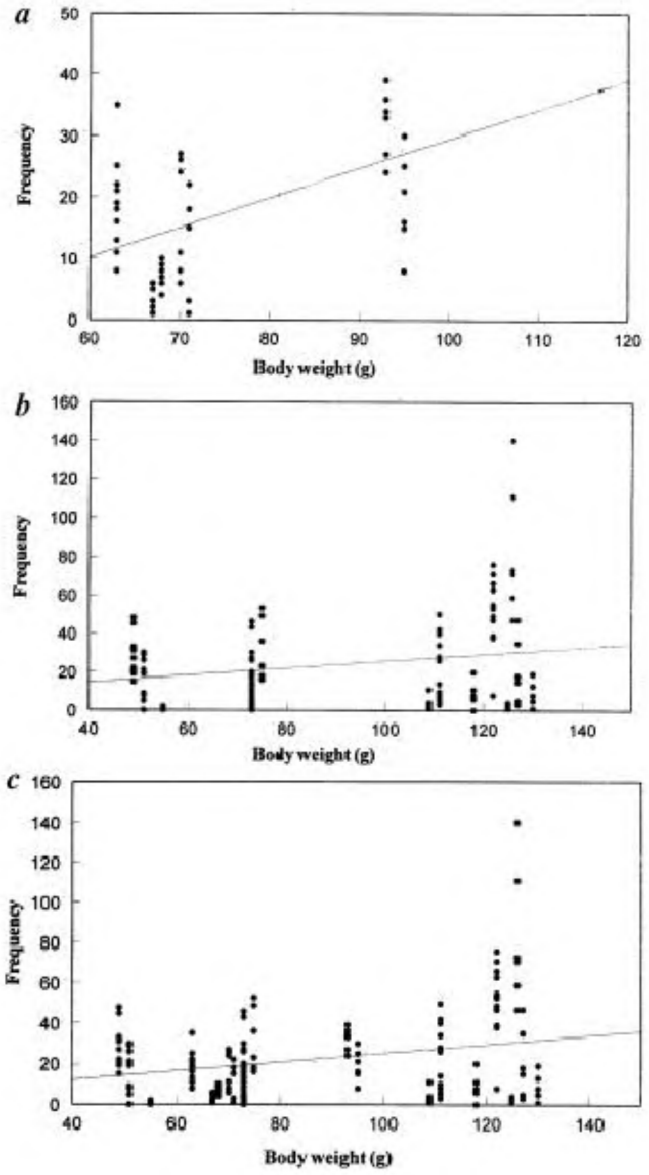

Figure 2. Correlation between body weight and frequency of visits to the photic zone (*a*) in fishes of the first experiment; (*b*) in fishes of the second experiment; and (*c*) when data from both experiments are combined. A positive correlation is detected between the two variables.

predator. Hence it dares to venture more often to the lighted zone. Another reason might be that being hardy, the largest fish is also better-suited to bear the rigours of the photic zone, which might be considerable for a nocturnal fish. This behaviour could not be attributed to the phenomenon of photoperiod preference, because experimental fishes were not provided with such an option. Further, the phototactic responses of the members of each group having lesser body weight were significantly different from those exhibited by the heaviest ones. In conclusion, it can be said that the phototactic behaviour in *C. batrachus* exhibits a dominant–subordinate relationship, being a function of body size, and that the frequency to the photic zone is directly proportional to the size and weight of the fish.

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Elephant temporal gland ultrastructure and androgen secretion during musth

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We have investigated the ultrastructure of the temporal gland of the Asian elephant (*Elephas maximus*) in the musth condition. We find that the organelles are highly evolved for the production of the androgen, testosterone which is reported to be very high in the Asian male elephant in full musth. The mitochondria bear cristae which are profuse and tubular, and occur along with many Golgi bodies. There is hypertrophy of smooth endoplasmic reticulum. All the structures involved in the production of androgen, as in the Leydig cell or the cells of the adrenal cortex, are thus

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found in abundance. Cellular structures also seem singularly evolved for the secretion of androgen and its degradation products.

ELEPHANTS are unique in that they possess a temporal gland behind each eye which, in male Asian elephants, exudes an oily secretion during certain periods of the year when the animal is said to be in the musth condition. Swelling of the temporal glands, unpredictable aggressive behaviour, a state of torpor and urinary incontinence characterize this period[1]. In this state, their capture and maintenance are hazardous tasks. Even in medieval times, subjugation of elephants in musth was considered as an act of valour worthy of being recorded for posterity in art form (Prince Aurangzeb controlling the *mast* elephant, Sidhkar[2]). Even now, male elephants are an integral part of temple festivities and are maintained by many temple trusts, especially in Kerala. It is also not uncommon to read newspaper reports of mahouts being killed by captive musth elephants or wild elephants in musth harming human interests. Male and female African elephants (*Loxodonta africana*) may have temporal gland secretions throughout the year, but the musth condition with characteristic behaviour is observed only in the adult male[3]. Physiologically, musth is characterized by increased testosterone concentration both in the serum and in the temporal gland secretion, with the parameter being up to ten times high in the Asian male on average compared to the African male in full musth[4]. The aggressive behaviour associated with musth seems to enable males in such condition to achieve a higher reproductive success in the wild, since they are able to compete successfully with larger males, which are not in musth. As a consequence, the condition seems to have been selected for during evolution. It is also known that within a group, when older males are in musth, there is inhibition of the condition in younger males (V. K., pers. obs.; Tamil Nadu Forest Department records). This observation has been made use of in controlling aggressive behaviour in younger males in African elephants in the wild[5]. In spite of the connection between musth and elephant behaviour, there have been no studies on the ultrastructural pathology of the gland, due to the necessity of obtaining a fresh sample for these studies when the animal is most aggressive. Early histological studies from culled and naturally dead elephants concluded that both mammary and musth glands are derived from apocrine sweat glands[6]. The present work reports, the ultrastructure of the gland during full musth in the Asian elephant in relation to androgen secretion, which has an important effect on man–elephant conflicts, elephant society, individual reproductive success and the evolution of the species *in toto*.

For our study, we obtained biopsy samples from an adult male aged around fifty years named Subramaniam, who is maintained at the Mudumalai Wildlife Sanctuary in Tamil Nadu. His diet includes cooked ragi (*Eleusine coracana*) with supplements prepared at the elephant camp in Mudumalai, along with natural forest vegetation. He came into musth on 1 November 2000 and when we obtained the biopsy from the left temporal gland on 27 December 2000, there was prolific flow of musth secretion. In an earlier episode of musth in 1991, he had killed his mahout. For obtaining the biopsy sample, he was sedated by an intramuscular injection of 250 mg xylazine. The sample was immediately fixed in cacodylate-buffered glutaraldehyde and subsequently in osmium tetroxide according to standard procedure, washed, dehydrated and embedded in Spurr resin. Semi-thick sections were stained with toluidine blue and ultrathin sections with lead citrate and uranyl acetate. Ultrastructure was examined with a JEOL 1200 EXII transmission electron microscope at suitable kilovolts. (Subramaniam suffered no ill effects following the operation, apart from being rather sleepy for a couple of days and a slight shortening of the total musth period. In subsequent years, he again came into the musth condition and is reported to have mated successfully.)

A semi-thick section shows an alveolus of the gland with a lumen (Figure 1). The cells are columnar and show preferential staining. Cellular debris is seen in the lumen. In some sections, free nuclei also were noticed. Myoepithelial cells occur at the periphery. The preferential staining seen here is noticed with the electron microscope stains also (Figure 2 *a*). The darker cells are connected by desmosomes, show more microvilli and have many secretory vacuoles compared to the lighter cells, which are, however, more contrasty with the nuclei and cell organelles stained well in comparison to the clearer cytoplasm. Some of the clear cells have ruptured plasma membranes. Mitochondria have become pyknotic

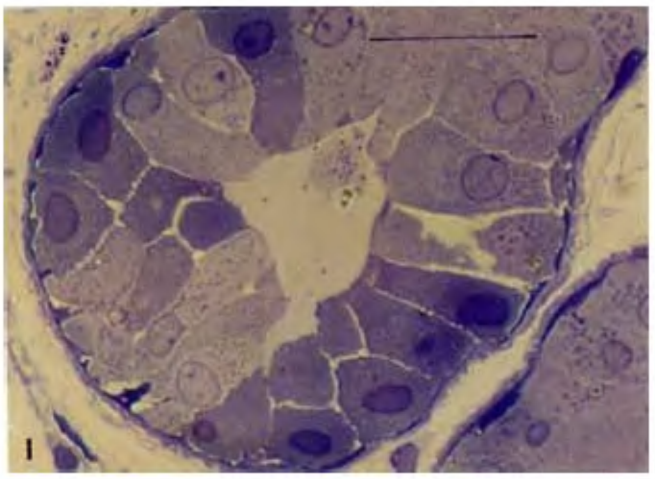

Figure 1. Optical micrograph of semi-thick section stained with toluidine blue showing gland alveolus with dark and light columnar cells and cell debris in lumen. Myoepithelial cells are seen at the periphery. Bar = 25 μm.

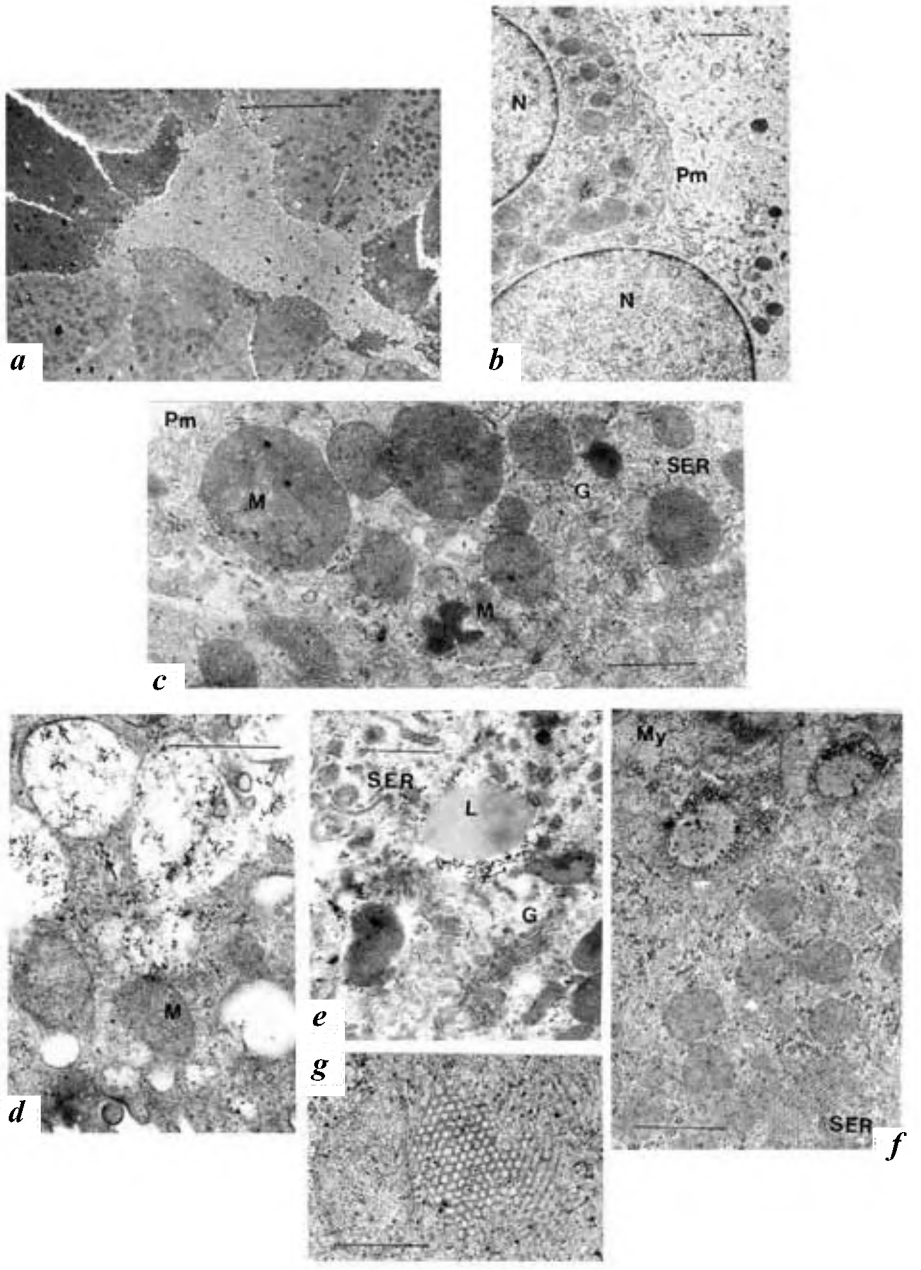

Figure 2. Transmission electron micrographs. ***a***, Dark and light columnar cells. Vacuoles and microvilli are more extensive in dark cells. Light cells more contrasty with mitochondria stained well; bar = 5 μm; ***b***, Nucleus (N) of a cell in the lumen along with another cell whose plasma membrane (Pm) had burst. Pyknotic and normal mitochondria in lumen, bar = 1 μm; ***c***, Region near bursting Pm, cristae in mitochondria (M) are fine, tubular and extensive. Golgi bodies (G) and smooth endoplasmic reticulum (SER) are seen. Some M are associated with lipid vacuoles and are turning darker; bar = 0.5 μm; ***d***, Vacuoles and microvilli of dark cells. Dark excretory products (arrows) accumulate at the edge of the vacuoles and seen to be excreted; bar = 0.5 μm; ***e***, Lipid droplets (L) in a cell with ruptured Pm. Note abnormal shape of L. Secretory product associated with dark M and L; bar = 0.5 μm; ***f***, Dark cell with myoepithelial cell (My) at the top with its muscular processes and normal M with septal cristae. Hypertrophic SER seen at the lower side; bar = 0.5 μm; ***g***, Magnified view of hypertrophic SER showing honeycomb structure; bar = 0.2 μm.

and appear free in the lumen (Figure 2*b*). Apart from their numbers, the most striking feature of the mitochondria is the large number of tubular cristae (Figure 2*c*). Tubular cristae are characteristic of steroid-producing cells like the Leydig cell and the cells of the adrenal cortex[7], but the cristae seem much more numerous here. This feature and the smooth endoplasmic reticulum (SER) which can synthesize cholesterol and the Golgi

bodies seen, can be related to the testosterone production and secretion by these cells which can be as high as 800 ng/ml in the musth secretion[4]. In the cytoplasm of both the dark and light cells, many mitochondria are found to be closely associated with lipid vacuoles and these are found to be more intensely stained. In the dark cells, normal and darkly stained mitochondria are seen while in the light cells mitochondria are mostly dark. This may be the result of a process in the conversion of cholesterol into pregnenelone, the precursor of testosterone, which occurs within the inner cristae through cytochrome P450 (ref. 7). The final hormone is produced by a series of transport steps, back and forth, between the mitochondria and SER. The pyknotic dark mitochondria may represent a later stage in the conversion process. Small, dark secretory products seen in the cytoplasm seem to accumulate at the secretory vacuole edges and get dispersed in the dark cell vacuole (Figure 2 *d*). It has been suggested that vesicular secretion may not be occurring in steroid-secreting cells[7]. Microvilli noticed here might also have a secretory role[7]. Dispersed particulates, as seen in Figure 2 *d*, can be seen even in the cytoplasm

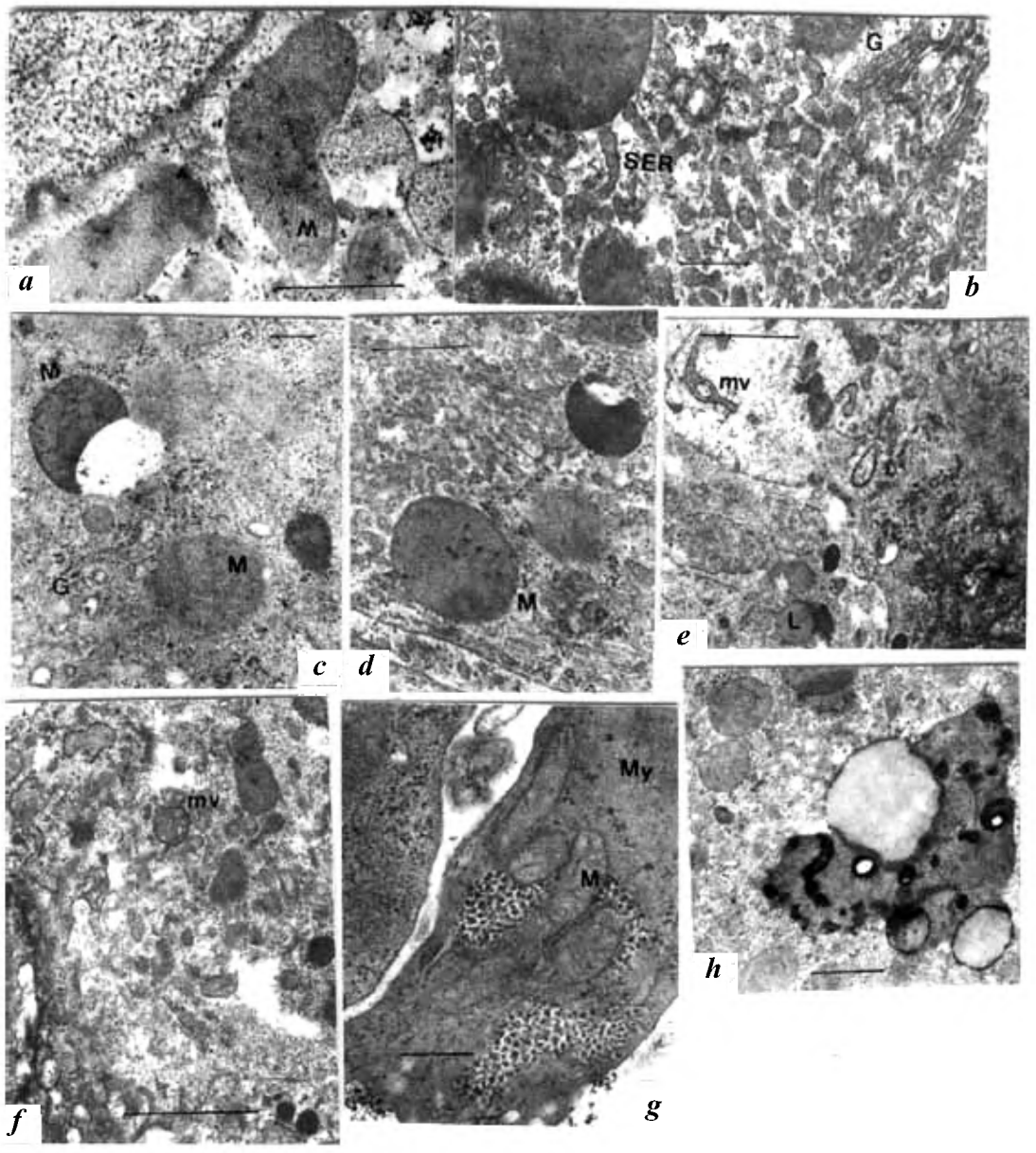

Figure 3. ***a***, M with tubular cristae. Part of nucleus with fibrous lamina; bar = 0.5 μm; ***b***, M, G and SER in burst cell; bar = 0.5 μm; ***c***, M associated with lipid vacuole and part of a G. M with various intensities of staining; bar = 0.2 μm; ***d***, Normal M and one associated with lipid turning dark; bar = 0.5 μm; ***e***, G vesicles with multivesicular bodies (mv), L and M, bar = 1 μm; ***f***, Formation of mv in various stages; bar = 1 μm; ***g***, Myoepithelial cell with muscular processes and M with septal cristae; bar = 0.5 μm. ***h***, Lipofuscin granules; bar = 1 μm.

of the lighter cells which have burst (Figure 2 *e*). There is some deformation of the organelles in the cytoplasm, like the lipid droplet here. This can be attributed only to the muscular contraction of the alveoli by the myoepithelial cells. The darker cell organelles show less deformation, but they also indicate high cellular activity. Most nuclei show chromatin margination and a fibrous nuclear lamina (which may structurally help retaining nuclear integrity as the cell is subjected to mechanical force). The SER in the dark cells shows, in places, a parallel array or honeycomb structure (Figure 2 *f, g*), a feature which has been reported in cells undergoing hypertrophy[8]. The bursting of the plasma membrane does not indicate necrosis, as the nuclei have not become swollen or pyknotic, nor is there swelling of mitochondria which tend to become pyknotic. In some Golgi vesicles (and cytoplasm), multivesicular bodies are seen, as also lipofuscin granules which are indicative of autophagy[7]. Dihydrotestosterone, a degradation product of testosterone and a more potent androgen, has also been reported in musth secretions[4]. Some alveoli, where the cells remained inactive, were also noticed. The cells here did not take up electron stain and just a few nuclei got stained. Additional evidence for the above discussion is presented in Figure 3.

The features noticed suggest the following. For steroid production, the cells go into a hypertrophic state with increase in numbers and tubular cristae of mitochondria, concomitant increase in SER and Golgi bodies with many secretory vacuoles, microvilli and changes in nuclei. Secretory products build up and they are sent out. Autophagy of lipids also takes place. When the limit of steroid synthesis is reached, microvilli disappear, the plasma membrane breaks and cellular debris enters the lumen along with the testosterone and dihydrotestosterone produced, as a result of the contractile forces exerted by the myoepithelial cells. This needs to be compared with mammary gland development, where epithelial stem cells in alveoli differentiate suitably for the production of milk protein and fat. It is interesting that in the temporal gland of the elephant they develop into androgen-secreting cells. The role of mitchondria and its changes during the production of androgen offers scope for biochemical investigations. It has been reported that younger males have in their musth secretion compounds which are honey-smelling (termed *moda* musth), which is tolerated by the older elephants and which in ancient Sanskrit literature is described as capable of attracting honey bees[9]. The present study also invokes the need to compare Asian musth gland ultrastructure with the younger male of the Asian elephant in moda musth, as also the male and female African elephants during normal temporal gland secretion activity and in musth. Along with the physiology involved, these studies can help in reducing human–elephant conflicts arising out of musth activity and perhaps even pave the way for a better understanding and treatment of endocrine diseases.

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Constructing 3D phylogenetic trees

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Lens crystallins are highly conserved, tissue-specific proteins. Crystallins from eight vertebrates were compared on the basis of their isoelectric points, molecular weights and immuno-crossreactivity against polyclonal antibodies using 3D plots, to estimate 'star' and 'network' Euclidian distances between species. The phylogenetic trees obtained were tested by bootstrap and fixed in the 3D space by multidimensional scaling and a new sequential positioning method. 3D trees using 'network' distances give the best fits.

THE vertebrate eye lens contains tissue-specific (α, β and γ or δ) crystallins, which have been compared[1–22] for

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