Scrotal melanins in bats (Chiroptera): description, distribution and function

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Several species of bats contain pigment granules within the scrotal skin, tunica vaginalis, or tunica albuginea surrounding the testis and/or epididymis. Seventy-two species of bats, representing 49 genera were examined for the presence of such pigmentation. Histological, chemical and spectrophotometric tests were performed and confirmed the pigment as melanin. Melanin was found only in the families Pteropidae, Megadermatidae, Myzopodidae and Vespertilionidae. A strong correlation exists between scrotal pigmentation and roosting in locations where the bats are exposed to solar radiation. Melanin pigmentation in the scrotal region appears to be an adaption protecting male germinal tissue from the harmful effects of ultraviolet radiation. In one species, Lasiurus italicus, melanin deposited within the scrotal skin appears to have a social/reproductive communication function.

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Introduction

Numerous hypotheses attempting to explain the adaptive significance of pigmentation in vertebrates have been proposed. Burtt (1981) categorized these hypotheses into three major groups: 1) physiological structural functions; 2) coloration that affects vision; and 3) coloration that affects the animal’s visibility to other animals. The physiological structural category includes the following suggested functions: radiation exchange, absorption of heat, nutrition, water balance, chemical defence, mechanical protection, camouflage, and radiation shielding (Hunsaker & Johnson, 1959; Daniels, Post & Johnson, 1972; Hamilton, 1973; Branda & Eaton, 1978; Hoppe, 1979; Barrowclough & Sibley, 1980; Burtt, 1981). Pigmentation may serve more than one function,

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and it may be difficult to distinguish between primary and secondary functions. Melanin is that most common dark pigment in vertebrates. It has been demonstrated that melanin is an effective ultraviolet radiation shield. Porter (1967) showed that the melanized peritoneum of the body wall of several species of lizards is an effective shield against ultraviolet radiation, and that the heavily-pigmented skin of antelope ground squirrels (Ammospermophilus leucurus) is an effective radiation shield. Timm & Kermott (1982) described cutaneous and subcutaneous melanins that served as complementary ultraviolet radiation shields protecting the brain in a diurnal African rodent, Rhabdomys pumilio. They demonstrated that each of the two pigmented layers absorbed maximally at different ultraviolet wavelengths, providing greater total protection than either melanin layer would alone.

In addition to general skin pigmentation, one pattern that emerges time and again in most male mammals possessing a scrotum is heavily pigmented scrotal skin. A black scrotal sac is found in the Artiodactyla, Primates, Carnivora, and the rodent families Sciuridae, Cricetidae and Muridae. In these animal groups the pigment is contained within the scrotal skin, and could function either as an ultraviolet radiation shield or in intraspecific communication, or both. In a few marsupials, black pigmentation has been reported from a subcutaneous layer of the scrotum, the tunica vaginalis (Bolliger & Carrodus, 1939; Finkel, 1945; Biggers, 1966; Barnes, 1977; Stonehouse & Gilmore, 1977). Pigmentation in the tunica vaginalis of bats was first noted by Racey (1974) and Racey & Tam (1974) who proposed that the degree of pigmentation might be used to assess age and reproductive status in the European pipistrelle (Pipistrellus pipistrellus).

Herein, we report several species of bats in three families possessing a heavily pigmented tunica albuginea and tunica vaginalis, and species in two families possessing heavily pigmented scrotal skin.

We became interested in the distribution and function of this pigmentation in individual bats and its incidence throughout the order Chiroptera. In this report we provide detailed descriptions of the pigmented tissues covering the testis, epididymis, ductus deferens, spermatic cord and scrotal skin in several species of bats. Additionally, we test the hypothesis that the pigment is melanin, we discuss its ecological and evolutionary significance in Chiroptera, and hypothesize that it functions in absorption of ultraviolet solar radiation.

**Materials and methods**

We dissected and examined fresh or preserved specimens of 72 species of bats in 15 families for the presence of pigmentation in the tunica albuginea, tunica vaginalis, and scrotal skin surrounding the testis and/or epididymis. For those species with pigmented tunicae, we recorded distribution and degree of pigmentation. Species examined are listed in Table 1. Specimens we examined are housed at the Field Museum of Natural History, Chicago, Museum of Natural History, University of Kansas, Lawrence, or Museum of Zoology, University of Michigan, Ann Arbor. Additionally, G. L. Forman, P.A. Racey and J. D. Smith provided unpublished information on several other genera.

Tissue samples of *Pteropus giganteus*, Lavia frons, *Lasionycteris noctivagans* and *Lasius arboris* also were examined histologically after formalin fixation. Sections were stained with haematoxylin and eosin, with the Fe⁺⁺ Ion Uptake Reaction of Melanin (Lillie), and controlled for haemosiderin and melanin, both with and without the picric acid-acid fuchsin counterstain of Van Gieson (Lillie & Fullmer, 1976: 526). Attempts were made to dissolve the pigment in the following solvents: acetone, ammonium hydroxide, chloroform, ethanol, formalin, glacial acetic acid, toluene, unleaded gasoline, water and xylene. Tissue samples of the tunica
### TABLE 1
Families, genera and species of Chiroptera surveyed for the presence of pigmentation, surrounding the testis and epididymis; pigmentation not present except as noted (see text for details)

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1 Data and or specimens provided by G. L. Forman and J. D. Smith
2 Data provided by P. A. Racey
vaginalis of *Lasionycteris noctivagans* and *Lasiusus borealis* were analysed in the ultraviolet range (210–400 nm) on a Cary 17 Spectrophotometer (see Timm & Kermott, 1982).

Species described in detail below include: *Lavia frons*—SUDAN: Equatoria; Torit, 2000' (9 males). ZAIRE: Ituri Dist., Catchpoles' Farm (1 male). *Myzopoda aurita*—MADAGASCAR: Tulear; Bemangidui, 72 km N Fort Dauphin (1 male). *Eptesicus fuscus fuscus*—USA: Illinois; Cook County, Chicago (1 male); La Salle County, Peru (1 male); Wisconsin; Milwaukee County, Milwaukee (1 male); Walworth County, Lake Geneva (1 male). *Eptesicus fuscus pallidus*—MEXICO: Coahuila; Las Delicias, 3000' (1 male). *Lasionycteris noctivagans*—USA: Illinois; Cook County, Chicago (11 males, 1 female). *Lasiusus borealis blassevilli*—PARAGUAY: Dept. Paraguari, Parque Nacional Ybycuí (1 male). *Lasiusus borealis borealis*—USA: Illinois; Cook County, Chicago (7 males, 1 female). *Lasiusus borealis frantzii*—COSTA RICA: Prov. Alajuela, Volcan Poas, elevation 2000 m (1 male). *Lasiusus cinereus cinereus*—USA: Illinois; Cook County, Chicago (1 male); North Dakota; McHenry County, J. Clark Salyer National Wildlife Refuge (1 male). *Lasiusus cinereus villosissimus*—CHILE?: (1 male). *Lasiusus ega ega*—BOLIVIA: (1 male); Beni; San Joaquin (1 male). *Myotis lucifugus lucifugus*—USA: Alaska (1 male); Illinois; Cook County, Chicago (1 male). *Myotis macrotarsus*—BORNEO: Lahad Datu Dist.; Semporna (2 males).

**Observations and results**

The species of bats we examined showing pigmentation of the scrotal skin or tunicae (see Table I) are discussed in detail below.

**Pteropidae**

*Pteropus giganteus*

Three specimens of *Pteropus giganteus* were examined. In two specimens the scrotal skin was dark brown to black in colour, especially in the region just over the testes. The third specimen was missing the scrotal skin. In all three specimens the tunica vaginalis immediately surrounding the testes appeared translucent and free of pigmentation. However, the tunica vaginalis covering the epididymides and the tunica albuginea surrounding each testis were darkly pigmented.

Microscopic examination of the sectioned tissues showed numerous dark pigment granules scattered throughout the entire thickness of the tunica albuginea of the testis and the tunica vaginalis capsule of the epididymis. Additionally, a few pigment granules were observed scattered throughout the connective tissue septa surrounding the seminiferous tubules. These pigment granules appeared dark brown to black with haematoxylin and eosin, and dark green with the Lillie stain.

A single zoo specimen (locality unknown) was similar to the specimens described above, except the degree of pigmentation in all tissues was less.

*Pteropus vampyrus*

One specimen of *Pteropus vampyrus* was examined; the scrotal skin was missing. The parietal layer of the tunica vaginalis was clear and unpigmented. The tunica albuginea contained dark brown pigment surrounding the entire testis.
Lavia frons

Ten specimens of *Lavia frons* were examined. The scrotal sacs of eight adult males were unusually prominent and pendulous, a condition which appears to be unique within the Chiroptera. The scrotal skin, however, was heavily pigmented and appeared brown in colour (Plate I). Two subadult males showed no pigmentation of the scrotal skin, or of the underlying tissues covering each testis and epididymis. In the adult males, the tissues surrounding each testis and epididymis also were unpigmented. In some specimens the entire scrotum was pigmented, but in others only one-half to two-thirds of the scrotum was pigmented, and in these later cases the pigment was confined to the most ventral dependent portion of the sac. This scrotal skin pigmentation did not grade into the skin of the rest of the scrotum or of the lower abdomen, but showed a sharp demarcation zone separating the pigmented scrotum from the white skin over the rest of the body.

Histological examination of the skin, testis and epididymis showed that the pigment was found only within the skin. The heaviest concentration was present in the epidermis; a lighter pigmented layer was found at the base of the dermis (Plate II). The tunica albuginea and vaginalis were unpigmented. The pigment appeared dark brown to black with haematoxylin and eosin, and dark green with the Lillie technique.

Myzopodidae

*Myzopoda aurita*

One specimen of *Myzopoda aurita* was examined. The skin of the scrotum was white. The tissues surrounding each testis were unpigmented. However, the tunica vaginalis surrounding each epididymis contained scattered dark brown pigment granules, especially dense at the posterior tip.

Vespertilionidae

*Eptesicus fuscus*

Five male specimens of *Eptesicus fuscus* were examined, four adults and one subadult. The scrotal skin was very light and without pigmentation. The tunica vaginalis was perfectly clear over most of the testis and epididymis; however, a light scattering of pigment granules was observed in the tunica vaginalis at the posterior end of the epididymis (3 specimens), or over the anterior one-third of the testis itself (2 specimens).

*Lasionycteris noctivagans*

Eleven males and one female of *Lasionycteris noctivagans* were examined. In males, the scrotal skin was without pigmentation. As in the species of *Lasiusus* examined, *Lasionycteris* possesses a pigmented tunica vaginalis with no pigment in the tunica albuginea. In five males, the tunica vaginalis covering the posterior one-third of the testis and the epididymis contained a moderate amount of dark brown to black pigment. The tunica vaginalis covering the anterior two-thirds of
PLATE 1 External genitalia of an adult male *Lavia frons* (Megadermatidae) (FMNH 66641) from Torit, Sudan showing the penis with the pendulous, darkly pigmented, scrotal sac.
The testis contained only a light scattering of pigment granules. In Lasiomys the epididymis does not extend as far posteriorly as in Lasiurus.

One subadult male with undescended testes was similar except that the pigmentation of the tunica vaginalis was light and uniformly distributed throughout.

In five males, two of which were young of the year and the other three adults captured in the autumn, the testes were retracted, being almost entirely contained in the inguinal canal. The epididymides in all cases were surrounded by a heavily pigmented, nearly black, tunica vaginalis. However, the testes themselves were not covered by pigmented tissue. It appeared as if the tunica vaginalis containing melanin had remained in situ and the testis had retracted cephalad toward the body cavity.

Histological examination showed the pigment confined to the tunica vaginalis alone. The spectrophotometric scans showed considerable absorption in the UV range.

The female examined showed no evidence of pigmentation around the ovaries, uterus and fallopian tubes.

*Lasiurus borealis*

We examined the reproductive tracts of 10 specimens of Lasiurus borealis, nine males and one female. External examination of the scrotal region of the nine males gave no evidence of skin pigmentation. Upon incision and reflection, the scrotal skin appeared translucent to white in color, but a few scattered pigment granules were seen in the posterior-most part of the scrotal skin of some individuals. The tunica vaginalis appeared heavily pigmented in all specimens.
PLATE III. Cross-section of the epididymis of an adult male *Lasiurus borealis* (Vespertilionidae) from Chicago, Illinois showing the darkly pigmented tunica vaginalis (1) surrounding the unpigmented tunica albuginea (2) and ductus epididymis (3) (40 ×; H & E stain). Spermatozoa are present in the ductus epididymis.

posterior two-thirds covering the epididymis and posterior one-half of the testis was very heavily pigmented and appeared black in colour. The anterior one-third covering the anterior one-half of the testis and extending around the spermatic cord was less heavily pigmented with the degree of pigmentation tapering off anteriorly. In one adult breeding male the tunica vaginalis covering the posterior two-thirds of the testis appeared brown in colour, but the portion covering the posteriorly projecting epididymis was solid black. Anteriorly, the portion of the tunica vaginalis covering the anterior one-third of the testis contained only scattered pigment granules, with the pigment disappearing completely just anterior to the testis. In one non-breeding adult male and one young male, the tunica vaginalis covering the entire testis, epididymis and spermatic cord was completely black in colour.

The tunica vaginalis easily peeled off the testis. All the pigmentation was contained in this membrane. The testis itself, including the tunica albuginea capsule, was pure white in colour. The epididymis extended along the testis in a medial groove. In adult breeding males, the epididymis was highly convoluted and extended posterior to the testis. The two testes and epididymides within the scrotal sac were widely separated, with each testis and epididymis surrounded by its own tunica vaginalis.

Histological microscopic examination of the testis and epididymis showed a heavily pigmented tunica vaginalis surrounding the epididymis and testis (Plate III). The pigment appeared dark
brown to black with haematoxylin and eosin, and dark green with the Lillie technique. Spectrophotometric scans showed maximal absorption in the UV range.

In one adult breeding male, the scrotal area appeared dark externally, with the colour due to the underlying dark tunica vaginalis. The skin itself was unpigmented.

The single adult female examined showed no evidence of pigmentation around the uterus, fallopian tubes or ovaries.

*Lasius cinereus*

Three specimens of *Lasius cinereus* were examined. A subadult and adult male had scrotal skin very light in colour, but a few scattered pigment granules were noted in the posterior portion. The tunica vaginalis was heavily pigmented (black) covering the elongate epididymis (projecting posteriorly as long as the testis) and posterior one-third of the testis. Anteriorly, the tunica vaginalis pigmentation became lighter, gradually tapering off in intensity, but extended anteriorly all the way up into the spermatic cord, disappearing just prior to the inguinal ring. The second adult male was nearly identical to adult males of *L. borealis*. As in *L. borealis*, the pigmentation was contained only in the tunica vaginalis and not the tunica albuginea.

*Lasius ega*

Two adult males of *Lasius ega* were examined. The skin was without pigmentation of any kind. The epididymis was long and extended far posteriorly. The amount of pigment in the tunica vaginalis was much less than in the above species, and appeared brown in colour, not black. Again, the degree of pigmentation decreased from posterior to anterior.

*Myotis lucifugus*

Two specimens of *Myotis lucifugus* were examined, both adult males with the testes in the scrotal sac. The skin of the scrotum was white in both specimens. The tunica vaginalis surrounding the epididymis contained a faint to moderate amount of scattered pigment granules. The tunica vaginalis surrounding each testis was unpigmented.

*Myotis macrotarsus*

Two specimens of *Myotis macrotarsus*, one adult and one subadult male were examined and possessed a lightly pigmented tunica vaginalis surrounding the epididymis, but not the testis, similar to that of *M. lucifugus* as described above. The scrotal skin was unpigmented.

All other species examined (see Table I) showed no pigmentation of the scrotal skin or the tissues surrounding the testis or epididymis.

**Discussion**

We have presented evidence that the pigmentation surrounding the testis and epididymis and in the skin of these bats is melanin. Grossly, the material was very dark in colour, and under the microscope was granular and appeared dark brown to black with haematoxylin and eosin. In identical sections cut from the same tissue blocks and stained with the Fe$^{2+}$ Ion Uptake Reaction of Melanin (Lillie), the pigment granules and layers stained dark green. This procedure is a
standard histochemical technique indicating the presence of melanin. The material was insoluble in standard solvents and was bleached by hydrogen peroxide. Additionally, on the spectrophotometric scans, we observed considerable absorption in the UV range. These various tests confirm that the pigments observed are melanin.

The tunica vaginallis propria testis is an outpocketing of the abdominal peritoneum which forms a double-walled serous cavity surrounding each testis, epididymis and spermatic cord. The tunica vaginallis consists of two layers, the outer or parietal layer, and the inner or visceral layer. Both lie immediately under the skin, from which they easily separate. The tunica vaginallis tightly encases the testis, epididymis and spermatic cord, but is easily separated from them. The tunica albuginea is the connective tissue capsule of the testis, itself lying directly beneath the tunica vaginallis.

The mammalian testis serves two functions, the production of the male gametes (spermatozoa) and the male sex hormones (androgens). The extreme sensitivity of the testis to the damaging effects of radiation has long been known (Albers-Schönberg, 1903; Bergonie & Tribondeau, 1904, 1905, 1906). Albers-Schönberg (1903) demonstrated that X-radiation immediately killed all sperm, prevented further spermatogenesis, and that irradiated males were permanently sterile. The A spermatogonia are the most sensitive cells. Radiation damage can vary in severity from chromosomal damage in spermatocytes and spermatids to total destruction of the gametes.

Why is it that several species of bats have melanin deposits in the scrotal region, whereas most species do not? Melanin deposits are present in both suborders, the Megachiroptera and the Microchiroptera. In the Microchiroptera, melanin was found in three of the 14 families examined. Thus, it does not appear to be a phylogenetically determined character, but seems to have evolved independently at least three times in the Chiroptera. In the megachiropteran family Pteropodidae, we see melanin in the tunica albuginea, tunica vaginallis over the epididymis, and in the scrotal skin only in the genus Pteropus. It was not observed in any other megachiropteran. In the microchiropteran family Megadermatidae, melanin was present only in the monotypic genus Lavia, and then only in the scrotal skin. The tunicae were unpigmented. In the microchiropteran families Myzopodidae and Vespertilionidae, melanin deposits are seen only in the tunica vaginallis surrounding the testis and epididymis. There was no pigment in the skin or tunica albuginea.

We believe there are several reasonable hypotheses that need to be explored to explain the presence of the melanin deposits observed in bats. These would include: 1) thermoregulatory function; 2) visual communication; 3) ultraviolet radiation shield; and 4) no adaptive value. Because the melanin in bats is so localized in the scrotal region, we feel that other hypotheses as mentioned in the Introduction are untenable.

One hypothesis is that the melanin has a thermoregulatory function, regulating absorbency of solar radiation (Hunsaker & Johnson, 1959). Melanins within the skin and hair may function in a thermoregulatory capacity, regulating absorbency of solar radiation. However, it is difficult to conceive how melanin beneath the skin could function in thermoregulation. Porter (1967: 294) concluded that the pigmented peritoneum in reptiles was 'insignificant in thermoregulation' because only a small fraction of the incident solar energy penetrated to that level. We concur. In the Myzopodidae and Vespertilionidae, melanin is found only in the underlying tunica vaginallis. In Pteropus melanin is present in the tunica albuginea, the tunica vaginallis over the epididymis, in addition to the scrotal skin. It seems unlikely there is any thermoregulatory function in these deep lying tissues.

If the pigmented scrotal skin serves a thermoregulatory function it would be as 'black body radiator'. In this sense, the skin pigment, and the underlying pigmented tissues as well, may act as black body radiators to assist in cooling the testis. However, black body radiation theory predicts
results opposite of those deemed beneficial to spermatogenesis, namely, a transfer of heat from the environment during the heat of the day to the testes, and away from the testes during cold nights.

Externally visible pigmentation may serve a communication function. Melanins in the tissues underlying the skin are not externally visible, and are thus eliminated. However, the pigmentation present in the scrotal skin of *Lavia*, and in *Pteropus*, may serve such a function, especially since the pigmentation is confined to the scrotum itself. In *Lavia*, the scrotum is pendulous, a condition that is atypical in the Chiroptera. The combination of a pendulous scrotum, lack of pigmentation in non-breeding subadults, and heavy, brown to black pigmentation in adult breeding *Lavia frons* strongly suggests a social/reproductive communication function in this species. In pteropids the scrotum is but sparsely covered by dark hair, thus allowing the pigmentation of the scrotal skin to be externally visible.

We cannot discount a social/reproductive communication function for this pigment. However, the pigmentation in the tunica albuginea and tunica vaginalis over the epididymis are not externally visible and a radiation shield function is more likely than visual communication.

These pigmented tissues may have no selective value, representing a by-product of other physiological processes or functions, or of accidental occurrence. We find this hypothesis difficult to accept because the characteristic has evolved independently more than once. In the bats reported here, the melanin is found in different tissues in different groups, indicating different evolutionary pathways to the same functional result. We feel that our survey of the Chiroptera and experimental results strongly support the hypothesis of an ultraviolet radiation shield function for these melanized tissues.

It is well known that melanin forms an effective ultraviolet radiation shield. Radiant energy is absorbed in melanin by raising electrons from the lower energy states to higher ones. Tissues most susceptible to radiation damage in vertebrates are nervous and reproductive tissues (Porter, 1967; Burtt, 1979). In reproductive tissue, the A spermatogonia show extreme sensitivity to, and are easily killed by, short wave-length ionizing radiation (Setchell, 1978). In the bats examined that had these melanized tissues, the melanin was concentrated over the testis where spermatogenesis occurs and the epididymis where sperm are stored.

Bats are primarily nocturnal, spending the daylight hours at a diurnal roost site. Bats select a wide variety of sites for their roosts which often include caves, hollow trees and logs, holes in the ground, cliff crevices, and in vegetation. Most of these sites are in dark, secluded, protected places. Numerous species of bats do roost in vegetation (Timm, 1987). However, most of these roost low within the canopy, in places that also provide seclusion and protection. A few species of bats, however, roost at the tops of the canopy hanging upside-down. This includes bats in the family Pteropidae (*Pteropus*), Megadermatidae (*Lavia frons*), Myzopodidae (*Myzopoda aurita*), and Vespertilionidae (*Lasionycteris* and *Lasiurus*). Both *Lasionycteris* and *Lasiurus* are considered ‘tree bats’, roosting exclusively in trees (see Nowak & Paradiso, 1983, and references included therein).

In the tree-roosting pteropids, megadermatids, myzopodids and vespertilionids, the scrotal region is directed upwards and outwards, and thus exposed to solar radiation. Consequently, the melanin present in the described tissues would provide significant protection from the effects of ultraviolet radiation upon the developing spermatogonia.

The exceptions to this correlation of pigmented scrotal tissue to roosting site are the majority of vespertilionids, including the genera *Eptesicus*, *Myotis*, *Plecotus* and *Pipistrellus* (Table I). Like most bats, these species roost in dark, secluded places. They showed light pigmentation in the tunicae vaginales. This may represent a primitive, phylogenetically determined character in the
Vespertilionidae, and we suspect that this character may be widespread within the family.

Has natural selection in these bats fashioned a response to some environmental pressures, resulting in the evolution of melanin deposits in the tissues surrounding the testis, epididymis and associated structures? We think the answer is yes, and the environmental factor is ultraviolet radiation impinging upon the gonadal tissue of these certain few tree-dwelling species.

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