about one per cell, if such associations were randomly distributed among the chromosomes (and no striking departure from such randomness appeared) about 2 percent of cells would be expected to show an association involving the two chromosome 6 centromeres. Thus very little if any synapsis occurred between the homologous centromeres and adjacent chromomeres which the two fragments of chromosome 6 carried in common. Any important specific role of the centromere in the initiation of synapsis is therefore questioned. The opposite situation in which regions devoid of centromeres successfully synapse is a common occurrence in structural heterozygotes.

It is also of interest that the isochromosomes formed chiasma bearing O or ∞ shaped configurations at diakinesis. Since these foldback chromosomes were presumably univalent for the centromere region, it seems unlikely that it could have functioned as a center of repulsion for diplotene separation and terminalization (*1*). Yet this separation apparently operated normally. Objections to the theory of terminalization by centromere repulsion have also been raised recently by Godward (*2*).

If the ear from the plant carrying the abnormality described above includes the aberrant sector, progeny may be produced which contain a normal chromosome 6 and the two isochromosomes 6. It is of interest in such plants to what extent and frequency each of the isochromosomes will foldback to synapse with itself at meiosis and to what extent it will pair with the homologous region of the normal chromosome or remain unpaired (*3*).

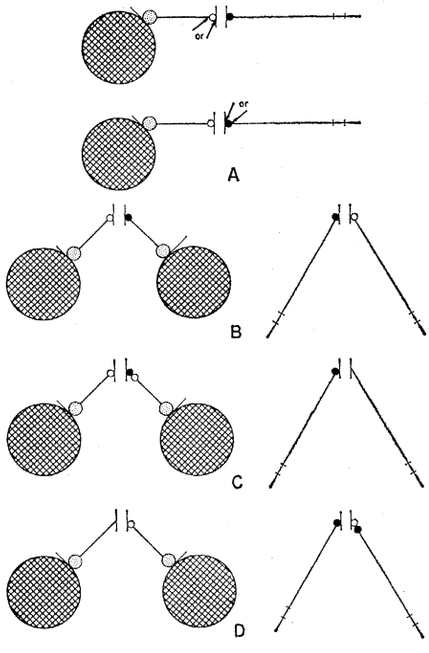

Fig. 2. *A*, Semidiagrammatic representation of normal chromosome 6 homologues. Possible positions of the x-ray induced breaks which led to the formation of isochromosomes are indicated by arrows. The centromeres are the blank regions set off by cross bars. Only the chromomeres adjacent to the centromeres are shown, and these are represented differently on the short arm and long arm sides so that they can be distinguished in *B*, *C*, and *D* (the chromomere on the short arm side is an open circle, on the long arm side, a black circle). The large crosshatched circles represent the nucleolus, the smaller stippled circles are the nucleolus organizer and crosslines near the end of the long arm are small knobs. *B*, Reunion chromatids in the case in which both breaks are separated from the centromere by one chromomere. *C* and *D*, The two cases of reunion chromatids in which one break is immediately adjacent to the centromere and the other break is separated from it by one chromomere.

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28 May 1962

Communication with Queen Honey Bees by Substrate Sound

Abstract. A caged queen honey bee, installed in an observation hive which already contained a virgin queen, piped in response to artificial piping which was played to it through the substrate. The experiments which followed this observation provide the first direct quantitative evidence that sound, at least in the range of 600 to 2000 cycles per second, is perceived by honey bees and that information is transmitted through sound from one bee to another.

Whether or not honey bees (*Apis mellifera* Linnaeus) perceive sound and use this sound in communication is a question which has long been a matter of dispute. Although there is some indirect evidence that honey bees receive sound (*1*), the statement of Snodgrass in 1910 ". . . that no one has yet produced any actual evidence that bees perceive sound" (*2*) has remained essentially unchallenged. At least, there is no existing evidence that there is exchange of information between bees by means of sound. The recent suggestion that foraging bees use sound in communicating distance of a food source to other workers in the hive increases the value of an answer to this question (*1*, *3*). The following experiments show how an artificially produced substrate sound, patterned after that produced by a free virgin queen, elicited a response from a caged virgin. The response, in turn, was similar to that produced by a virgin forcibly contained in her cell by worker bees.

A honey bee colony in a one-frame observation hive raised its own queen from an egg. Five days after emergence of the first virgin queen, another virgin (obtained by mail from northern California) was installed in the observation hive without having been removed from the standard wooden mailing cage. Sound was generated by a Hewlett-Packard oscillator, amplified, and played to the bees through a converted pillow speaker, Calrad PS-10, which had been removed from its plastic case. This speaker was securely fastened to the outside of the hive at the end of the top bar of the frame which contained the honey comb. Thus the speaker was 5 to 10 cm from the queen, which was in a cage on the top bar of the frame.

Queen piping, which had been recorded 3 months earlier from a 5-day-old virgin, was analyzed on a Sonagraph (Kay Electric Company model 662-A). The analysis is shown in part *A* of Fig. 1, where frequency is displayed on the ordinate and time on the abscissa; the relative intensity within one tracing is indicated by the darkness of tracing. When the pattern was imitated by means of a telegraph key connected between the amplifier and transducer, a response was obtained from the caged bee. A record of a successful stimulus and the bee's response to it are shown in the parts *B* and *C* of Fig. 1, respectively. The intensity of the stimulus was approximately equal to that of loud queen piping. The caged queen in the hive usually piped within 5 seconds after the artificial piping ceased.

To eliminate the possibility of coincidence, my assistant listened to the caged queen for a 10-second period each minute, on the minute, for a total of 1 hour. For 30 of these observations, at random, I imitated a queen piping just before the 10-second listening period. The assistant then tallied +

or 0 if the caged queen piped or did not pipe during that period. When sound was played, the queen piped 24 times out of the 30 imitations offered; at the 30 intervals when no sound was offered the queen did not pipe at all. For these figures, $p < .005$ by the chi-square method.

To determine whether the stimulus was airborne or substrate borne, the experiment was repeated with the vibrator suspended in the hive 0.5 cm above the queen cage. Now when the sound was played the queen piped two times out of 30 times when sound was played and three times out of 30 when no sound was played. Again, by the chi-square method, $.50 < p < .75$.

Since the intensity of airborne sound which actually reached the queen in the second experiment must have been greater, the success in the first experiment must have been due to reception of substrate sound; this finding is in agreement with previous suggestions (*1*).

To eliminate the possibility that this second finding resulted from cessation of response by the queen, the first type of experiment was repeated with the transducer in its first position outside the hive. Results were comparable to those obtained in the first experiment.

With the one set of experimental conditions, this type of stimulus elicited the same response for six consecutive days, after which time the caged queen no longer responded. In the meantime, a crude frequency response range was obtained by playing the same pattern at various frequencies, the order of which was selected at random; those responses which occurred within 10 seconds after discontinuance of sound were tabulated. The most consistent response was in the range of 600 to 2000 cy/sec, with some infrequent responses outside this range. Repeated attempts to obtain a response at the fundamental frequency (430 cy/sec) of the imitated sound failed. This result does not agree with the conclusion of Woods that the fundamental frequency of queen piping is predominant and that harmonics of this sound are artifacts of recording technique (*4*). We plan further experiments with frequency response with instruments which will permit greater control of transducer quality and of the energy transmitted by the substrate at different frequencies.

At no time were tape recordings of queen piping played to the hive. Such experiments await a more thorough analysis of queen piping to permit more accurate interpretation of any results obtained, for queen piping varies considerably in harmonic content and emphasis.

Some attempt was made to vary the pattern played to the queen. Although some variation from the pattern shown in Fig. 1*A* (a 1-second pipe followed by several pipes of less than ½ second) produced a response, queens would not respond if the initial pipe was much shorter than a second or if less than five short pipes followed the initial pipe. Also, if a long pipe was followed by two to three short pipes, and then by a long pipe again, queens would not respond.

Interestingly enough, the queen piping which we successfully imitated is a variation of that commonly referred to as "tooting." This is the sound normally produced by a queen which has recently emerged from her cell. The response sound we obtained is similar to that commonly referred to as "quacking," the sound normally produced by fully developed queens forcibly contained in their cells by worker bees. This is the more interesting since the responding queen in these experiments, although already emerged from her cell, was contained in the queen cage within the hive. An imitation of "quacking" did not result in a response from her, even if the sound was played within the frequency range of reception.

These results indicate that a degree of specificity of the sound pattern is required for a given response, and therefore they furnish direct evidence that bees exchange information by means of sound waves (*5*).

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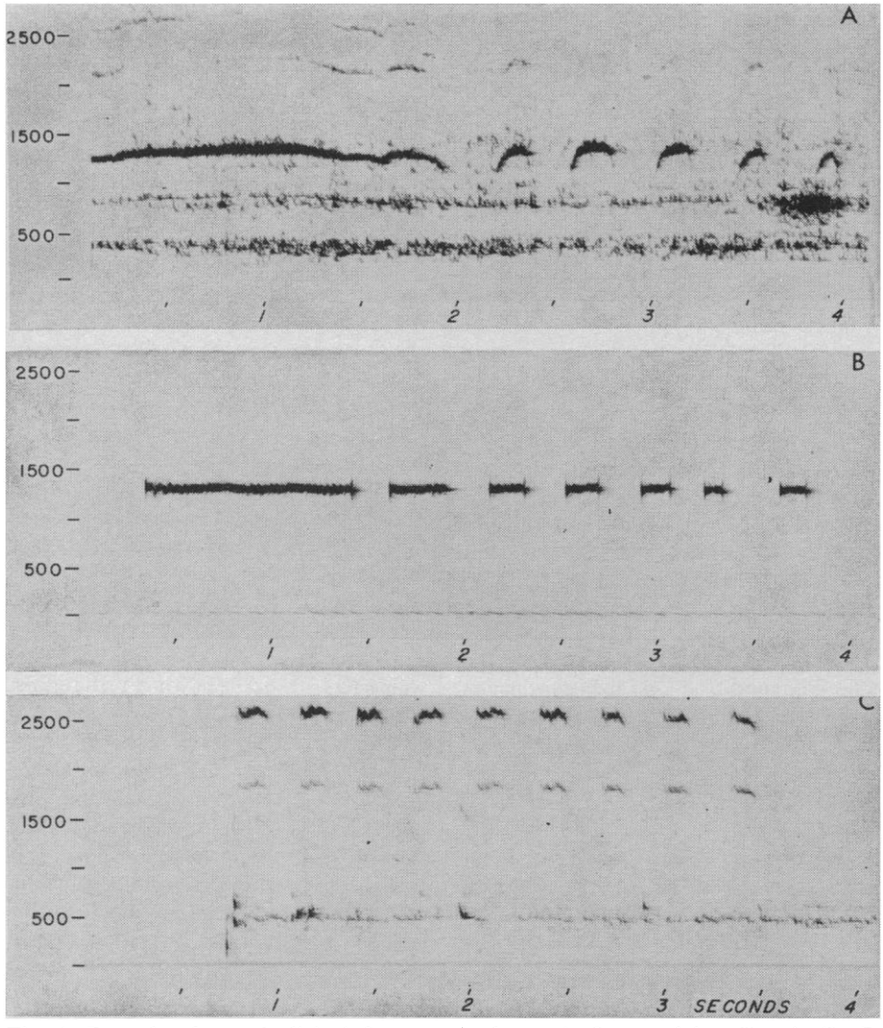

Fig. 1. Records of sound elicited from queen bees. *A*, Queen piping ("tooting"). *B*, Artificial "tooting." *C*, Response piping ("quacking"). Ordinates, frequencies in cycles per second; abscissas, time.

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Virus Neutralization Test in a Capillary Tube

Abstract. A simple and accurate virus titration method which requires extremely low numbers of cells per test vessel is described. Inhibition of metabolism was the criterion for 50 percent end point analysis when capillary tubing was used as the cell culture container. Virus neutralization titers were obtained within 24 hours.

Cell culture techniques have evolved to the extent that they may be used routinely in diagnosis of certain virus diseases. The most widely used virus neutralization tests are based either on cytopathic changes or inhibition of metabolism (*1*). Currently, these tests have the disadvantage of requiring special facilities for carrying cell lines, or if cells are purchased from commercial sources, tests are limited for economic reasons. The technique described here requires only a small number of cells in each test vessel and allows a neutralization titer to be made with cells harvested from only one or two tube cultures (*2*).

The culture vessels were prepared from capillary tubing of outside diameter 1.3 mm, wall thickness 0.2 mm, and length 40 mm. They were sterilized by autoclaving without prior cleansing.

Stock cell cultures were grown in screw-cap test tubes (15 by 125 mm) in Eagle's medium with 10 percent calf serum (*3*). Incorporation of antibiotics in all media, checks for media contamination, and constant use of normal precautionary measures to maintain asepsis resulted in a low incidence of contamination of the test vessels. The occasional contaminated capillary tube could be identified by appearance alone. Suspensions of cells were prepared by trypsinization of the cell layer; for this purpose, 0.06 percent trypsin in Hanks balanced salt solution, in which there was no added calcium or magnesium, was used. The number of viable cells was determined by a hemacytometer count of neutral red stained preparations, and total number of cells with an electronic counter. The cells were then suspended in Eagle's medium with 2 percent calf serum, at *p*H 7.6, so that each 0.02 ml of final volume contained 600 cells. The number of cells determined the length of time which was required before the phenol red in the medium changed to yellow.

If the titer of the virus was to be determined, tenfold serial dilutions were made in Eagle's medium, and aliquots of each dilution were mixed with equal proportions of the cell suspension. The mixture of cell and virus was then drawn by capillary action into the capillary tubes. This was facilitated by preparing the mixture in Wassermann tubes so that the suspension could then be brought to the lip of the tube to charge the capillary. Each end of the filled capillary tube was then sealed, by capillary action, with a 2-mm plug of mineral oil. The tubes were kept horizontally at 36°C in a dry atmosphere incubator until the control tubes reached the proper *p*H for end point readings. A simple tube holder was devised by stapling white adhesive tape to cardboard so that the tubes could be stuck to its surface in dilution groups.

Virus titers and serum neutralization titers could be obtained with an accuracy comparable to that obtainable by other methods which depend upon a 50-percent end-point analysis. The color difference between tubes with viable cells and those with cells destroyed by virus was marked, so that the end points were distinct. This color difference was enhanced because the medium became more basic as CO_2 was gradually lost through the oil seal in tubes which did not contain metabolizing cells.

The greater the number of metabolizing cells the more rapid was the conversion to an acid reaction. In fact, conversion could be observed in about 6 hours if there were 2000 cells in each tube. Large numbers of cells both caused a rapid appearance of end points and were responsible for a concurrent decrease in the sensitivity of the test. Thus, by careful adjustment of the number of cells, fairly accurate end points could be obtained in 24 hours. The most accurate titers were obtained when there were 300 cells in each tube and there was a lapse of 3 to 6 days before final readings were made.

In titrations of type III poliovirus, using HeLa cells grown in 15- by 125-mm tubes, the end points based on cytopathic cell response compared favorably with titers obtained by the capillary tube method. A sample of type III poliovirus antiserum indicated approximately the same titer by each method.

This technique, because of its speed and economy, could be used with the ordinary equipment of a bacteriological diagnostic laboratory to increase the use of virus neutralization tests in the diagnostic laboratory (*4*).

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