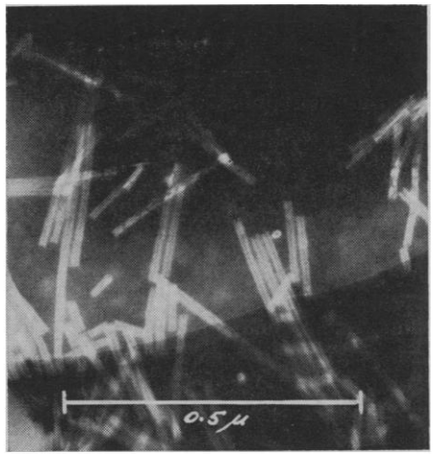

Fig. 1. Tobacco mosaic virus protein negatively stained at *p*H 5.3. The region at the bottom is supported by a carbon film, while the rest of the preparation has dried over a hole in the supporting membrane.

fectious virus particles are formed (*1*). The electron micrograph (Fig. 2) of the negatively stained aggregated PM2 protein may help to explain this behavior. The aggregated PM2 protein forms open, flexuous, helical structures rather than compact rods. The integrity and biological activity of tobacco mosaic virus RNA are normally stabilized when the RNA is sheathed in viral protein which thus shields it from the deleterious action of nucleases. Presumably, the open structure of aggregated PM2 protein could provide no such shield. Alternatively, the PM2 protein may have no affinity for virus RNA.

The electron micrographs of aggregated PM2 protein also show some single strands. We believe this may be an artifact of negative staining because micrographs of shadowed preparations show rope-like flexuous particles of a diameter characteristic only of double strands. Moreover, the particles are considerably longer in the shadowed preparations, indicating some breakage from negative staining with phosphotungstic acid (*4*).

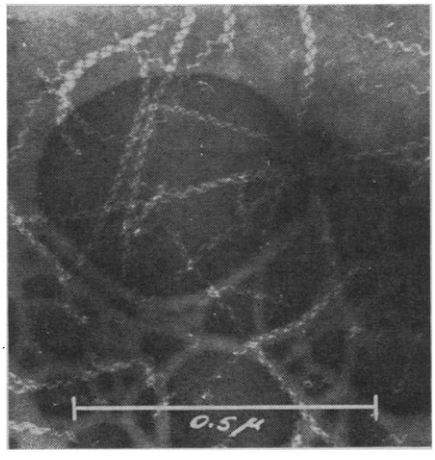

Fig. 2. An electron micrograph of PM2 protein negatively stained at *p*H 5.3. The protein particles extend over a network of holes in the membrane.

The increased resolution within the "holey" regions on both micrographs is of interest. Huxley and Zubay (*5*) have reported a reduction of flattening of *Escherichia coli* ribosomes and of turnip yellow mosaic virus particles accompanied by an increased visualization of structural detail where the droplet of phosphotungstic acid dries unsupported over the holes in the carbon film. The double strands of PM2 protein are most obvious in this region and appear to be narrower than where supported by the film. Outside of the hole the PM2 strands may be flattened during drying onto the membrane. Conversely, tobacco mosaic virus protein, does not show different dimensions in regions outside of the hole (Fig. 1).

Some of the PM2 helixes appear as right-handed coils whereas others seem left-handed. This is apparently an optical artifact, because in shadowed preparations all the particles are oriented in one way (*6*).

For negative staining with phosphotungstic acid we followed the procedure of Brenner and Horne (*7*). A 0.1-percent solution of either PM2 or tobacco mosaic virus "A" protein (*8*) was dialyzed in water at *p*H 8 for 4 hours at 2°C against 0.1*M* ammonium acetate adjusted to *p*H 5.3 with acetic acid. The aggregated protein was mixed with an equal volume of 2 percent phosphotungstic acid adjusted to *p*H 7, and sprayed onto carbon-coated screens. The screens contained holes and "pseudo holes" in the formvar membrane when prepared according to Harris (*9*). Before application of the specimens, the formvar was removed with chloroform.

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4. Most particles in shadowed preparations are more than 5 μ in length, which explains why these aggregated preparations exhibit a very marked viscoelastic effect, characteristic of high molecular weight polymers.
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6. If you consider that you are viewing the particle end on, then the strands would be coiling clockwise as they approach you.
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10. We thank Albert Siegel and Morris Cohen for their advice and interest. This work was supported in part by a grant from the National Science Foundation, by Atomic Energy Commission contract AT (11-1)-873, and grant award GM06327 of the National Institutes of Health. University of Arizona Experiment Station Technical Paper No. 865.

20 January 1964

Sensitivity of Female Inbreds of Cucumis sativus to Sex Reversion by Gibberellin

Abstract. *Two female inbred cucumbers were developed by substituting gene* Acr *for* acr *in the genetic backgrounds of the monoecious races Marketer and Tokyo, which exhibit weak and strong male tendency respectively. Marketer females are resistant and Tokyo females are sensitive to sex reversion in response to treatments with gibberellin* A_3. *Resistance and sensitivity of this type appear to depend upon the genetic system which controls sex tendency.*

The monoecious races of cucumber, *Cucumis sativus* L., are male early in development and female later. They vary greatly in sex tendency, depending on rate of sex reversion during plant development. Gene *Acr* accelerates the rate of sex reversion (*1*) and is often associated with complete femaleness (*2*).

Certain treatments with gibberellic acid or gibberellins increase male tendency in monoecious races (*3*) and induce staminate flowers in female inbreds (*1, 4*). However, most female inbreds, *Acr Acr*, are relatively resistant to sex reversion by gibberellic acid.

Previous results (*1*) suggested that some females might differ from each other in degree of resistance, depending on the potential sex tendencies of their genetic backgrounds. Hence, we attempted to synthesize resistant and sensitive female inbreds.

Two monoecious inbreds of the varieties Marketer and Tokyo were selected for synthesis. Marketer *acr acr* exhibits relatively weak male tendency, and Tokyo *acr acr* is strongly male

(Fig. 1). The objective was to develop two female inbreds by substituting gene *Acr* for *acr* in the genetic backgrounds of both varieties. The source of *Acr* was the variety Shoigon. The female inbreds of Marketer and Tokyo were obtained through eight to nine backcrosses (*5*) which were followed by self-pollination of *Acr acr* and *Acr Acr* individuals with the aid of gibberellic acid. We expected that Marketer female *Acr Acr* would be highly resistant and Tokyo female *Acr Acr* would be sensitive.

In testing the resistance of these female inbreds we used Merck's Gibrel (sample No 61-RTS-1051), a potassium salt of gibberellin A_3. The results of one of our tests are presented in Table 1. This test was conducted under greenhouse conditions from 12 August to 1 October 1963. The plants were grown in 15-cm (6-inch) pots, in soil, and arranged in a randomized block design of ten single-plant replicates for each treatment and control. The treated seedlings were sprayed once with an aqueous solution of gibberellin when the midrib of the first true leaf reached a length of about 2.5 cm. Flower primordia were not differentiated at this stage. Greenhouse conditions provided a 16-hour photoperiod, day temperature of 23° to 27°C, and night temperature of 15° to 19°C. During the photoperiod the plants received supplementary light of about 11×10^3 lu/m^2 (10^3 ft-ca) from fluorescent tubes (Sylvania F96T12CWVHORL).

In a similar test the same inbreds were grown in Hoagland No. 2 nutrient solution to which gibberellin was added (*6*) only once when the seedlings reached the first true leaf stage. The initial gibberellin concentrations were 0, 10^{-5}, 10^{-4}, and $10^{-3}M$. The plants were raised in growth chambers which provided a 12-hour protoperiod; light of 33×10^3 lu/m^2, 90 percent of this intensity coming from fluorescent tubes and 10 percent from incandescent lamps; day temperature 24° ± 1°C and night temperature 18° ± 1°C as recorded at a given location in each growth chamber. The results of this experiment were similar to those obtained in the spraying experiment: Tokyo females differentiated staminate flowers in response to all treatments, but Marketer females exhibited complete resistance.

Table 1. Sensitivity and resistance of cucumber females *Acr Acr* to sex reversion in response to spraying of seedlings with gibberellin A_3. Data are based on the first 25 leaf axils along the main stem of each plant (ten plants per treatment and control).

Concn. of spray (ppm)	Leaf axils per plant (mean No.)	
	Reproductive	Staminate
Females of Tokyo background		
Control	22.9 ± 1.0	0.0
250	20.7 ± 1.6	4.6 ± 2.4
1000	19.0 ± 1.4	13.0 ± 2.6
4000	19.3 ± 1.3	15.0 ± 2.9
Females of Marketer background		
Control	21.4 ± 2.0	0.0
250, 1000, 4000*	18.4 ± 3.1	0.0

*Combined.

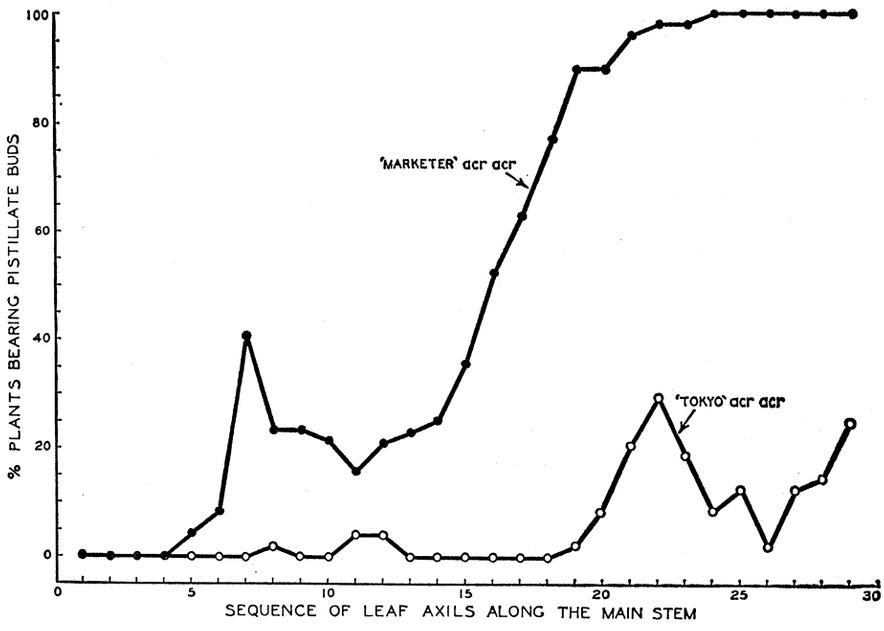

Fig. 1. Sex expression in two monoecious inbreds of cucumbers during plant development (*5*). Data are based on 48 plants per inbred.

Marketer female inbred can be induced to differentiate a few staminate flowers by applying two sprays of gibberellin, each 2000 ppm or more. The two sprays are applied 7 days apart when plants are in the seedling stage. Such a treatment adversely affects growth. In contrast, Tokyo females differentiate more staminate flowers in response to only a single spray of 250 ppm of gibberellin, applied at a comparable seedling stage, and this treatment does not adversely affect plant growth. Further, unlike most female inbreds, Tokyo females can revert even when treated in later stages of plant development.

Sex reversion in treated females of Tokyo occurred not only on the main stem (Table 1) but also on many laterals. Some of these laterals appeared 3 weeks after the seedlings were sprayed.

Our results suggest that resistance and sensitivity of these female inbreds depend upon the genetic system governing sex tendency. Two additional facts strengthen this view. First, Marketer *Acr acr* is female and Tokyo *Acr acr* is monoecious (*5*), indicating an interaction between *Acr* and genes for sex tendency. Second, female inbred GY-7 (*1*), which has a genetic background intermediate in sex tendency between Marketer and Tokyo, proved to possess intermediate sensitivity.

It is evident that treatments with gibberellic acid or gibberellins can reveal marked differences in sex potentialities between some cucumber females which are otherwise indistinguishable.

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