chimpanzee ($2n = 48$). Knowledge of the significance of the chromosome number of the chimpanzee in terms of primate evolution is dependent, among other things, on a detailed analysis of the karyotype as well as upon the chromosome numbers and karyotype analysis of the other anthropoid apes.

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References and Notes

1. C. H. Yeager, T. S. Painter, R. M. Yerkes, *Science* **91**, 74 (1940).
2. J. H. Tjio and A. Levan, *Hereditas* **42**, 1 (1956).
3. We wish to express our appreciation to David Bodian and Philip Sartwell for providing the specimens, and to Neal Nathanson for assistance in obtaining the material.
4. Colcemid (Ciba Pharmaceutical Products, Inc., Summit, N.J.).
5. C. E. Ford and J. L. Hamerton, *Stain Technol.* **31**, 247 (1956).
6. W. Welshons and L. B. Russell, *Proc. Natl. Acad. Sci. U.S.* **45**, 560 (1959).

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Induction of Staminate Flowers on Gynoecious Cucumbers with Gibberellin A_3

Abstract. Staminate flowers induced on gynoecious plants permitted the establishment and increase of inbred lines bearing only pistillate flowers. This method of altering sex expression has practical applications in developing F_1 hybrids, and is useful in studying the physiology and genetics of sex expression.

Completely female (gynoecious) and predominantly female types of sex expression found in certain Japanese and Korean varieties of cucumbers have been recognized as sources of genes that might be utilized to develop female inbred lines for hybrid seed production (*1–3*). Environment and growth regulators alter sex expression in monoecious varieties (*4–6*), and there is wide genetic variability in sex expression (*1–3*, *7*). Stimulation of pistillate flower production with l-naphthalene acetic acid (*4*), and inhibition of pistillate flowers with gibberellin (*6*, *8*), are of special interest in our efforts to utilize genetic variability in sex expression for hybrid seed production.

Beginning in 1954 with a gynoecious segregate found in the Korean variety Shogoin (PI 220860), we developed a number of inbred lines segregating up to 80 percent of gynoecious plants. These inbred lines were used as parents in hybrid seed production by rogueing monoecious segregates from the female parent rows grown in an isolated seed plot provided with a monoecious pollen parent (*3*). This method of seed production is practical and has produced hybrids with a high degree of heterosis. In the U.S.S.R., Mescerov (*2*) independently proposed an almost identical technique for production of F_1 hybrid seed, and likewise observed a striking heterotic effect in his hybrids.

In most of our segregating lines, some predominantly female plants (50 percent or more of the nodes bearing pistillate flowers) were difficult to distinguish from gynoecious plants at the ten-node stage, when seed plots were rogued. The most troublesome were those that developed only one or two staminate clusters after rogueing. This type of predominantly female plant was infrequent, but still a source of some contamination in seed plots. In an effort to eliminate staminate flowers on predominantly female segregates, several chemical growth substances, including gibberellin A_3 (*9*), were applied in 1958 to the foliage of field-grown cucumber plants.

The late developing staminate flowers on predominantly female plants were not completely eliminated. However, gibberellin treatment induced staminate flowers on some plants that would have remained gynoecious. In one line, 17 out of 83 nontreated plants bore staminate flowers compared with 25 out of 33 following foliar applications of 250 parts per million gibberellin. There were 12.4 staminate flowers per 100 nodes on gibberellin-treated plants and 1.3 on the controls.

One completely gynoecious hybrid arising from a cross of gynoecious × predominantly female provided uniform gynoecious plants for further tests in the greenhouse in the fall of 1958. No staminate flowers were produced on control plants, while increasing staminate flower production was observed as the concentration of gibberellin was increased from 250 to 1500 parts per million, and as the number of applications was increased from one to four. Plants receiving four weekly applications of 1500 parts per million produced an average of seven nodes bearing staminate flowers. The induced flowers were normal and produced abundant pollen. Many successful pollinations were accomplished. Homozygous gynoecious inbred lines have been developed through five generations of self-pollination with pollen from staminate flowers induced on gynoecious plants.

Field experiments in 1959 demonstrated a wide range of effective induction treatment and a high tolerance to gibberellin in gynoecious lines. The data from one trial show an increasing response to gibberellin in concentrations up to 5000 parts per million (Table 1). Considerable vegetative distortion followed the 5000 parts per million treatment but many of the plants produced normal lateral branches. No serious vegetative injury resulted from a single application of 2000 parts per million.

In 1959 two 80-ft rows of a gynoecious inbred line, MSU 713-5, were grown in a screen isolation cage provided with a small hive of bees. One row was used to determine the number of foliar applications of 1500 parts per million necessary for adequate pollen and seed production under field conditions. Treatments, replicated three times on four-plant plots, were begun at the second true-leaf stage and ranged from one to four in number. The repeat applications were made at weekly intervals. At least 30 nodes of each plant were examined and classified for sex expression. The nontreated row of 74 plants produced no staminate flowers on more than 2000 nodes classified. A single initial application resulted in nine staminate flowers per 100 nodes and an average of 2.3 per plant. Two, three, and four applications resulted in 45, 66, and 71 staminate flowers per 100 nodes, respectively. Each application affected only two to four nodes per plant, beginning at about node 7 for the first applica-

Table 1. Staminate flower induction by foliar applications of gibberellin A_3 on gynoecious cucumber line MSU 713-21 grown in the field, 1959.

Treatment (ppm)	Total plants	Plants with one or more nodes bearing staminate flowers	Total nodes classified	Total nodes bearing staminate flowers	Staminate flowers per 100 nodes
Control	15	0	417	0	0.0
1000*	14	4	308	7	2.9
1000†	16	8	425	16	4.7
1500*	13	7	283	10	5.3
1500†	16	13	406	27	8.4
2000*	12	6	267	13	5.6
2000†	15	12	359	25	10.9
5000‡	15	10	337	23	9.5

* Applied at first true leaf stage and again 7 days later. † Applied at first true leaf stage and again 14 days later. ‡ Single application at the first true leaf stage.

tion, node 13 for the second, node 20 for the third, and node 27 for the fourth. Intervening nodes bore pistillate flowers and all treated plants reverted abruptly to gynoecious habit. The 118 plants grown in this isolation cage produced 4.2 lbs of seed, demonstrating that ample pollen was provided by two, three, or four weekly foliar spray applications of gibberellin at 1500 parts per million.

In a genetic analysis of sex expression there are obvious advantages in being able to self gynoecious plants in segregating generations and to establish homozygosity for gynoecious habit. In established gynoecious inbreds there is evidence of genetic variability, in that some plants have remained gynoecious even after two applications of 2000 parts per million gibberellin (Table 1). Other gynoecious plants have produced staminate flowers following two applications of 250 parts per million. It is possible to use gibberellin as a selection device to classify plants for ease of staminate flower induction, and subsequently determine how such plants differ genetically.

Sex expression in *Cucumis sativus* can be explained on the basis of response to specific chemical substances synthesized by the plant. These syntheses are controlled by genes. Some genotypes depend upon environment for their expression while others are not sensitive to environment. It is possible that staminate flower production depends upon synthesis in the plant of a gibberellin-like growth substance, and that this synthesis does not occur in gynoecious plants. The establishment of a wide range of sex types under close genetic control should provide material for precise studies of biochemical and physiological mechanisms involved in sex determination (*10*).

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References and Notes

1. S. Kumasawa, M. Matumoto, K. Fujeida, *J. Hort. Assoc. Japan.* **24**, 1 (1955).
2. E. T. Mescerov, *Vestn. Sel'skokh. Nauke* (*J. Agr. Sci.*) **6**, 43 (1957).
3. C. E. Peterson and J. L. Weigle, *Mich. State Univ. Agr. Expt. Sta. Quart. Bull.* **41**, 960 (1958).
4. F. Laibach and F. J. Kribben, *Ber. deut. botan. Ges.* **62**, 53 (1949).
5. J. B. Nitsch, E. Kurtz, Jr., J. Liverman, F. W. Went, *Am. J. Botany* **39**, 42 (1952).
6. S. H. Wittwer and M. J. Bukovac, *Econ. Botany* **12**, 213 (1958).
7. Oved Shifriss and Esra Galun, *Proc. Am. Soc. Hort. Sci.* **67**, 479 (1952).
8. Esra Galun, *Phyton* **13**, 1 (1959).
9. The gibberellin A_3 used in the experiments was an emulsifiable formulation of potassium gibberellate supplied by Merck & Company, Rahway, New Jersey.
10. This report is article 2626 from the Michigan Agricultural Experiment Station.

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In vitro Studies of Single Isolated Beating Heart Cells

Abstract. Rat heart cells, separated by trypsin treatment and grown attached to glass in a liquid medium, exhibit periodic contractions similar to a whole beating heart. The rate of beating, which is up to 150 beats per minute, is affected by cardiac drugs and by metabolic substrates and inhibitors.

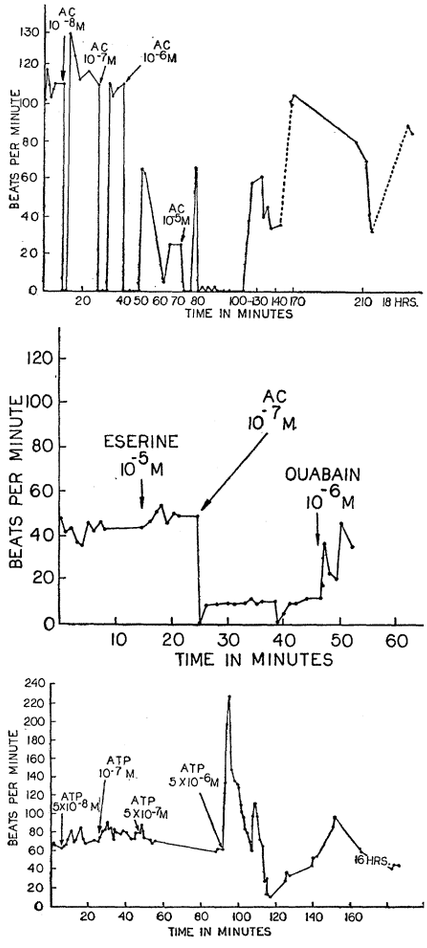
As part of a continuing study of the process of so called "dedifferentiation" in mammalian cell cultures, the cells of the rat heart were separated and grown in vitro. Upon examination of the cultures, it was seen that several of the separated cells exhibited a distinct and rhythmic contraction similar to a heart beat. Some of these cells were observed to beat for as long as 40 days.

The cultures were prepared by separating the cells of coarsely minced young rat heart by incubation with 0.10 percent trypsin for 30 to 60 minutes at 33° to 36°C (*1*). The resultant cell suspensions were centrifuged and suspended in a modified Puck's medium (*2*). The suspensions were made dilute enough so that contact between cells in the cultures was minimized. At first the cells were spherical, but within a few hours many of the cells settled to the bottom of the petri dish and became attached to the glass.

After 3 days at 37°C in an atmosphere of 5 percent CO_2 and 95 percent air, the cells appear to be either typical fibroblasts or myofibrils. Both of these types have been observed to beat. The cells are attached at first by a simple flattening at the bottom cell surface. Eventually the cells may flatten entirely to form either a symmetrical or an irregular shape. Long ameboid or thin filamentous processes increase the irregularity in the shape of the cell. The upper surfaces of the beating cells bulge upwards spherically or are stretched flat between what appear to be the strongly attached processes. It should be emphasized that the morphology of these cells varies and that no correlation can be made between their ability to contract and their anatomy. The lack of a definite histological identity, however, does not alter the fact that they are clearly functional heart cells. Most of the cells contain only one nucleus, although some multinuclei have been observed. In most cases only a small proportion of the cells have been observed to beat and the beating ranges all the way from intermittent, irregular twitches to steady, deep, rhythmic contractions at rates up to 150 per minute. Most of the cells, however, are within the range of 30 to 80 beats per minute. When two or more single beating cells have been observed in the same microscopic field they appear to beat independently.

During the first few days the increase in area covered on the glass seems to be due to the spreading out of the cells. Within a week an increase in the number of cells occurs. After three transfers, in which dilute trypsin was used to detach the cells from the glass, the suspensions were counted in a hemocytometer. An over-all fourfold increase in the number of cells has been observed. After transfer of these cells to another dish with enough dilution so that separate cells were again seen, no beating cells were found, and at no time in any of the transferred cultures were beating cells found.

The effect of certain drugs was tested on these cells to determine how the behavior of the cells compares with that of the intact heart. Figure 1 shows the



Figs. 1–3. Effect of cardiac drugs and metabolic substrates and inhibitors on rate of beating of rat heart cells. The substances were added at times shown by arrows, with final concentrations indicated. Fig. 1 (top), effect of acetylcholine (AC). Fig. 2 (middle), effect of eserine and AC. Fig. 3 (bottom), effect of adenosine triphosphate (ATP).