equally. 300 c/sec, though picked up by these leaves less than 200 c/sec, was equally effective. With the rise in the frequency above 300 c/sec, the interruption of the communication and mating in the leafhoppers declined until at 1200 c/sec as many as 70% males reached the females to mate with them. A decrease in the sound intensity level of the notes to less than 70 db in the air resulted in a decreased pick-up of the notes by the leaves and, hence, in a decline in the interruption of mating.

The acoustic communication and mating in the rice brown planthopper on rice plants was also interrupted, mostly by 200 c/sec and less by 300 or 400 c/sec (table).

In view of the above, 200 c/sec would be quite a suitable sound frequency for use in pest management programs since it is less disturbing and more effective than other notes at a medium aerial sound intensity level (72–76 db). The next requirement would be to produce the desired note at an almost constant intensity for a long time. For this purpose, 2 types of audio-frequency oscillators were tested: type I (Eastern Electronics, Faridabad, India) generated pure notes (figure 3,B) whereas type II was an electronic tuner (Bina Musical Stores, Delhi, India) which generated various notes with their respective harmonics (figure 3,C).

The 200 c/sec note (pure) from the type I oscillator, although picked up by the cotton leaf (figure 3,B (III)) more than the note from the harmonium, did not interrupt the acoustic communication and mating in the leafhoppers (table), possibly because the note lacked harmonics. However, the same note with harmonics from the type II oscillator, which was picked up equally well by the cotton leaf (figure 3,C (III)) but very little by the rice plant (figure 3,C (IV)), was quite effective in this respect over a period of 5 as well as 30 min (table). A combination of 200 and 300 c/sec was effective up to 4 h. Thereafter, even if the sound was stopped, the interruption of the acoustic communication and mating in the leafhoppers persisted for the next 4 h, only 26.6% males reaching the females for mating during this period.

The present observations thus suggest the possibility of using musical sounds for the control of these insect pests by interrupting their acoustic communication and mating. However, for such an application of sound, it would be necessary to select suitable frequencies and intensities of sound and to program their presentation schedule in such a manner as to minimize noise pollution and maximize the interruption of the acoustic communication.

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Effect of cell synchronization techniques on polyamine content of HeLa cells

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Summary. Synchronized cultures of mitotic HeLa cells were obtained by different protocols and the polyamine content of these cells determined. It was found that the method of synchronization can significantly change the polyamine content of the mitotic cells, and can also alter the time course of polyamine accumulation during the subsequent cell cycle.

There is considerable evidence that polyamines are involved in the maintenance of cell growth[3,4]. Changes in the rate of cell proliferation are always paralleled by changes in polyamine levels[5–8] and it is of great interest, therefore, to examine how polyamine content changes during the fundamental unit of cell proliferation, the cell cycle. To obtain sufficient cells to analyze biochemical parameters during the cell cycle, it is necessary to use a synchronization technique[9]. However, many of the standard techniques are known to produce unbalanced growth[10,11] or cause irreversible damage to the cells[12], and it is therefore important to determine whether such techniques can perturb polyamine content.

Materials and methods. Human HeLa-S3 cells were routinely grown in suspension culture at 37 °C, in Eagle's minimal essential medium[13] supplemented with 5% (v/v) fetal calf serum. Different synchronization protocols were used to synchronize the cells. Protocol 1: medium from subconfluent cultures of cells was aspirated to remove dead cells. The dishes were washed with 5 ml prewarmed medium and then 15 ml prewarmed medium was added. After 2 h the dishes were shaken to dislodge mitotic cells, the medium removed and the cells collected by centrifugation. Protocol 2: 4 million cells were plated out into 140 mm petri dishes and incubated for 12 h at 37 °C. The dishes were shaken and the medium aspirated to remove floating cells. The dishes were washed with 5 ml prewarmed medium, 15 ml prewarmed medium was added and the cells synchronized by incubating the dishes in an atmosphere of 95% N_2O:5% CO_2 under 5×10^5 Nm^{-2} pressure at 37 °C for 3.5 h[14]. The mitotic cells were removed by gently shaking the dishes. Protocol 3: thymidine was added to cells grown in suspension culture to produce a final concentration of 2.5 mM, and left for 19 h. The cells were centrifuged, resuspended in fresh medium without thymidine and seeded into petri dishes. The cells were left for 5 h before a 7.5 h nitrous oxide arrest was commenced. Protocol 4:

Table 1. Polyamine content of mitotic HeLa cells selected by different protocols

Synchronization protocol	Polyamine content (nmoles/million cells)		
	Spermine	Spermidine	Putrescine
1	1.9±0.2	3.7±0.3	0.73±0.1
2	2.0±0.4	4.0±0.4	0.86±0.1
3	1.2±0.1	2.0±0.3	1.40±0.2
4	4.0±0.4	6.0±0.4	ND

Each value is the mean of at least 6 determinations±SEM. ND, not determined. Polyamine content was determined by the dansylation method[16].

Table 2. Cell cycle parameters of HeLa cells synchronized by different protocols

Time after release from synchronization block (h)	Protocol 2 [^{3}H]-thymidine incorporation (dpm/10^5 cells)	Mitotic index (%)	Protocol 3 [^{3}H]-thymidine incorporation (dpm/10^5 cells)	Mitotic index (%)
0	18± 3	97	21± 1	95
3	20± 1	1	28± 2	1
6	133± 8	0	67± 8	0
9	350±37	0	283±31	0
12	267±26	0	267±31	0
15	151±16	4	148±10	1
18	100± 8	41	62± 4	36
21	-	63	-	56

The values for [^{3}H]-thymidine incorporation represent the mean of 6 determinations± SEM. The mitotic indices represent the percentage of 1000 cells scored (0.1 µg/ml colcemid was added to the cultures at 12 h after release from the synchronization block to accumulate cells in mitosis).

Table 3. Accumulation of polyamine during the cell cycle of HeLa cells synchronized by different protocols

Time after release from synchronization block (h)	Polyamine content (nmol/million mitotic cells seeded) Protocol 2 Spermine	Spermidine	Putrescine	Protocol 3 Spermine	Spermidine	Putrescine
0	2.0±0.4	4.0±0.4	0.86±0.1	1.2±0.1	2.0±0.3	1.4 ±0.2
3	2.2±0.5	3.8±0.2	0.80±0.2	1.2±0.2	1.9±0.3	1.3 ±0.2
6	1.8±0.4	4.1±0.5	0.76±0.1	1.1±0.1	2.2±0.1	0.55±0.2
9	2.2±0.2	4.7±0.3	0.78±0.2	1.3±0.2	2.0±0.1	0.80±0.1
12	2.4±0.4	5.8±0.5	1.0 ±0.1	1.2±0.2	1.9±0.3	0.75±0.1
15	3.2±0.3	6.8±0.6	1.2 ±0.2	1.4±0.2	2.5±0.2	0.95±0.1
18	3.6±0.3	6.9±0.4	1.2 ±0.1	3.0±0.4	5.2±0.4	1.1 ±0.1

Each value represents the mean of at least 6 determinations± SEM. Polyamine content was determined by the dansylation method[16].

thymidine was added to cells in suspension culture to produce a final concentration of 2.5 mM and left for 17 h. The cells were centrifuged and resuspended for 9 h in medium without thymidine before having thymidine added again and being left for 19 h[15]. The cells were again centrifuged, resuspended in medium without thymidine and left for 5 h before adding colcemid (0.1 µg/ml) for 12 h to produce a culture containing at least 95% mitotic cells.

Results and discussion. The polyamine contents of mitotic HeLa cells, synchronized by different protocols, are summarized in table 1. This demonstrates that the polyamine content of the cells was dependent on the method of cell synchrony. Cells selected by the 'shake-off' method (protocol 1) can be regarded as unperturbed mitotic cells, and the similarity between the polyamine content here and that of cells synchronized by protocol 2 suggests that the latter causes minimal disturbance of polyamine metabolism. However, other methods of synchronization involving excess-thymidine blocks, which are known to alter RNA content[11], and more prolonged metaphase arrest (to increase the yield of mitotic cells), have significant effects on cell polyamine content.

To determine whether the synchronization techniques could also affect the accumulation of polyamine during the subsequent cell cycle, cells obtained by protocol 2 (which appears to give unperturbed mitotic levels of polyamine) were compared with cells synchronized by protocol 3. Cells obtained by both techniques exhibited similar cell cycles, as measured by the time course of ^{3}H-thymidine incorporation into acid-precipitable material and by the occurrence of a second wave of mitotic cells (table 2). The accumulation of polyamines during the cell cycle after release of the mitotic blocks is summarized in table 3. In protocol 2 cells, the major increase in polyamine content occurred between 9 and 15 h after release of the block, with spermidine and spermine increasing 1.8 times but putrescine only 1.4 times. The inability of polyamine content to double during the cell cycle may have been due to some cell death. However, in protocol 3 cells, the major increase in polyamine content did not occur until after 15 h, with both spermidine and spermine increasing 2.5 times but with very little change in the putrescine content.

In conclusion, it would appear that vigorous synchronization protocols which yield high numbers of synchronized cells can significantly alter the polyamine content of the cells and also seriously affect both the extent and the time course of the subsequent accumulation of polyamine during the cell cycle. Synchronization techniques can be used to increase the yield of mitotic cells only if they are applied for short periods of time, as observed with the 3.5 h nitrous oxide block (protocol 2) of HeLa cells.

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