

mice are heavier than "agouti" A^{vy} -mice because of a larger fat and water content of the carcass and liver. Differences between the mean fat and water content of "clear" and "agouti" carcasses and livers (data for both sexes pooled in each category) were significant ($P < .01$), while the non-fat dry weights of carcasses and livers of the two phenotypes were not statistically different ($P > .05$). There was no difference in the rate of bone growth, as indicated roughly by increase in tail length, between the "clear" and "agouti" phenotypes (Table 1).

Variability of expression of the A^{vy} phenotype and the correlation of coat color with body and liver composition suggest that A^y and A^{vy} alleles have similar effects on the synthesis of hair pigment as well as on fat metabolism.

However, the characteristics of the "agouti" A^{vy} phenotype indicate that these effects of the A^{vy} genotype are more easily modified than those of the A^y genotype.

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Interspecific Transfer of the "Sex-Ratio" Agent of *Drosophila willistoni* in *Drosophila bifasciata* and *Drosophila melanogaster*

Abstract. *The maternally transmitted "sex-ratio" condition in several species of Drosophila appears to be due to infection by a microorganism of the genus Treponema. Drosophila bifasciata is an exception, since no microorganism has been found in the "sex-ratio" strains of this species. Normal D. bifasciata can be infected by injection of the hemolymph of a "sex-ratio" strain of D. willistoni containing treponemas. The progenies of the infected D. bifasciata, up to and including the F₄ and F₅ generations, have numerous treponemas in their hemolymph. Their progenies are, however, not unisexual, although both females and males are infected. The hemolymph of these D. bifasciata injected in D. melanogaster females causes typical "sex-ratio" symptoms in the progenies of the latter.*

A cytoplasmically inherited "sex-ratio" condition has been found in several species of *Drosophila*, namely, *D. bifasciata* (1), *D. prosaltans* (2), *D. willistoni* and *D. paulistorum* (3), *D. nebulosa* (4), *D. equinoxialis* (5), and perhaps others. The females carrying this condition produce mainly or exclusively daughters in their progenies. About 50 percent of the eggs deposited

by "sex-ratio" females, presumably representing the male zygotes, die. Malogolowkin and Poulson (6) and Malogolowkin, Poulson, and Wright (7) showed that the agent responsible for the "sex-ratio" condition can be transmitted to females originally free of this condition, by injection of the oöplasm of the eggs of "sex-ratio" females, or by injection of their hemolymph (8). Poulson and Sakaguchi (9) then discovered that the infective agent is a microorganism belonging to an apparently new species of the genus *Treponema*. Treponemas can be seen in the hemolymph of the flies from the "sex-ratio" strains of all the species tested, excepting *D. bifasciata*. The agent responsible for the "sex-ratio" condition in the last named species remains to be found by microscopic observation. While the treponemas apparently responsible for the "sex-ratio" condition can be transferred by injection from species to species, the "sex-ratio" agent of *D. bifasciata* appears uninfecious

(10), either inter- or intraspecifically. It cannot be easily inactivated by x-rays (11) or γ -rays, as it can be in *D. willistoni* (12). Poulson and Sakaguchi (13) showed, however, that *D. bifasciata* can be infected with the "sex-ratio" agent of *D. willistoni*. The experiments described here are concerned with transfer to *D. bifasciata* of the infectious agent in the hemolymph of *D. willistoni* and its subsequent testing in *D. melanogaster*.

A "sex-ratio" strain of *D. willistoni* used in this study was obtained from B. Sakaguchi; it is descended from the original culture of Ch. Malogolowkin. The normal strain of *D. bifasciata* was collected by Moriaki, Okada, Ohba, and Kurokawa at Akkeshi, Japan, in 1952. Females of *D. bifasciata* were injected with the hemolymph of the "sex-ratio" *D. willistoni*; the females were transferred at 4-day intervals to fresh culture bottles. The proportions of the females and males were determined in the successive broods. Several pair matings were arranged from each brood, and in the F₂, F₃, F₄, and F₅ progenies the frequencies of the two sexes were determined. The hemolymphs of at least five females were examined under a phase-contrast microscope in most lines in F₂, F₃, and F₄ generations. All the experimental cultures were kept at 25°C.

The eight injected females of *D. bifasciata* produced a total of 372 females and 164 males in the F₁. The percentage of males is, consequently, 30.6 percent, which is significantly different from 48 percent of males in the control cultures. The total count in the F₂ generation was 549 females and 277 males, or 33.5 percent males; the counts in F₃, F₄, and F₅ were 2427 females and 826 males, 4356 females and 1631 males, and 4064 females and 2433 males, which means that the frequencies of the males were 25.4, 27.2, and 37.5 percent, respectively. This is consistently and significantly below the control frequency of males. Detailed data (14) show that some males appeared in almost all broods, at least in those in which an appreciable number of flies were produced. The numbers of the

Table 1. The hemolymph of the progenies of *D. bifasciata* injected with "sex-ratio" of *D. willistoni*.

Sex	Flies examined (No.)	Treponemas		
		+	—	Few
<i>F₂ generation</i>				
♀	67	67	0	0
♂	10	10	0	0
<i>F₃ generation</i>				
♀	81	74	2	5
<i>F₄ generation</i>				
♀	187	150	16	21
♂	16	12	3	1

Table 2. Tests in *D. melanogaster*.

Species of <i>Drosophila</i>		Progenies	
Donor	Recipient	Unisexual	Normal
<i>bifasciata</i>	<i>melanogaster</i>	102	14
<i>willistoni</i>	<i>melanogaster</i>	11	0

flies whose hemolymph was examined with phase-contrast microscope for *Treponema* are shown in Table 1. Almost the entire progeny of the *D. bifasciata* females injected with *Treponema*-containing hemolymph of *D. willistoni* were infected, and the infected females have transmitted the parasites to their progenies, at least up to and including the F₄ generation. Also, *D. bifasciata* males withstand the infection better than do *D. willistoni* males; only a minority of the males of the former species die, and the remainder are both viable and fertile.

The question now is whether *Treponema* which has lived for one fly generation or more in *D. bifasciata* preserves its original properties, or whether it becomes in some sense a less virulent strain. This was tested by injecting the hemolymphs of the infected *D. bifasciata* and *D. willistoni* into Oregon-R females of *D. melanogaster*. The progenies of the latter females, produced after 3 to 5 days of incubation, were examined for unisexual, or almost unisexual, progenies. The results are shown in Table 2. The *Treponema*, although

relatively innocuous to *D. bifasciata*, is still able to infect and to produce the usual effect, unisexual progenies, in *D. melanogaster*.

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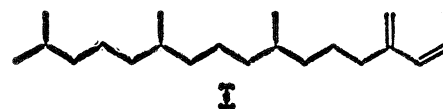
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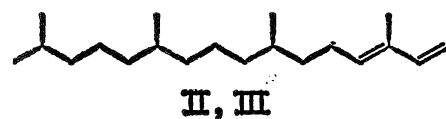
spectrometry, a molecular weight of 278; catalytic hydrogenation of the individual hydrocarbons yields phytane (2,6,10,14-tetramethylhexadecane; molecular weight 282). Phytane was synthesized as a reference compound by hydrogenation of phytadienes that were obtained by catalytic dehydration of phytol (3). The identity of the two products was shown by their retention indices (Table 1) in gas chromatography and by their infrared and mass spectra.

The olefins thus characterized as four isomeric phytadienes were separated by small-scale preparative gas chromatography and were further examined by infrared and ultraviolet spectroscopy and by ozonolysis. Ozonides were prepared from very small (microgram) quantities of the olefins in a thin film free of solvent, at the temperature of dry ice. They were studied by oxidation to the acids, by catalytic or triphenylphosphine reduction to the aldehydes, or—with the best yields—by pyrolysis in the gas chromatographic inlet system at 200°C. The products were identified by their retention indices, and the type of substitution was determined from the infrared spectra of pure samples trapped from the effluent of the column.

The infrared spectrum of compound I shows terminal vinyl and terminal methylene unsaturation, and the ultraviolet absorption indicates a monosubstituted conjugated diene. Ozonization followed by oxidation and esterification produced a methyl ester of a C₁₇ acid. In infrared spectrum and retention index compound I is identical with synthetic neophytadiene (I) (3).



The presence of a trisubstituted ethylene structure conjugated with a terminal vinyl group is indicated by the infrared spectra of compounds II and III.



The ultraviolet spectra are those of conjugated dienes and both compounds yield upon ozonolysis the identical C₁₆ aldehyde. Thus II and III must be the two possible geometric isomers of

Phytadienes in Zooplankton

Abstract. Four isomeric phytadienes have been isolated from mixed zooplankton of the Gulf of Maine. The chemical structures suggest that the mixture is derived by dehydration of phytol (presumably by acid catalysis), which is present in the diet of the zooplankton.

Chromatographic analyses of bulk zooplankton extracts from the Gulf of Maine yielded a saturated hydrocarbon fraction consisting predominantly of pristane (2,6,10,14-tetramethylpentadecane) (1, 2) and an unsaturated fraction containing a complex mixture of olefins. We wish to report the struc-

tures of four closely related olefins with gas chromatographic retention indices between 1900 and 2000 (Table 1), measured on a polar column (3.5 percent Carbowax C20M on Chromosorb G, acid washed, treated with dichloromethylsilane).

All four compounds have, by mass

Table 1. Gas chromatography and spectra of phytadienes and derivatives. Gas chromatogram temperature 4 deg/min, 1.8 m by 0.3 cm (outside diameter) steel column; 3.5 percent RTV 502 (filter free) on Chromosorb G, acid washed, DCMS treated; 3.5 percent Carbowax C20M on same; w, weak.

Compound	Retention index		Spectra (maxima)	
	RTV502	C20M	Ultraviolet (mμ)	Infrared (cm ⁻¹)
I	1841	1919	224	3095,1630(w),1590,991,906,896
II	1863	1951	234	3095,3020,1630,1590,987,904,835(w)
III	1878	1979	228	3095,3020,1640,1600,989,893,850(w)
IV	1901	2004	232	3030,1640,1620,964,830
Phytane	1812	1786		
C ₁₆ aldehyde	1561	1810		
C ₁₆ aldehyde	1668	1931		
Me ester of C ₁₇ acid	1858	2118		