metry, microcephaly, ectopic hearts, and umbilical hernias.

We cannot estimate the frequency of malformations since many of the embryos were already dead and too disintegrated for study when the mothers were killed. From those litters which received 10^{-1} dilution of tissue culture virus most of the survivors were abnormal. The occurrence of a widespread viral infection of the fetuses was indicated by the numbers of intranuclear inclusion bodies in all of the mesenchymal tissues of the fetus, especially those in the region of the somatic mesoderm on the 13th day of gestation. There was an apparent reduction in the amount of skeletal muscle tissue. Other inclusion bodies, typical of H-1 virus infections (*9*), were noted in the smooth muscle cells of the wall of the aorta, the endodermal cells of the wall of the developing gut, the heart, the notochord, the lung, and in cartilage cells in the ribs and vertebral bodies.

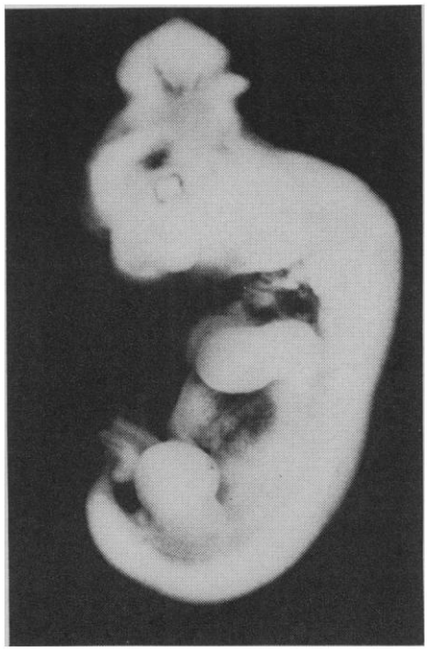

Fig. 1. Eleven-day-old hamster fetus whose mother received H-1 virus on the 7th day of gestation. Early exencephaly (× 7).

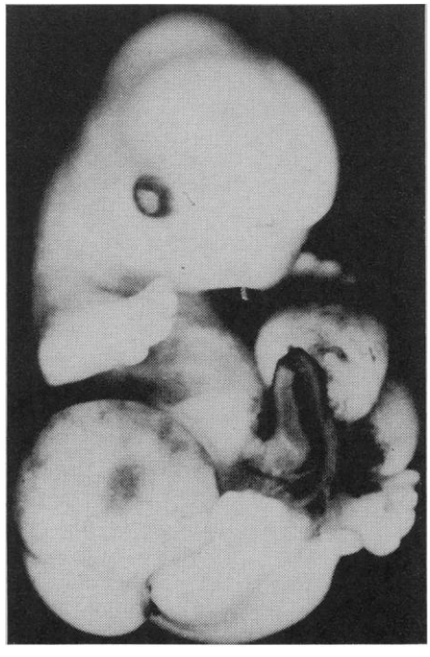

Fig. 2. Thirteen-day-old hamster fetus. H-1 virus (10^{-1}) on the 6th day of gestation. Marked bilateral abdominal herniation of liver tissue (× 5).

The H-1 virus recovered from placental and uterine tissues of a hamster inoculated on the 10th day of gestation and killed 3 days later had a titer of 10^{-3}. Virus was recovered in dilutions of 10^{-1} in similar tissues obtained from animals killed at 7 days.

In a pregnant hamster inoculated on the 7th day of gestation and killed 4 days later, H-1 virus was demonstrable in the placenta at a titer of 10^{-6} and, in the fetus, in a titer of 10^{-2}. While titers of recovered virus were not particularly high they did show, taken in conjunction with the finding of numerous inclusion bodies, that viral invasion of the fetus did take place.

Our results are not dissimilar to those associated with malformations following infection with rubella during human pregnancy. Selzer (*11*) has reported finding inclusion bodies and isolated rubella virus in tissues of a 20-mm human embryo from a mother in whom onset of clinical rubella occurred 10 days before abortion. Another parallel is afforded by cytomegalic inclusion disease. This condition is characterized by the presence of characteristic cytoplasmic inclusions and a persistent chronic viruria in human abortuses and newborns (*3*). Fetal infection with this virus undoubtedly occurs in the uterus.

Previous work in this laboratory showed that—like H-1 virus—a strain of rat virus was capable of penetrating the placenta. It did not, however, exert any embryocidal or teratogenic effect (*8*). Mumps virus used under similar experimental conditions (*12*) failed to penetrate the placenta in detectable amounts, and was without effect on the embryo. Herpes simplex virus was tried in an additional set of hamster experiments. It also failed to reach the embryo after intravenous injection of the mother, although the hamster fetus was susceptible when this agent was injected directly into the uterus (*13*). Thus, in our experience with various agents, H-1 virus has been the only one to penetrate the placenta and proliferate in the fetuses with production of congenital anomalies.

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18 June 1964

Preferential Segregation of Chromosomes from a Trivalent in Haplopappus gracilis

Abstract. *Crosses between plants of* Haplopappus gracilis (n = *2*) *and a race with three pairs of chromosomes (tribivalens) gave a highly fertile five-chromosome hybrid. In both races the chromosomes with the satellites appear homologous, but the other two tribivalens chromosomes pair with the A chromosome (without a satellite) of* H. gracilis. *Disjunction from the resulting trivalent is preferential: the A chromosome goes to one pole and the two tribivalens chromosomes to the other.*

Haplopappus gracilis (Nutt.) Gray is a small, annual composite found in arid and semi-arid habitats of the southwestern United States and northern Mexico. Interest in this species derives from its low chromosome number of $n = 2$ (*1*) and its consequent value as a tool in certain radiation, physiological, and cytogenetic studies. Cytological study of the species has shown that

each chromosome can be recognized throughout mitosis and meiosis, beginning with early prophase (*3*). In a recent study (*3*), I demonstrated that *H. gracilis* is of aneuploid origin, derived from an ancestral species with four pairs of chromosomes, *H. ravenii* Jackson or a very similar taxon, by unequal reciprocal translocations and loss of centromeres. I suggested also that an intermediate race with three pairs of chromosomes in the descending aneuploid series was probably no longer extant. However, recent collections of *H. gracilis* from Arizona yielded plants with three pairs of chromosomes which may represent the missing number in the descending aneuploid series of $n = 4$ to $n = 2$. This three-paired entity will herein be referred to as "tribivalens" without implying any taxonomic status.

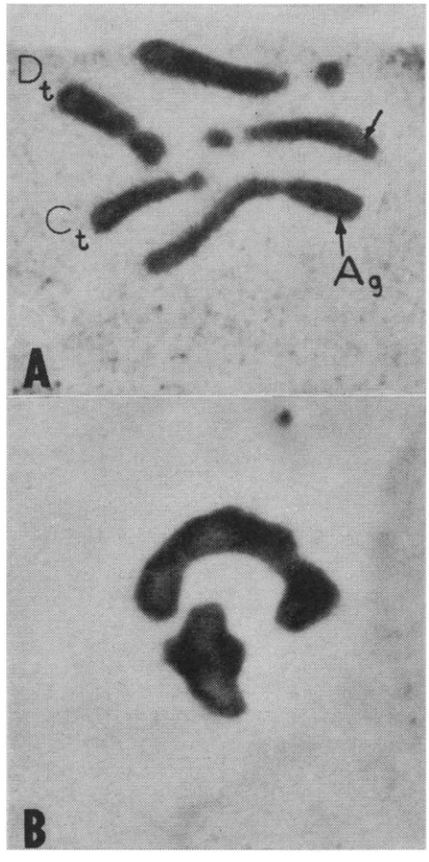

Fig. 1. *A*, Mitotic metaphase in a root-tip cell of *H. gracilis* ($n = 2$) × tribivalens ($n = 3$). The long arm of A_g is slightly stretched in this preparation. C_t and D_t are about equivalent in total length to A_g. The top arrow designates one of the two chromosomes with satellites which may be either B_g or B_t. *B*, Metaphase I in a microsporocyte of *H. gracilis* × tribivalens. The trivalent A_g-C_t-D_t is at the top of the metaphase plate. Chromosome A_g occupies the central position of the trivalent with C_t and D_t connected by chiasmata to either arm. C_t and D_t centromeres are directed downward while the centromere of A_g is pointed upward.

Chromosome counts from several populations show the sporadic occurrence of tribivalens and a limited number of its hybrids with *H. gracilis*. However, only one very weak tribivalens plant has been grown. One head was crossed to a race of *H. gracilis* before the plant died, and one vigorous F_1 hybrid grew to maturity.

The five-chromosome hybrid produced about 89 percent stainable pollen and 73 to 95 percent viable seed per head with an average of 85. Normal *H. gracilis* produces 94 to 99 percent stainable pollen and the same range of viable seed. The fertility of the pollen of a tribivalens plant collected in the field was 91 percent.

A preliminary study of meiosis in the hybrid has shown that the nucleolar organizing chromosome of *gracilis* (B_g) is essentially homologous with that of tribivalens, designated B_t. Chromosome A_g of *gracilis*, however, regularly pairs with the other two chromosomes of tribivalens, designated C_t and D_t. A mitotic metaphase in the hybrid shows (Fig. 1*A*) that the total length of C_t and D_t is about equivalent to chromosome A_g of *gracilis*, suggesting that tribivalens is, indeed, the missing number in the aneuploid series. Nevertheless, this is speculative until more detailed cytological and morphological studies are completed.

Of immediate interest here is the type of meiotic segregation observed in a limited number of microsporocytes produced by the hybrid *gracilis* × tribivalens. The bivalent B_g-B_t disjoined normally and apparently at random with respect to the other chromosomes at anaphase I. I could not, however, distinguish between B_g and B_t in either mitotic or meiotic stages. Chromosomes A_g, C_t, and D_t regularly formed a trivalent, with C_t and D_t each pairing with an arm of the metacentric A_g.

At metaphase I, a limited number of configurations showed A_g oriented toward one pole with C_t and D_t toward the other (Fig. 1*B*). In 14 metaphase-II plates, chromosome A_g and either B_g or B_t were at one plate, while C_t, D_t, and either B_g or B_t were at the other. From these data, preferential disjunction from the trivalent A_g-C_t-D_t was indicated, and further cytological work was curtailed so that the remaining flowers could be used to test this.

Subsequently, the five-chromosome hybrid was crossed reciprocally to races of *H. gracilis* from New Mexico and Arizona. Seed harvested from these crosses were dried at room temperature for several days and then germinated in Petri dishes. Seedling root tips were placed in 0.002*M* 8-hydroxyquinoline for 1 hour, hydrolyzed for 10 minutes in 15 percent HCl, and stored in Carnoy's fixative until used. Somatic chromosome counts were made with a phase-contrast microscope, propionocarmine being used as a weak stain.

On the basis of chance alone, one would expect disjunction from the trivalent A_g-C_t-D_t to be A_g/ C_tD_t, A_gC_t/ D_t, and A_g D_t/ C_t in equal frequencies. However, this did not occur. A test cross of the hybrid as the

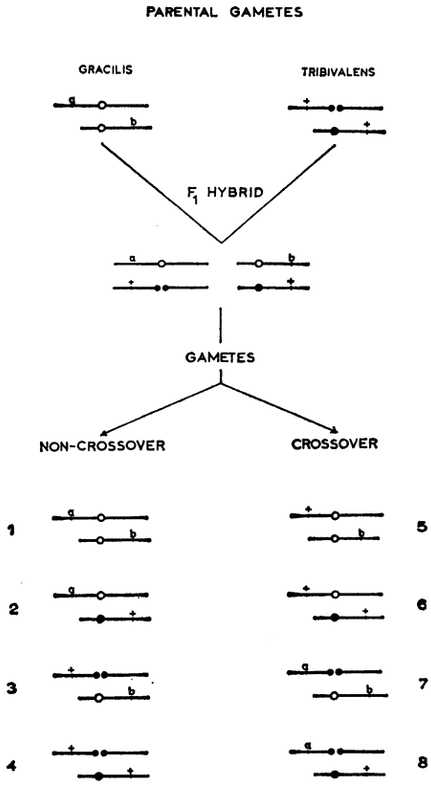

Fig. 2. A theoretical cross of *H. gracilis* × tribivalens. Mutant genes *a* and *b* are introduced on chromosomes A_g and B_g respectively in the gamete of *gracilis*. Centromeres of *gracilis* chromosomes are white and those of tribivalens are black. Crossovers between B_g and B_t are not considered.

pistillate parent with *H. gracilis* (Ariz.) as the staminate parent yielded 57 plants with $2n = 4$ and the *gracilis* karyotype and 48 plants with $2n = 5$ and the hybrid karyotype. A chi-square test for a 1 : 1 ratio of two- and three-chromosome eggs from the hybrid gives a probability $> .30$. A cross of *H. gracilis* (N.M.) as the pistillate parent and the hybrid as the staminate parent produced 106 plants with $2n = 4$ and the *gracilis* karyotype and 66 plants with $2n = 5$ and the hybrid karyotype. Both crosses thus demonstrated preferential segregation from the trivalent, with A_g going to one pole and C_t and D_t to the other.

Cytological studies of microsporocytes during meiosis in the hybrid showed no mechanism for the selection of two-chromosome gametes and pollen stainability was high (89 percent). Therefore, the deviation in the expected 1 : 1 ratio of $2n = 4$ and $2n = 5$ plants ($p < .01$) with the New Mexican *gracilis* race as the pistillate parent must be due to competition between two- and three-chromosome pollen grains. Whether the competition process acts at the time of pollen germination or during growth through the style is unknown.

Preferential segregation from a trivalent with and without chiasmata formation is known for the sex chromosomes of both animal and plant species (*4*). Preferential disjunction from a trivalent composed of two acrocentrics and a large metacentric genetically equivalent to, and derived, by previous centric fusion of the two acrocentrics was predicted more than 20 years ago for *Drosophila* (*5*). This was later demonstrated in hybrids between *D. americana* subspecies *americana* and subspecies *texana* which showed regular disjunction of two acrocentrics from a genetically equivalent metacentric without loss of fertility (*6*).

Because *H. gracilis* has only two pairs of chromosomes and because one of these undergoes preferential disjunction in the *gracilis* × tribivalens hybrid, a technique exists for determining which of the two linkage groups of *gracilis* is carrying a recessive marker gene. This technique is effective only if there is less than 50 percent recombination between the marker and the centromere of either C_t or D_t. Crossovers between B_g and B_t can be ignored if these chromosomes undergo random disjunction.

A theoretical cross of *H. gracilis* × tribivalens with recessive markers on the chromosomes of *gracilis* is shown in Fig. 2. Expected results of testcrossing the F_1 as the pistillate parent to *gracilis* homozygous for the markers may be summarized as follows. If the marker is introduced on A_g and no recombination occurs between the marker and the centromere, two equal classes of test cross progeny are expected: four-chromosome plants homozygous for the marker and five-chromosome wild types. Crossover classes would yield four-chromosome wild types and five-chromosome plants homozygous for the marker. If the marker is introduced on B_g, equal numbers of the following classes, including crossovers, should occur: four-chromosome plants homozygous for the marker; four-chromosome wild types; five-chromosome plants homozygous for the marker; five chromosome wild types.

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Chromosome Puffs in Drosophila Induced by Ribonuclease

Abstract. *Ribonuclease induces many puffs in salivary gland chromosomes of* Drosophila busckii. *The left arm of chromosome 2 was analyzed in detail; in this arm seven puffs were induced in a definite sequence.*

Chromosome puffs in dipteran salivary glands are structural modifications believed to be associated with gene action (*1*). Since RNA is synthesized in chromosome puffs (*2*), a series of experiments has been undertaken to attempt to elucidate puff structure and function in the presence of ribonuclease. This enzyme is known to enter cells, including salivary gland cells of *Drosophila*, and produce a number of alterations (*3, 4*). We have observed that ribonuclease can induce a large number of puffs.

Salivary glands of *Drosophila busckii* larvae reared at 25°C were excised and incubated in Ephrussi-Beadle solution (*5*) containing 3 or 5 mg of ribonuclease per milliliter (*6*). For the control, glands were incubated in Ephrussi-Beadle solution alone.

After about 1 hour of incubation, the nucleolus disappears almost completely—only a small remnant is found

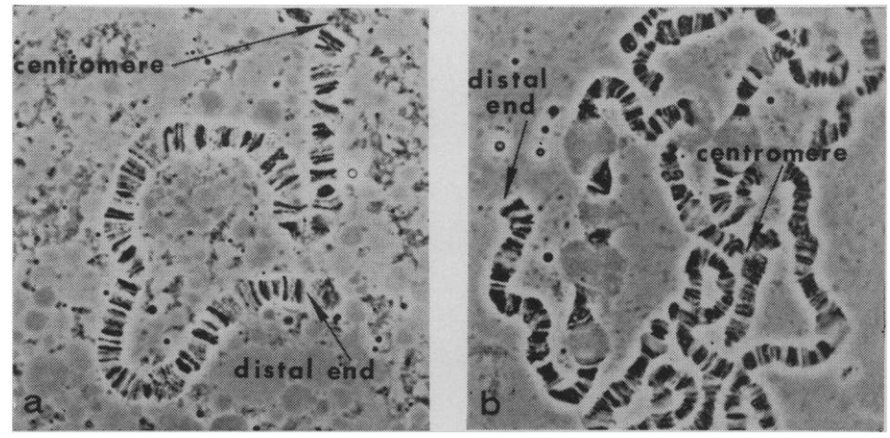

Fig. 1. Left arm of the second chromosome of *D. busckii*. *a*, A salivary gland smear in 45 percent acetic acid after 5 hours' incubation in Ephrussi and Beadle solution. *b*, A salivary gland smear after 5 hours' incubation in Ephrussi and Beadle solution containing 5 mg of ribonuclease per milliliter.