

Lactation in male fruit bats

SIR — Although male lactation is physiologically possible¹, it is isolated and rare. It has been observed in domesticated mammals^{2,3} and in humans^{4,5}, but it has not been reported in wild, free-ranging species. Here we report lactation by males in a population of Dayak fruit bats, *Dyacopterus spadiceus* (Chiroptera: Pteropodidae), in the Krau Game Reserve, Pahang, Malaysia.

In July and August 1992, we captured 18 *D. spadiceus* in mist nets 8–30 m above the ground in the subcanopy of a lowland rainforest at Kuala Lompat in the game reserve. Of the 13 males that were captured, 10 were judged to be mature based on fully-ossified wing joints and descended testes. Each of the mature males also had functional mammary glands from which small amounts of milk were ex-

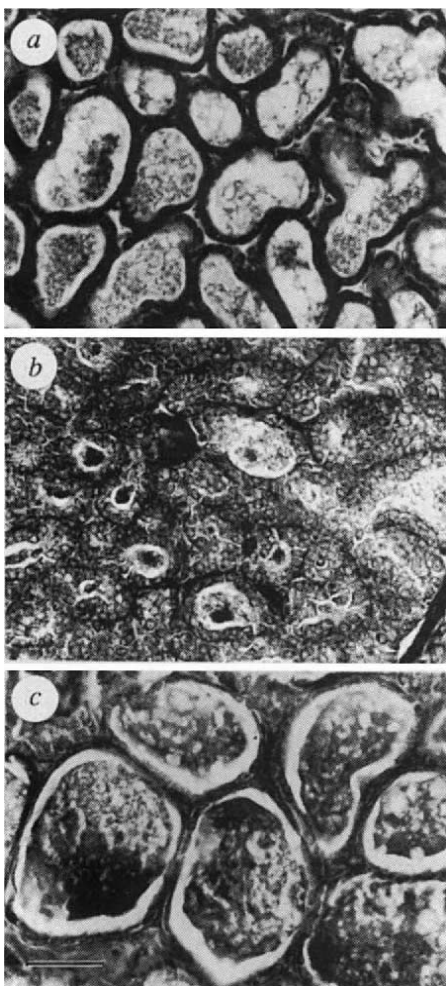


FIG. 1 Histological sections of mammary gland alveolar tissue in a lactating female *D. spadiceus* (a), a non-lactating female (b) and a lactating male (c). The glands were fixed by immersion in 10% buffered formalin, passed through a dehydrating series of ethanols followed by xylene, and embedded in Paraplast Plus. Sections were cut to a thickness of 10–15 μm and stained with Delafield's hematoxylin and eosin. Bar, 50 μm .

pressed. Among the five females that we captured, three were mature, but milk could be expressed from only one in response to manual palpation.

Although some milk was expressed from each of the adult males, the amounts were relatively small. From two males captured in late August, only 4–6 μl were obtained, compared to 350 μl from the right mammary gland of a single adult female. The nipples of the males were smaller and less cornified than those of the females, suggesting little if any suckling activity by the young.

We examined histological sections of the mammary glands and testes of three males, and the mammary glands of two females (one of which was lactating), using light microscopy (Fig. 1). The secretory tissue of the lactating female (a) was thicker and better developed than in the non-lactating female (b). In the males, secretory tissue occupied a layer similar in total thickness to that of the non-lactating female. In two of the males, most alveoli were more distended than in the lactating female, ranging in diameter from 80–140 μm (c). The distension of the alveoli, as well as the consistent presence of secretory material, suggests that milk may have accumulated in the alveoli as a result of not being suckled. In the third male that we examined, the alveoli were less distended, resembling those of the lactating female.

Large mammary ducts were conspicuous in males. These ducts contained secretory material and coursed centrally towards the nipple from the peripherally located alveolar tissue. Multiple lactiferous ducts entered the nipple and opened onto its surface (Fig. 2).

The histological appearance of the testes in the two males with the more distended alveoli was consistent with active spermatogenesis. All stages of developing germ cells were observed in the seminiferous tubules, and these tubules had patent lumina. The significance of testicular histology in the third male was uncertain, as germ cells in all developmental stages were present, but there were few seminiferous tubules with open lumina.

Male lactation may not occur in all populations of *D. spadiceus* or it may vary seasonally. For example, from another sample of 17 individuals netted in Septem-

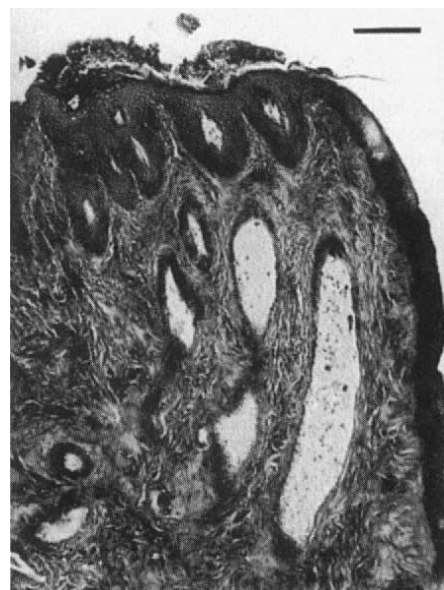


FIG. 2 Vertical section of the nipple of a lactating male *D. spadiceus*, illustrating lactiferous ducts. At the tip of the nipple (top), material expressed from these ducts appears to have adhered to the epithelium during fixation. Bar, 200 μm .

ber 1992 in Sabah, six were mature males with enlarged testes, but none was obviously lactating, nor were the eight apparently mature females. In contrast, one of four mature males caught in August 1993 in Perak was lactating, as was one of the five mature females captured at the same time (two females were pregnant).

Male lactation has been reported only in highly inbred domestic animals^{2,3} or in humans in association with hormone treatments or pathological conditions^{4,5}. Reports of gynaecomastia^{5–7} (breast development) in human males indicate that given sufficient levels of oestrogen and progesterone, mammary glands will undergo hypertrophy and hyperplasia. Elevated circulating oestrogen levels may result from: liver malfunction, which can hinder its inactivation and clearance; from 5 α -reductase deficiency, in which failure to convert testosterone to dihydrotestosterone provides excess substrate for aromatization of androgen to oestrogen; or from dietary or topical exposure to phytoestrogens. Another possibility is that in male *D. spadiceus* mammary glands contain an aromatase system that converts circulating testosterone into oestrogen, in the same way that the hypothalamus of a neonatal male converts circulating testosterone locally to oestrogen during hypothalamic masculinization. If, in addition, there are high circulating levels of progesterone derived from the adrenal gland, as in some species of *Pteropus* (L. Martin, personal communication) and circulating levels of prolactin are adequate, an endocrine status conducive to lactation can be imagined.

Studies on circulating hormones in this

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bat will help clarify the physiological basis for male lactation. On theoretical grounds, functional male lactation would be most likely to evolve in monogamous species¹, in which males share in the care of the young and have high certainty of paternity. Studies of the social structure of *D. spadicus* are required to determine whether they fit these criteria, and whether they actually provide young with milk.

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Antediluvian DNA research

SIR — Lindahl, in a Review article¹ and in Scientific Correspondence², has discussed the limitations of techniques to recover DNA from ancient and fossil samples, in this context citing my PCR amplification of part of a chloroplast gene from a 17-million-year-old fossil leaf³ as being “incompatible with the known properties of the chemical structure of DNA.” His criticisms are based on controlled *in vitro* studies of rates of DNA depurination and subsequent chain breakage under physiological solvent conditions under high temperature^{4,5}. From these experimental trials, he extrapolated rates of depurination and suggested that they are inconsistent with long-term preservation of DNA. It is unfortunate that Lindahl either ignored or was unaware of various empirical reports that are consistently incompatible with his expected properties of DNA decay. Some of these reports were pointed out by G. Poinar, in a reply to Lindahl’s Scientific Correspondence².

The most consistent finding from various studies on ancient DNA is that the rate of decay of DNA is not linear over time. Pääbo⁶ reported that, in DNA extractions from Mammalian soft tissue ranging in age from 4 to 13,000 years old, the extent of the size reduction of preserved DNA was independent of age, the preponderance of the damage occurring immediately post-mortem due to autolysis. Rapid desiccation appeared to improve preservation, although subsequent oxidative damage to the thymines occurred in all samples over time. The amount of ancient DNA in bones is not appreciably different in 200- and 9,000-year-old

samples⁷, and DNA preservation in ancient seeds is clearly not linear⁸. Within a shorter time frame, there is variability in DNA preservation which was not correlated with age⁹.

Of more direct relevance to the question of preservation of plant fossil DNA from the *Clarkia* deposits, Soltis *et al.*¹⁰ reported amplification of a 1,320-base pair fragment from a *Taxodium* *Clarkia* fossil leaf extraction. The analysis of the sequence clearly demonstrated that the sequence was not a contaminant sequence from an extant sample and showed a high degree of similarity to the expected congener.

Lindahl’s reference to the work by Sidow *et al.*¹¹ must also be assessed in its full context. Sidow *et al.* failed to amplify chloroplast sequences from five leaf samples and one acorn, only two of which had DNA that was visible under ethidium bromide staining. They were successful in amplifying a mixed population of apparent bacterial DNA fragments. This success, however, did not correlate with the presence or absence of high molecular mass DNA, as all the samples, including those not having observable DNA and those derived from the adjacent shale without fossil leaf material, could successfully be amplified. The only conclusions that can be drawn from this work are that not all samples are readily amplifiable for chloroplast sequences under the conditions used, and that DNA from soil bacteria is also found in soil-derived samples.

In my view, the most distressing aspect of Lindahl’s analysis is his failure to mention that genic DNA contains information that can be analysed by evolutionary systematics. The presence or absence of contaminating DNA is irrelevant to the question of the persistence of fossil DNA, as long as contaminants can be clearly identified. When there is contaminating DNA, analysis of the derived sequence readily identifies the data as false-positive. Even in the few cases in which the contaminating sequence cannot clearly be ascribed to exogenous sources, unexpected phylogenetic relations will indicate a false-positive. It is clear that presently used criteria for validation of ancient DNA are conservative both because of experimental design and because of the stochastic processes of nucleotide substitution, and thus will have a greater tendency towards rejecting true results than towards accepting false-positives¹².

Theory is important for generating testable predictions, and the validity of a theory is determined by how well it is supported by empirical results. The reverse of this process, establishing the validity of empirical results by determining how well they fit theoretical expectations, is at best arrogant, and at worst, regressive. It is clear from a growing body of empirical studies that the preservation

of DNA is not simply a function of time. Microenvironmental conditions within preserved tissues and organelles are likely to be vastly different from physiological conditions, making simple extrapolations from *in vitro* studies uninformative.

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Chimpanzee DNA profiles on trial

SIR — The capture and trading of great apes has been banned in 112 countries since a CITES meeting in Washington in 1973. Despite this agreement, about 1,000 chimpanzees are deported annually from Africa to Europe, the United States and Japan¹. Private owners (zoos, circuses) disguise this illegal trade by simulating births in captivity and by replacing adults with young animals. Measures to identify captive chimpanzees and control their numbers have so far had little effect. Genetic identity tests — the obvious solution — have never been used because of the unavailability of highly polymorphic markers and the inherent difficulties of obtaining blood samples (in most cases, blood is withdrawn under narcosis).

The use of highly polymorphic DNA markers has been proposed², in combination with the noninvasive procedures of genomic sampling^{3,4}. However, some of these markers (restriction-fragment length polymorphisms, multilocus and single-locus minisatellites) require large amounts of DNA, are labour-intensive and their use as identification profiles is controversial. Others (dinucleotide-repeat microsatellites⁵) are prone to errors in genotype diagnosis. If the standards of reliability and statistical parameters demanded for forensic analyses in humans⁶ were adopted for *Pan troglodytes*