sequently suggested as means of communication[4-7]. VANDERPLANK[8], after having observed that mating calls stimulate copulation, proposed that specific sounds may play a role in mating of tsetse flies. So far no experimental proof has been put forth showing that sound is used by the tsetse fly for communication.

In the present study we show that ultrasonic components (30-70 kHz) of the sound produced by *Glossina morsitans* are consistently different in character for mating as compared with feeding behavior. Furthermore, the character of the sound produced by males and females is different. We hypothesize on this basis that high frequency sounds may play an important role in communication among tsetse flies. Sounds in the frequency range above that audible to man are for several reasons more favorable for communication among insects than are lower frequency sounds; but these have not been investigated earlier in the tsetse fly.

Sounds from *Glossina morsitans* were recorded on magnetic type under laboratory conditions using Brüel & Kjaer $^1/_4''$ microphone (type 4135), Brüel & Kjaer microphone amplifier (type 2618) and a Precision Instrument tape recorder (type PI 6200) operated in direct modes with a tape speed of 37.5 inches per sec. A highpass filter (cut-off frequency 470 Hz) was inserted between the microphone amplifier and the tape recorder in order to remove low frequency noise. The recording system hence had a flat frequency characteristic between 470 Hz and 100 kHz (within less than $\pm$ 3 dB). One or more tsetse flies were kept in a small box covered with a nylon net from which the microphone was kept at a distance of 2–3 cm. The flies were fed by placing the box on one of the ears of a rabbit.

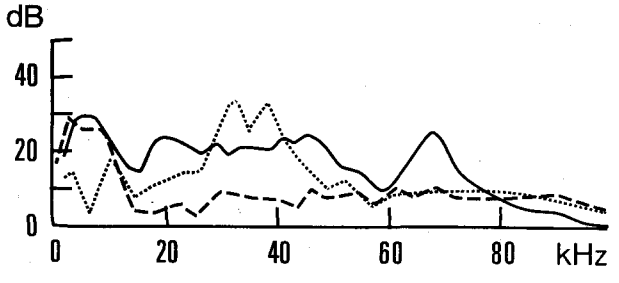

Fig. 3. Spectrograms of mating sounds (solid lines), feeding sounds; male (dashed line), female (dotted line) made from the tape recording using an audiospectrum analysis system with the tapes played back at 3.75″, i.e. 1/10 of the recording speed. The analyzer had a bandwidth of 250 Hz corresponding to a real analysis bandwidth of 2500 Hz. Integration time of the analyzer 400 msec corresponds to 40 msec real time. Zero dB corresponds approximately to 50 dB SPL, (decibel sound pressure level, logarithmic measure of sound pressure with a reference of 0.0002 μbar (2×10^{-5} N/m^2)).

Oscillograms of 2 typical mating sounds are shown in Figure 1, A and B, on 2 different time scales. The complete signal is seen in the upper trace, whereas the lower traces show the signal in the frequency band between 47 and 82 kHz. It follows from the lower traces that the sounds contain considerable energy in the high-frequency range and that this energy appears periodically as brief bursts. The periodicity varies from a few hundreds to about 1000 sec. Despite the individual variability, it is a consistent finding that mating sounds, in contrast to feeding sounds, are rich in high-frequency energy (Figure 1). It is not known whether the recorded mating sounds were produced by the male or the female. Recording from single females in connection with feeding shows that high-frequency components are much less significant than was the case during mating. In males (Figure 2) such high-frequency components are highly unusual.

The frequency spectrum of mating sounds has a significant peak in the high-frequency range (Figure 3), the exact location of which varies from time to time. The intensity of these high-frequency sounds is of nearly the same value as that of the low-frequency sounds. Figure 3 also shows spectra of typical sounds produced after feeding by a female and a male. The double peak between 35 and 40 kHz is characteristic for sounds produced by females in connection with feeding. The lack of high-frequency components in the sound produced by males is evident from this graph.

In short, the results show that the foundations for acoustic communication among tsetse flies (*Glossina morsitans*) indeed exist. To learn the reception and behavioral response of the tsetse fly to such sounds will be the purpose of future studies.

Summary. The spectrum of the sounds produced by the tsetse fly *Glossina morsitans morsitans* extends to above 80 kHz and the energy distribution between 20 and 70 kHz is related to behavior.

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Determination of the Total Number of Dissociated Cells Obtained from the Cerebral Hemispheres of Chick Embryos at Various Ages

Dissociated cells from the cerebral hemispheres of the chick embryo have been cultivated by a number of workers[1-3]. Development of the cultures seems to depend on various factors including the kind of substrate on which the cells grow, the composition of the nutrient medium, the age of the embryo at dissociation and the number of cells cultivated. This means that, while the effect of any one of the above parameters on the culture is being investigated, the others should be kept as nearly constant as possible. Because of cellular proliferation taking place in the developing embryo, the total cell count changes rapidly. Consequently one should have a reasonable idea of the number of cells at various ages, if

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The number of cells/mm³ in the original cell suspension and the total number of cells in the cerebral hemispheres of different age embryos

Age (days)	4	6	7	8	10	12
Cells/mm³	988	8,867	11,900	20,600	47,800	43,000 [a]
	770	6,467	15,700	19,200	47,200	38,000
	929	8,461	16,500	17,000	48,300	30,900
	577	8,781	13,900	19,300	51,900	31,300
		5,846	11,800	22,000	46,600	34,900
		5,878	16,300	22,000		36,900
			18,400	21,900		
				20,100		
Mean	816	7,383	14,928	20,262	48,360	35,850 [a]
SD	159.4	1,340.7	2,303.2	1,647.8	1,859.7	4,151.2
Total cells/2 cerebral hemispheres						
	1.6×10^6	14.8×10^6	29.9×10^6	40.5×10^6	96.7×10^6	143.4×10^6

[a] Only 1 hemisphere/2 ml used.

the number of cells at the start of culture is to be constant for all experiments. This paper reports the increase in total cell count during the development of chick embryo cerebral hemispheres.

Materials and methods. Cells from the cerebral hemispheres of 4-, 6-, 7-, 8-, 10- and 12-day-old chick embryos were used. The embryos were removed from the eggs and transferred to a Petri dish containing Earle's solution, and the cerebral hemispheres were dissected. The meningeal membranes were removed, except in the case of 4-day-old embryos where this was practically impossible. The hemispheres were placed on to a nylon sieve (48 µm pore size) over a Petri dish containing Hankes' solution, and passed through the sieve by gentle stroking of the tissue with a glass rod. Two hemispheres were used per 2 ml of the above solution, except in the 12-day-old embryo where one hemisphere per 2 ml was used. The suspension was then mixed thoroughly by gently rotating the Petri dish. A dilution of 0.04 ml/20 ml was made in Isoton (supplied by Coulter Electronics) and counted by means of a Coulter Counter Model F using a 100 µm orifice.

The Coulter Counter does not distinguish between living and dead cells, nor between single cells and clumps. It was therefore essential to do the counting as soon as possible after dissociation of the cells. The cell preparation was also examined microscopically, either before counting or immediately afterwards, to ascertain that no appreciable amount of clumps have been found. Scrupulously clean glassware was used, since impurities would also be counted as cells.

Results and discussion. The Table gives the number of cells/mm³ in the original cell suspension, and the total number of dissociated cells in the cerebral hemispheres for the various embryonic ages. The latter is graphically represented in Figure 1, from which it is evident that there is an extremely rapid increase in cell count from 1.6 million on the 4th day to 47 million on the 8th day (29-fold increase) and to 143 million on the 12th day (89-fold increase).

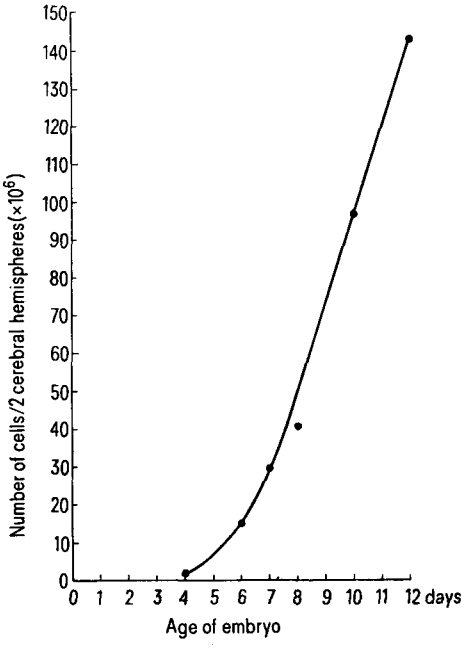

Fig. 1. The total number of dissociated cells in the cerebral hemispheres for the various embryonic ages.

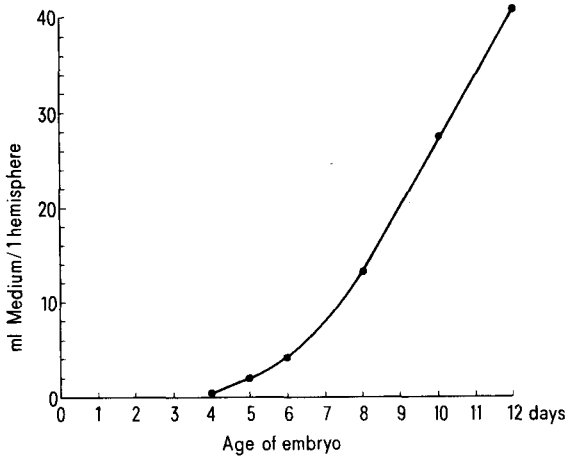

Fig. 2. The quantity of medium required per hemisphere to provide a density of 3,5 million cells/ml.

Our main research concerning the development of the central nervous system is performed on the cerebral hemispheres from chick embryos. For this purpose 7-day-old embryos have so far been used[3], but sometimes it may be of interest to vary the age of the embryo at the start of the culture to study certain aspects of differentiation. It has been found that when using 7-day-old embryos, optimum culture development is obtained by using 1 hemisphere per 8 ml medium with collagen as substrate, and 1 hemisphere per 4 ml when cultivating directly on a plastic surface (SENSENBRENNER, personal communication). From our results, these are equivalent to 3.5 and 7 million cells/ml medium respectively. To obtain the same density for all embryonic ages when cultivating on collagen, the amount of medium to be used was calculated for each case, and the results are shown graphically in Figure 2. These data can be used as a guideline when setting up cultures from embryos of different ages. When cultivating on plastic alone, the above quantities of medium should be halved[4].

Résumé. Le nombre total de cellules obtenues après dissociation du cerveau d'embryons de Poulet de divers âges a été déterminé au «Coulter Counter». La quantité de milieu nutritif nécessaire pour avoir un nombre constant de cellules dans la suspension a été calculée pour chaque âge embryonnaire, afin de pouvoir réaliser des cultures comparables.

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Suspension Culture of *Nigella sativa*

Cell suspension culture is an excellent system to study different cellular mechanisms. The behaviour of the suspended cells of *Nigella sativa* is reported here.

Materials and methods. Cell suspensions of leaf callus tissue of *N. sativa* (Fam. Ranunculaceae; $2n = 12$) were grown in 50 ml MURASHIGE and SKOOG's (MS) medium[1] supplemented with NAA (0.5 mg/l) and coconut milk (15% v/v from fresh green coconut) in projecting flasks (1 l) on revolving shaker (4 rev/min)[2] in dark at 25° ± 1 °C. The medium was sterilized at 121 °C for 20 min. Cells were counted in a counting chamber with dimensions of 50 × 20 × 1 mm and a capacity of 1 ml. The suspended cells in 10 random fields were counted at a magnification of ×100. Suspension culture was initiated by transferring 0.8–1 g 2-week-old callus tissue to the 50 ml liquid medium. During subculture, 5 ml of the suspension from the previous passage was inoculated to a count of 800–900 cells/ml at the onset of the experiments. Cells were subcultured at 30 day intervals. Chromosomes were stained with 1% acetocarmine directly. Coconut milk alone, or in combinations with NAA/2,4-D, was tested to see if there was any increase in the number of free cells.

Results. A good number of free cells were obtained in Ms + NAA (0.5 mg/l) + CM medium, whereas 2,4-D supported clone formation (Table I). In presence of casein hydrolysate or coconut milk, there was considerable increase in total cell number (Table II). A unique suspen-

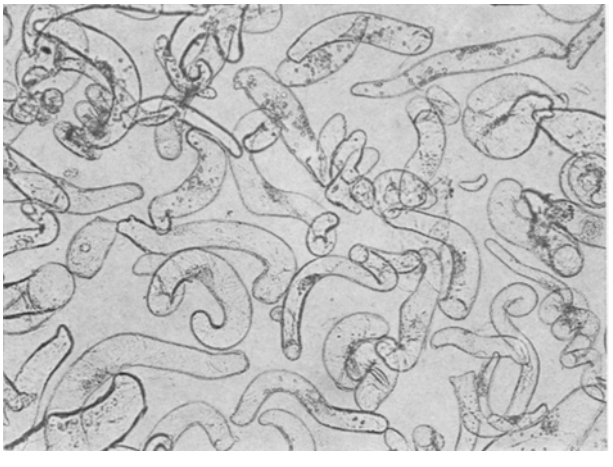

Fig. 1. Cell suspension of *Nigella sativa* with mostly elongated cells.

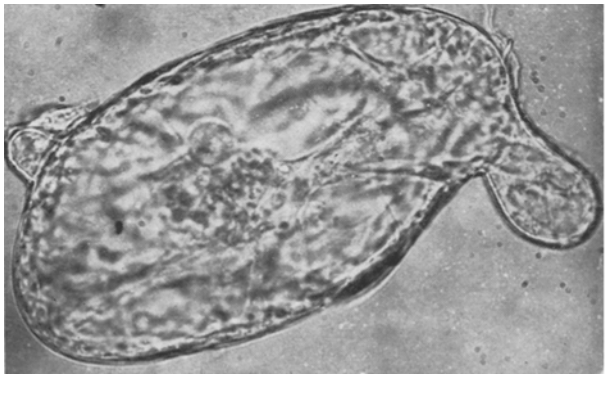

Fig. 2. A free cell of *Nigella* undergoing division by budding.

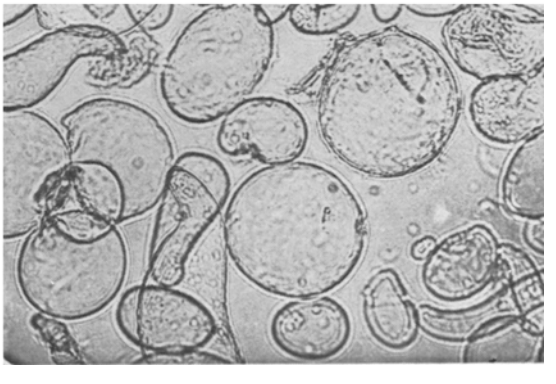

Fig. 3. Cell suspension of *Nigella* with a majority of spherical cells.

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