium, and uropatagium was similar to that in the interdigital web. However, the mesenchymal layer in the proximal parts of wing membranes contained muscle fibers and hair follicles.

Dermoptera

At the 20th embryonic stage of development, the flying lemur embryo demonstrated membranes in the brachiocephalic, lateral, and interdigital regions of both the thoracic and pelvic limbs.

During development, the gliding membrane and other integumentary tissues of the thoracic limb of the flying lemur mainly possess a single layer of cuboidal epithelial cells (fig. 2, A). Single flat epithelial cells were observed between the cells of the simple cubic epithelium. It appeared the two-layer epidermis formed as the number of flat epithelial cells increased. There were single spherical formations beneath the epidermis, represented by concentrically located cells containing nuclei. These formations are probably homologous to spherical glomeruli in bats.

Undifferentiated mesenchymal cells were located beneath the epidermis. These mesenchymal cells had nuclei and long processes, which formed a thick net together with collagen and elastic fibers. In the mesenchyme under the epidermis of the gliding membrane of the flying lemur embryo, capillaries and small blood vessels were observed. Some vessels had nucleated erythrocytes (red blood cells RBC).

Unlike the condition in bats, in which individual muscle bundles in the wing membrane are not highly localized, in the gliding membrane of flying lemurs, there was layer of myotubes, the precursors of muscle fibers, under the layer of undifferentiated mesenchymal cells (fig. 2, A–C). These structures were strictly ordered, directed proximodistally and splayed somewhat cranially. Nuclei were found in the central region of the myotubes.

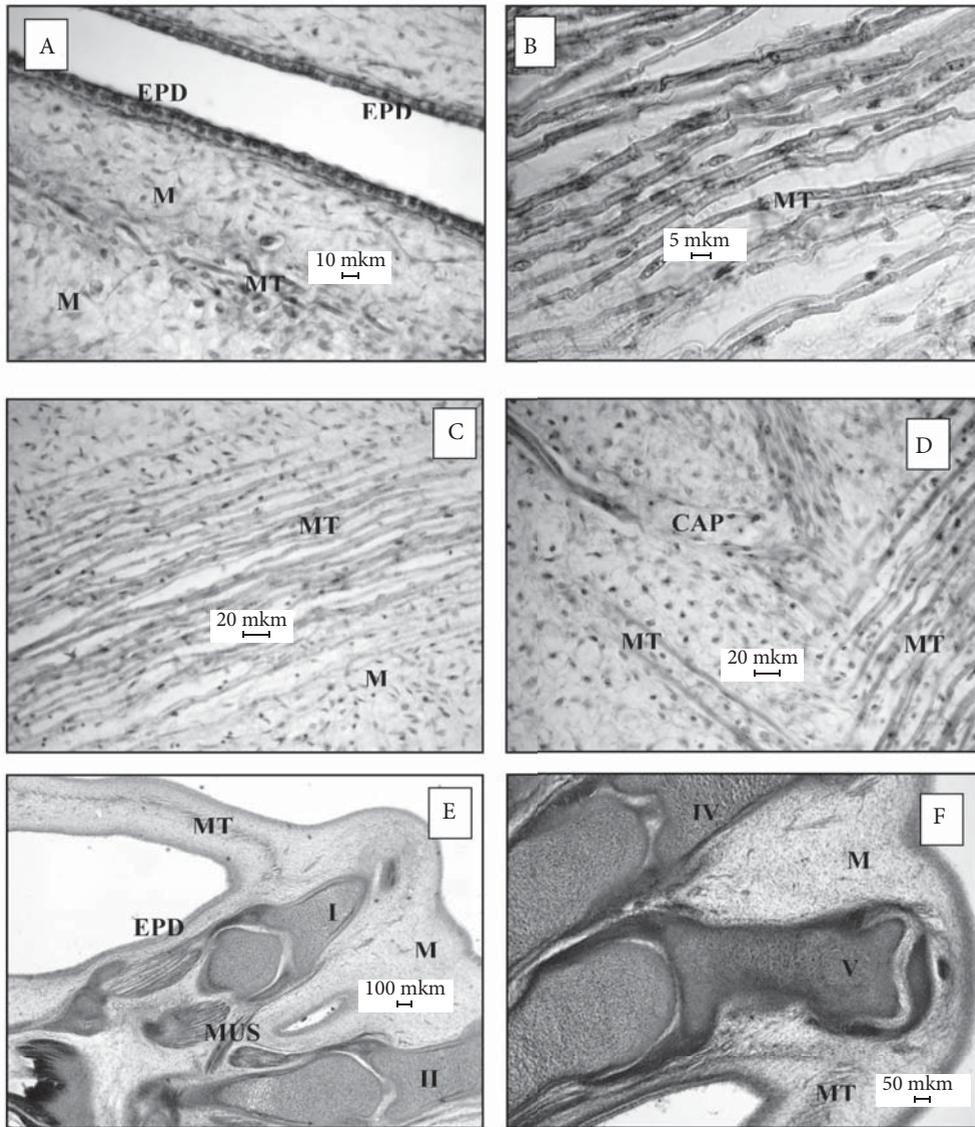


Fig. 2. Hand and wing membrane of embryo *Cynocephalus variegatus*, stage 20. Longitudinal sections. The right forearm. A, D, E, F — x400; B, C — x1000: A — longitudinal (below) and cross-section (from above) of the propatagium skin; B, C — the muscle tubes in the plagiopatagium skin; D — the two row of muscle tubes in the plagiopatagium skin; E, F — the chiropatagium skin. Epidermis (EPD), undifferentiated mesenchyme (M), blood vessel and blood capillaries (V and CAP), muscle tubes (MT), muscles (MUS), rudiments of digits I (I) and II (II); IV (IV) and V (V). Stained with Mallory's trichrome.

In brachiocephalic and lateral areas of the gliding membrane, under the cranially directed tubules there was another layer of myotubes deep to the cranially directed tubules, directed caudally (fig. 2, D). This created at least two layers of muscle fibers formed. It is probable that the multiple, different directions of muscle fibers are important for function of the skin-muscle structure.

It was significant that these structures (myotubes) were found in all areas of the gliding membrane of the flying lemur with the exception of the interdigital web (fig. 2, E, F).

At the 20th stage of embryonic development in the flying lemur, as in bats, cartilaginous anlagen of all skeletal elements of the thoracic limb were observed (fig. 2, E, F). How-

ever, the length of metacarpals and phalanges of the flying lemur, unlike in the bat condition, retained proportions typical for non-volant mammals. Phalanges of all five fingers of the thoracic limb of flying lemur were clearly evident. The first finger had two phalanges, and the others had three phalanges. The first finger was slightly opposed to the rest of the fingers and was strengthened by muscles. Bundles of muscle fibers and large blood vessels were observed along all of the metacarpals and phalanges of the flying lemur embryo.

Discussion

Some researchers believe that the patagia of mammals which use flapping flight and those that use gliding flight have significant similarities in morphology. They see differences between the wings only in the ratio of the areas of different parts of the flying membrane (Panyutina, Korzun, 2009).

A considerable amount of work is devoted to research on chiropteran locomotor organs. However, studying morphological features of bat wings failed to shed light on the problem of the origin of the wing membrane skin, which carries out flight.

V. E. Sokolov's fundamental monograph (1973) is devoted to the description of the structure of the integument of representatives of almost all mammal groups. The author describes the structure of the skin both the body and the gliding membrane (in its middle part and at the level of a body side) of the Malayan flying lemur *Cynocephalus temminckii*. It is noted that the skin of the body is thin, that the Malpighian layer of epidermis contains 1–2 rows of cells, and that granulosum and lucidum layers are absent. The thickness of the epidermis reaches 27–34 microns, and the thickness of its stratum corneum is 18–22 microns. The author states that the skin of the lower and upper surface of the flying membrane has the same structure as the skin on the body, except that there is no hypodermis. He also notes that flying lemurs, unlike other mammals that use gliding flight, have interdigital membranes on the thoracic and pelvic limbs.

The results of study of the morphology of the skin of the wing membrane in two bat species (*Eptesicus serotinus* and *Nyctalus noctula*), with measurement of individual layers, were given by the present author (Kovalyova, 2013). The minimum thickness of the flying membrane was found to be 20–25 microns. The maximum thickness of the epidermis was 8.0 microns, and the maximum thickness of its stratum corneum was 5.0 μm ; the thickness of the Malpighian layer, consisting of 2 layers of cells, was 2.0–3.0 microns.

There are no granulosum and lucidum layers, and no hypodermis or skin glands in the skin of the interdigital (chiropatagium) area or the distal parts of the plagiopatagium and uropatagium of the wing membrane in the bats studied. Also, there is no clear subdivision of the dermis into mesh and papillary layers. In the dermal layer of the membrane there are extensive fields with a dense network of capillaries. In the proximal parts of plagiopatagium and uropatagium there are separate hair follicles and skin glands, and the number of these structures increases close to the body.

Relatively recent studies have shown that in the skin of the hand in flying lemurs, as in bats, the dermis does not form papillae, and the border between the epidermis and dermis is almost a straight line. As a result, there are no dermal ridges, or papillary lines, on the fingers (Lemelin, 2000). It should be noted that the presence of these ridges is common to other mammals, particularly primates and tree shrews. Apparently, the absence of structures common for mammalian skin, in particular, muscle fibers, large blood vessels, skin glands, etc., leads to the smooth boundary between the epidermis and dermis.

Previously, the comparison of innervation and blood supply of the patagia in bats and gliding mammals, particularly the flying squirrel *Pteromys volans*, led to M. Kovtun to suggest: "...these structures are not homologous: while the bat membrane is a derivative mainly of the skin of extremities, the membrane of the flying squirrels is a derivative of the body skin" (Kovtun, 1984, p. 228).

The present study shows that in the flying lemur embryo, in a small interdigital area between the ventral and dorsal layers of the epidermis there is only the layer of mesenchymal cells. This structure is similar to the interdigital area in bats. It is likely that the similarity arises from similarity in the morphogenesis of these structures in bats and flying lemurs.

Developmental analysis shows that the flight membrane results from a lack of interdigital tissue cell death that normally occurs in most mammals (Adams, 2008; Cooper, Tabin, 2008; Farnum et al., 2008; Giannini et al., 2006; Sears et al., 2006, 2008; Weatherbee et al., 2006; Zou, Niswander, 1999). Similar research has yet to be conducted in flying lemurs. However, there is no reason to expect that flying lemurs are not subject to similar molecular gene mechanisms, so mesenchymal cells, initially forming the primordium of thoracic and pelvic limbs, remain between fingers to form the interdigital membrane.

The interdigital web of the flying lemur embryo is considerably smaller than that in bats at similar stages of embryonic development. This is mainly because the length of the bones of the manus in the flying lemur is less than in bats. In addition, hand muscles (*mm. interossei*) “fill” intercarpal gaps in the flying lemur, whereas in bats such hand muscles are relatively smaller, and the wing membrane is formed between metacarpals.

It appears that the length ratio of the skeletal elements of thoracic limbs in flying lemurs remains similar from early embryogenesis through ontogeny. In contrast, in bats bones of the hand continue to elongate through postnatal ontogenesis (Adams, 2008). In flying lemurs, unlike bats, there are well-differentiated muscle bundles between the metacarpal bones. The flying lemur has only 23 muscles (Panyutina et al., 2012, p. 268) in comparison with the “initial” 26 muscles available in the hand of the tree-shrew *Tupaia*. In bats the hand muscles have undergone profound changes. These muscles are poorly differentiated or reduced; their number varies from 7 to 12 (*ibid.*).

Thus, in flying lemurs the chiropatagium is represented only by the interdigital membrane. In bats the chiropatagium includes, besides the interdigital membrane proper, also the membrane formed between the metacarpal bones.

Conclusions

Formation of the brachiocephalic, lateral and tail membrane in flying lemurs and bats is the result of proliferation of integumentary tissues of the body, thoracic and pelvic limbs, and the tail. The interdigital membrane of these animals is the result of preservation of mesenchymal cells of the interdigital area during embryogenesis. Thus, the different parts of the flying membrane in bats and flying lemurs have different origins.

The comparative analysis of limb development in volant mammals using distinct forms of flight — gliding and flapping — show clear shifts in developmental program and resultant divergent morphogenesis.

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