

Table 1.—Progeny resulting from various testcrosses (repulsion combinations) utilizing mutants (markers) on each of the previously marked linkage groups for *Tribolium castaneum*.

Linkage group	Group marker	Marker-ivory	Phenotype		
			Wild	Marker	Ivory
II	pegleg	157	415	418	701
III	black	188	246	229	229
IV	sooty	85	109	74	90
V	jet	70	84	79	91
VI	micro-phththalmic	32	136	40	120
VII	Short antennae	44	99	31	93
VIII	antennapedia	59	106	63	96

groups have been marked (see Sokoloff and Dawson 1963 for a fairly complete literature citation). This note describes an eye-color mutation (*ivory*, *i*) which closely resembles but is nonallelic to *pearl*.

The *ivory* mutant was isolated from an X-rayed population under selection for large body size (strain LCS in Bartlett and Bell 1966). The eyes of this mutant are creamy-white and have a spectacled appearance reminiscent of *pearl*. Reciprocal crosses of *ivory* to *wild-type* yielded all *wild-type* offspring. Crosses among F_1 individuals and backcrosses of F_1 hybrids to *ivory* gave offspring characteristic of monohybrid Mendelian inheritance. In these matings, *ivory* exhibited complete penetrance and acted as an autosomal recessive gene. Viability of *ivory* homozygotes approaches that of the normal *wild-type*.

Linkage relationships with genes on the previously marked chromosomes have been carried out. Only the backcross data for repulsion crosses will be reported here (See Table 1). Since *ivory* is not sex linked, no tests for linkage group I were made. Five of the 7 linkage tests gave results statistically different from the expected 1:1:1:1 ratio for this type of cross. In 4 of the 5 significant cases (III, VI, VII, VIII) the discrepancies were due to shortages in the *marker* and/or *marker-ivory* classes. However, recombination data for linkage group II revealed shortages not only in the *marker* class but the *wild* class was less frequently observed than was *ivory*. Note in Table 1 that the *ivory* and *wild* classes have approximately equal frequencies in each of the other tests. The apparent conflict was resolved by comparing the total of the parental types (*ivory* and *marker*) with the total of the recombinant types (*wild* and *marker-ivory*). Under the assumption of no linkage, these totals should be equal. Chi-square tests were made on these totals and only the test for linkage group II showed a significant deviation from the expected values. Therefore, it was concluded that *ivory* is linked to *pegleg* on linkage group II. The data for reciprocal crosses are not presented but chi-square tests showed no significant heterogeneity owing to the sex of the parents. There are several ways of calculating the recombination between *pegleg* and *ivory*. The commonest method simply determines the percent of nonparental types in the backcross progeny. In the present case this calculation gives 33.8% recombination. Since low penetrance of the *pegleg* homozygotes decreases the numbers found in both *pegleg* classes (*pegleg* is misclassified as *wild-type*) this figure of 33.8% is probably inflated. A minimum estimate of recombination can be calculated from only the *pegleg-ivory* class of recombinants and the *ivory* class of parents. The use of these data (157/858) gives a recombination value of 18.2%.

Data were collected in this study for the F_2 progeny of

the *pegleg* and *ivory* crosses. The F_2 yielded 307 *wild-type*, 264 *ivory*, 55 *pegleg*, and 59 *pegleg-ivory* individuals. These data are highly significantly different from an expected 9:3:3:1 ratio. There are many less *pegleg* than would be expected with independent assortment, but many more *wild* than expected if linkage exists. Again misclassification of *pegleg* individuals is expected. Mather (1957) illustrates the use of the product method for the estimation of linkage with disturbed ratios. A recombination frequency of 28% was calculated with the product formula on the F_2 data. This recombination value is between the 2 previous estimates and is probably the best estimate.

Lasley and Sokoloff (1961) reported that *pearl* and *pegleg* were linked and they found 30% recombination. In the present study, reciprocal crosses of *ivory* and *pearl* gave *wild-type* offspring. The close resemblance between the 2 mutant phenotypes handicaps recombination study, but preliminary F_2 data suggest that *ivory* and *pearl* are closely linked and at approximately equal distance from *pegleg*. Precise estimation of recombination between these 3 loci in linkage group II must await the isolation of more useful markers.

The occurrence of this mutant (*ivory*) further emphasizes the need for study of possible homologies between mutants in other species, as well as the biochemical relationships between similar mutants of the same species. Biochemical study of the 2 eye mutants, *pearl* and *ivory*, might yield significant information concerning the biosynthesis of eye pigments in both *Tribolium* and other insects.

REFERENCES CITED

- Bartlett, A. C., and A. E. Bell. 1966. Changes in quantitative traits of *Tribolium* under selection and irradiation. Genetics. In press.
- Lasley, E. L., and A. Sokoloff. 1961. Section on new mutants. *Tribolium* Inform. Bull. 4: 16.
- Mather, K. 1957. The Measurement of Linkage in Heredity. John Wiley & Sons, Inc., New York.
- Park, T. 1937. The inheritance of the mutation "pearl" in the flour beetle *Tribolium castaneum* Herbst. Amer. Nat. 71: 143-57.
- Sokoloff, A., and P. S. Dawson. 1963. Linkage studies in *Tribolium castaneum* Herbst. IX. The map position of antennapedia, squint, short elytra and elbowed antenna. Can. J. Genet. Cytol. 5: 450-8.

A Method of Shipping Live Larvae of *Simulium vittatum* Long Distances (Diptera: Simuliidae)¹

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During studies on the biology and ecology of black flies (Simuliidae) at the Seney National Wildlife Refuge, Seney, Michigan, and at the Patuxent Wildlife Research Center, Laurel, Maryland, larvae of 3 species of black fly (*Simulium vittatum* Zetterstedt, *S. aureum* Fries, and *S. decorum* Walker) were collected and transported for distances of up to 50 miles in nonagitated jars of water. In each case the water used to transport the larvae was removed from the waterway where the particular larvae were taken. However, on numerous occasions it was found that larvae could not be kept alive in nonagitated water jars for more than 6 hr.

To my knowledge, no one has reported a method for transporting live black fly larvae long distances, although

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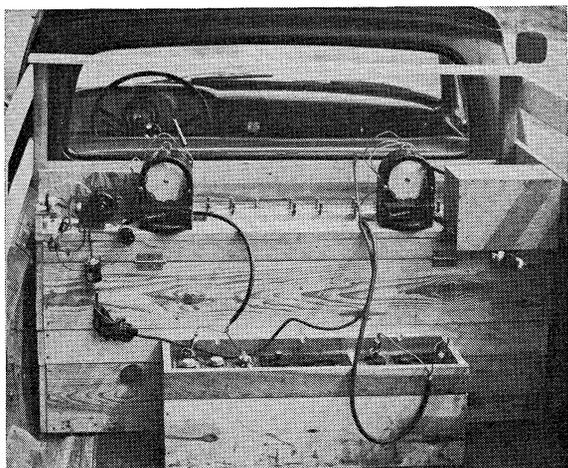


FIG. 1.—Front view of larval transport box on pick-up truck. Aquarium pump on the right side of the box is covered with a plastic-lined exterior plywood box to protect the motor from rain or snow. The tempscribes are used to record ambient and water temperatures.

Bequaert (personal communication, 1965) has had some success transporting them for short distances in wet moss.

My first attempt to transport live larvae long distances (Seney, Mich., to Laurel, Md.—1100 miles) was in 1964 (Tarshis 1965), when shipments of black fly larvae and eggs were transported in arctic hampers cooled with Scotch-ice refrigerants via a commercial passenger airline. The eggs came through in good condition, but none of the larvae were alive on arrival. Attempts to ship live larvae in packets of wet cotton via air mail, and first-class and fourth-class mail, from Seney to Laurel, also were unsuccessful. From these trials it was concluded that the only way successfully to transport live larvae the long distance from their breeding site to the laboratory would be to ship them by truck in a specially designed water tank containing equipment that would agitate the water in such a way that the larvae would not be harmed by the equipment. I have found that larval survival is not so much dependent upon water agitation for keeping the oxygen content of the water high as it is upon keeping the food particles in the water in constant motion. Water movement is essential to larval survival beyond 6 hr.

Agitating water in larval breeding jars was abandoned when it was found that a 50-lb. compressed air supply would last only 2 hr. More tanks of compressed air than practicable would have been needed for each trip from Michigan to Maryland. Gasoline-operated compressors for supplying air to tanks were not considered because of the safety hazard.

Since piston-driven aquarium pumps utilizing aquarium air stones had been used successfully in the laboratory for the agitation of water in black fly larval tanks, it was thought these same pumps might be used successfully in transporting the black fly larvae from Michigan. However, to use these pumps while transporting larvae, a 6-v d-c, $\frac{1}{32}$ hp motor had to be substituted for the regular 115-v a-c electric motor of the pump.

When traveling, the 2 pumps were connected to the car generator while the car was in motion. At night, each pump was powered by a 6-volt battery supplied for this purpose. The batteries could not be used continuously, for they have a life of about 12 hr, and the trip from Michigan to Maryland takes about 35 hr. An extra battery was kept on hand in the event of the failure of one of the others.

A wooden box, 22 in. wide, 53 in. long, and 20½ in. high, was used to house the air pumps and larval transport jars. The air lines from the air pumps were connected in series with air valves, 1 valve to each of 16

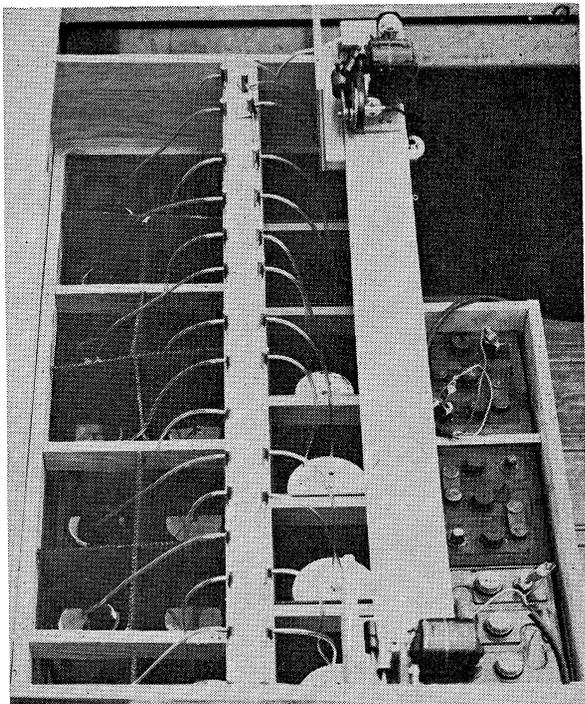


FIG. 2.—Top view of larval transport box, showing air pumps and plastic air lines from air valves.

2-quart jars and 2 valves to each of 6 1-gal larval jars. The air lines were placed through holes in the lids of these jars to prevent spillage of water from the jars while the truck was in motion. Two aquarium pumps were more than ample to supply air to the 22 jars used for transporting the larvae.

The utilization of this equipment has enabled me to transport without mishap several hundred thousand *S. vittatum* larvae in various stages of development, and the larvae developed normally in the Maryland laboratory into adult flies. As a result of this study, it now seems feasible to transport black fly larvae even longer distances than those I covered. Fig. 1 and 2 show the equipment used for the transportation of *S. vittatum* larvae from Seney, Mich., to Laurel, Md.

REFERENCE CITED

- Tarshis, I. B. 1965. Procurement and shipment of blackfly eggs. *Bull. Wildlife Dis. Ass.* 1: 8-9.

Chemoreception of Attractants from the Cotton Plant by Boll Weevils, *Anthonomus grandis* (Coleoptera: Curculionidae)^{1, 2}

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Most researchers believe that the organs of chemoreception in insects are situated chiefly in the antennae (Wigglesworth 1961). However, McIndoo (1926), though he did not conduct tests with attractants, con-

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² Mention of a proprietary product does not necessarily imply endorsement by the USDA.