and cutting out the major portion of the amnion. In most cases there was no hemorrhage. Amniotic contractions and yolk-sac movements were absent after this operation.

The embryo was allowed to remain undisturbed in the observation box for at least 30 minutes both after initial opening of the shell and shell membranes and after removal of the amnion. During these intervals the opening in the shell was sealed with parafilm. All operations were performed under sterile conditions.

Four 15-minute recordings were made on each of the 30 embryos, two before removal of the amnion and two after. Contractions of the amnion were recorded simultaneously with embryonic motility during the first recording of activity of each embryo. Embryonic motility and amniotic contractions were manually recorded on a Sanborn 4-channel polygraph. All observations were made through a binocular dissecting microscope. Only active (as opposed to passive swinging) somatic movements of the embryo were recorded. At the conclusion of the recordings each embryo was staged according to the Hamburger and Hamilton stage series.

Table 1 is a summary of the results. The mean percentage of activity refers to the total amount of time spent in activity by the embryo during a 15-minute recording period. Activity and inactivity phases have been defined previously (*8*); that is, if two activity phases are separated by 10 seconds or more they are treated as individual activity phases. If movements are separated by intervals from 1 to 9 seconds, they are considered as one activity phase.

At each age, the two values recorded with the amnion intact and the two with the amnion removed have been combined. This provides one mean value for recordings taken before removal of the amnion and one value for recordings taken after removal. With the Wilcoxon matched-pairs signed-ranks test and the sign test, no significant differences were found between the values for embryos with the amnion intact and those with the amnion removed.

Mean percentage of activity of the amnion was high at all stages that were studied. In fact, 22 out of the 30 embryos showed 100 percent amnion activity during the observation periods.

If contractions of the amnion and movements of the yolk-sac do, as Kuo suggests, stimulate active movements of the embryo, then the measure of the mean percentage of embryo activity should show a difference between the embryos with amnion intact and those with amnion removed. However, this is not the case. The discrepancy between these results and those of Kuo could be due to any of a number of factors: (i) the previously mentioned differences in methodology; (ii) the failure of Kuo to apply a statistical test of significance to his data; or (iii) the different ages of embryos used in the two studies. Kuo used 7-, 8-, and 9-day embryos whereas I used 9-, 10-, and 11-day embryos. However, the failure, in this investigation, to find any significant differences at the one age common to both studies (9-day) would seem to rule out the last possibility.

The cyclic aspects of motility (activity–inactivity phases) are also unaffected by removal of the amnion. The results are in agreement with experiments referred to above which showed that exteroceptive stimulation does not alter the periodicity of 11-day embryos. These results reduce the probability that sensory input (amnion contractions, yolk-sac movements, and exteroceptive stimuli), at least for the stages studied, is an important variable in cyclic, embryonic motility. The alternative hypothesis that cyclic motility is generated in the motor system is given further support.

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Chromosomes from Testicular Preparations of Lepidoptera

Abstract. *Typical elongate, beaded chromosomes have been observed in squash preparations of testicular tissue of the butterfly* Speyeria aphrodite *(Fabricius), the first demonstration of relatively uncondensed chromosomes in the Lepidoptera.*

No karyotypes with elongate chromosomes have so far been reported in Lepidoptera. To obtain chromosomes of a more "conventional" nature, as demonstrated in many other groups of animals, we have used the following technique.

The testis is removed from a living butterfly and is macerated thoroughly. The macerated tissue is then placed in a hypotonic saline solution, and the expanded tissue is transferred into a "soft" fixative that is composed of a mixture of methanol and glacial acetic acid (3 : 1, by volume). The fixed material is squashed between two

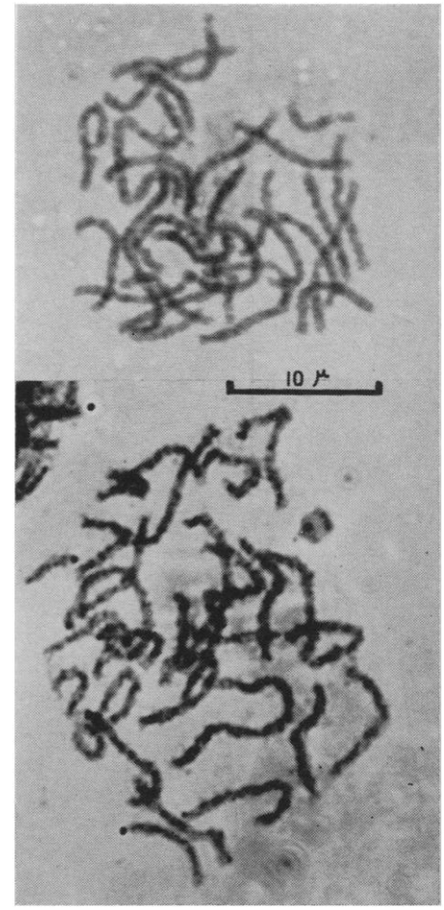

Fig. 1. Photomicrographs of two karyotypes from testicular squash preparations of *Speyeria aphrodite* (Fabricius) showing characteristic chromosomal morphology; the preparation was stained with Giemsa.

cover slips, and the cover slips are carefully separated. The tissue adhering to both cover slips is stained with Giemsa, and the two preparations are permanently mounted for study.

The testes of five butterflies [one male each of *Speyeria cybele* (Fabricius), *S. aphrodite* (Fabricius), *Pieris rapae* (Linnaeus), *Papilio glaucus* Linnaeus, and *Papilio polyxenes asterius* Stoll] were prepared in this manner. Only one of these specimens, *S. aphrodite*, yielded dividing figures showing detailed chromosomal morphology (*1*).

As shown in Fig. 1 (*2*), karyotypes of typical elongate, beaded chromosomes, comparable with those shown by other groups of animals, can be obtained from preparations of butterfly testicular material. Certain chromosomes appear to be traceable from one cell to another.

The chromosome number of the Rocky Mountain subspecies of *S. aphrodite* (*ethne* Hemming) has been recorded as $n = 29$ (*3*), based on sectional material. The present results indicate this number may be too high (probably $n = 27$ is correct). It is possible, though unlikely on morphologic grounds (*4*), that *S. aphrodite* and *S. a. ethne* may not be conspecific.

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Dieldrin: Extraction of Accumulations by Root Uptake

Abstract. *Certain forage crops can absorb and translocate the chlorinated hydrocarbon insecticide dieldrin from soil or sand. An extraction technique routinely used for analyses of residues does not quantitatively remove this internal chemical, but a method employing chloroform-methanol extraction leads to essentially quantitative recovery.*

Many halogenated pesticides are no longer recommended for direct application to forage crops because of their long persistance. It has recently been recognized that certain plants absorb these pesticides from the soil. Lichtenstein (*1*) has established that certain root crops absorb aldrin and heptachlor when grown in soils treated with these chemicals; others have demonstrated the presence of small quantities of dieldrin in alfalfa grown in soil treated with aldrin or dieldrin (*2*).

Most of the techniques currently used for extracting pesticides were developed to measure surface residues. The literature does not report the efficiency of extraction of residues solely within the plant. We now present data on the extraction of dieldrin absorbed through the plant-root system.

Our first experiment used Cl^{36}-labeled dieldrin (*3*) mixed with sand at 15 parts per million. Seeds of orchard grass, corn, and wheat and rooted alfalfa cuttings were planted in this mixture and the plants were grown for 2 weeks with the use of Hoagland's nutrient solution. Extraction and analyses by gas chromatography (*4*) and thin-layer chromatography confirmed the presence of radioactive dieldrin in the plant tissues.

A Research Specialties Co. model-600 gas chromatograph, equipped with an electron-capture detector, was used; flow rate of the nitrogen carrier gas was about 70 ml/min, with an inlet pressure of 1.56 atm. Two columns were used to aid identification of the compounds: one of 1.8 m, 3 percent QF-1 on Gas Chrom CLA; the other of 1.2 m, 5 percent DC-200 on Gas Chrom CLA. Temperatures of vaporizer, column, and detector were 250°, 225°, and 285°C, respectively.

It was found that extraction of radioactive plants by blending in a mixture of *n*-hexane–isopropyl alcohol (2:1) could not remove all label from tissues; this technique is used routinely in many laboratories for fresh plant materials. This failure stimulated the search for a better extraction method. After some trial and error, it was found that almost 100 percent of the radioactivity could be extracted from labeled plants by: (i) maceration and repeated blending of the plant material with *n*-hexane–isopropyl alcohol (2:1); and (ii) 12-hour reextraction of this tissue with a 1:1 mixture of chloroform and methanol in a Soxhlet extractor.

Several hundred plants grown under controlled environmental conditions in sand and soil containing unlabeled dieldrin have been extracted by this method and all extracts have been analyzed by gas chromatography. The identity of the compound, in both extracts, that was thus measured as dieldrin was confirmed by thin-layer chromatography and by mass spectrometry. In every instance the chloroform-methanol extract contained additional dieldrin.

Application of this double-extraction technique to field samples of fresh alfalfa, grown on soil containing dieldrin but not sprayed, revealed the presence of considerably more pesticide than had been detected by the usual extraction techniques. The upper portion of Table 1 shows the levels of dieldrin detected by the single- and double-extraction techniques. The hexane–isopropyl alcohol mixture removes on average only about 64 percent of the total extractable insecticide. Wheat, corn, and orchard grass grown under controlled environmental conditions have yielded similar results. Considerable variation in the efficiency of extraction was observed, depending on plant species and on concentrations of pesticides in the plants.

In the Pesticide Research Laboratory

Table 1. Dieldrin contents of three extracts from fresh alfalfa (four samples) and dry alfalfa hay (five samples); determined by use of a QF-1 column and confirmed with a DC-200 column. In parentheses are the percentages of total extractable dieldrin extracted in hexane and in 2:1 hexane–isopropyl alcohol (HI) extracts. Recoveries of dieldrin by extraction from material fortified with known quantities averaged 95 percent. CM, chloroform-methanol (1:1).

Dieldrin (ppm) in extracts		
HI	Hexane	CM
	Fresh alfalfa	
0.025 (69)		0.011
.010 (62)		.006
.049 (67)		.024
.007 (59)		.005
	Dry hay	
	0.020 (74)	.007
	.029 (66)	.015
	.084 (74)	.030
	.045 (70)	.019
	.078 (68)	.037