Sakaguchi, B. and S. Tsuchiyama. Kyushu University, Fukuoka, Japan. Self-induced interaction of D. equinoxialis sex-ratio spirochete.

Interactions among three "Sex-Ratio" (SR) spirochetes from D. equinoxialis, D. nebulosa and D. willistoni in the host of D. pseudoobscura have been demonstrated by Sakaguchi et al. (1968). The interaction phenomena occur between two different SR spirochetes among any of the three spirochete species when mixed infections in the same host were made. During the course of these experiments, we found interaction phenomena between the same SR spirochetes from D. equinoxialis.

To test the effects of superinfections of the D. equinoxialis SR spirochete, hemolymph of the SR strain of D. equinoxialis (ES) was introduced into young adult females. The females were members of the seventh generation of the artificially established SR strain of D. pseudoobscura (PES).

Results of the experiments, Table 1, showed that superinfections of ES hemolymph result in the production of some male progeny in the second brood and in successive broods. Males appeared in the offspring from 16 mothers out of 17 tested. On the other hand, the controls, which were not superinjected, produced very few male progeny. Furthermore, ES or PES females were homogenized in Drosophila saline and the homogenate then centrifuged at 5,000 g for 20 min. The supernatant was treated by heating for 10 min at 60°C. After the treatment it was quickly chilled and centrifuged again at 10,000 g for 20 min. The supernatant, which was almost free of spirochetes, was injected into the PES. The proportion of female offspring in successive broods in the experiment was gradually reduced in subsequent broods from 99% in the first brood and finally reached about 50% in the eighth brood (21 to 24 days after injection). In this experiment, ten females were injected and male offspring were produced from every mother.

As a control, 0.7% NaCl solution was injected into eight PES females. A few males appeared in every brood. The proportions of male offspring was higher in the early broods than in the later broods and all injected females produced some male progeny.

The progenies, from second to fifth generations, from females injected with ES hemolymph, the heat-treated supernatant, and NaCl solution were tested. The SR condition, production of only female offspring, was cured in the series with the heat-treated supernatant and showed normal sex-ratio. The other series did not show such marked effects.

The hemolymph of injected females and their progeny was examined by phase-contrast microscopy. Many morphologically abnormal, as well as a few clumps of the spirochetes, were observed in the females on the second day after injection with the heat-treated supernatant. However, these phenomena gradually disappeared and the spirochetes increased after successive days. The number of these spirochetes was reduced markedly after about 12 days and many abnormal spirochetes were observed. The spirochetes were observed in the hemolymph of male and female adults in the early broods (1 to 9 days) of the second generation. The number of spirochetes in their hemolymph showed difference between individuals, but the spirochetes almost disappeared in the later broods (15 to 21 days). Little effect of injection of the ES

Table 1. Progenies of females of SR pseudoobscura with equinoxialis SR spirochete injected with hemolymph and other materials.

Injected materials	BROODS (DAYS) 0-3	-6	-9	-12	-15	-18	-21	-24	-27	-30	-33	-36
-	0	99	81	63	72	76	77	98	120	93	53	79
% of ♀		97.9	100	100	98.6	98.7	100	100	100	100	100	100
ES hemolymph*	310	555	682	635	472	528	477	505	429	247	120	64
% of ♀	100	96.5	96.1	83.0	78.1	81.4	80.2	78.3	74.8	85.3	82.5	87.5
ES supernatant**	230	214	338	440	414	366	229	245	161	128		
% of ♀	99.1	75.2	56.2	58.9	57.2	57.1	55.0	48.2	50.9	46.1		
0.7% NaCl	227	113	108	65	123	74	14					
% of ♀	84.3	87.7	97.2	96.9	98.4	98.7	92.3					

*, ** see text

(Continued at bottom of next page)

Puro, J., T. Nygrén and M. Nuutila. University of Turku, Finland. Cytogenetic localization of Pc in Drosophila melanogaster.

Attempts at exact genetic localization of the "extra-sexcomb" genes, Pc and Scx, in the third chromosome of D. melanogaster have yielded unsatisfactory results (Hannah-Alava 1969, DIS 44: 75-76). It was only determined by Hannah-Alava that the order was Pc-Scx (not Scx-Pc as listed in Lindsley and Grell, Genetic Variations of D.m.) but until quite recently there has been a complete lack of cytological information on the position of these genes. Pc and Scx were earlier considered as pseudoalleles (Hannah and Strömnaes 1955, DIS 29:121-123) but if so, the distance between the subloci (about 0.3 of a unit, Hannah-Alava 1969) is greater than found for other complex loci. Because of the antennapedia-like phenotype of Pc, as well as semilethality of $Pc^2/Antp^{49}$ and lethality of Scx/Antp compounds, Hannah-Alava (1969) further suggested that both Pc and Scx could be functionally related, if not allelic, to Antennapedia mutants and, thus, possibly in the right arm between 83E and 84D. From the lethal interaction of $Antp^B$ and four X-ray induced revertants of Ns (Nasobemia) sharing a recessive lethal effect and having cytologically detectable change at 84B1-2 Denell (1972, DIS 48:45) concluded that Antp and Ns are allelic. Subsequently he has shown that these Ns revertants also fail to complement the recessive lethality of Scx. This he considers as evidence for Scx, too, being allelic, or possibly pseudoallelic, to the Antp mutants (Denell, Genetics in press). This implies that Antp as well as Scx and Ns are located at 84B1-2 in the salivary chromosome map. On the other hand, since Pc shows no lethal interaction with Scx and the revertants of Ns, Denell concluded that Pc is neither an allele of Antp nor of Scx.

This last conclusion is supported by evidence from our recent experiments (to be published in detail elsewhere) which demonstrate that Pc is in the left arm of the third chromosome. The results of three independent analyses are briefly reviewed here.

(1) It was shown by analyzing 79 recombinant chromosomes derived from crossing-over in the in-p interval from h th st cp in ri Pc^2 sr^{61j2}/eg rn^3 pP bx sr e^s ca compounds that Pc^2 is to the right of ri but to the left of both eg and rn^3. This together with the result of Holm et al. (1969, DIS 44:112) that eg invariably is associated with C(3L) chromosomes induced in homozygous eg/eg females suggests that Pc^2 is in 3L.

(2) In an attempt to induce compound-3 autosomes in th st cp Pc^2/+ females, eight new C(3L) chromosomes with, presumably, heterozygous Pc^2 and one with homozygous Pc^+ were recovered. At the same time, none of eight newly induced C(3R) chromosomes had the Pc^2 gene.

(3) The recessive lethality associated with Pc^2 was found to be suppressed in flies homozygous for Pc^2 but covered by Dp(3;2)FM27, a segregational derivative of an insertional translocation, T(2;3)FM27, comprising a piece of 3L - from 75A-B through 80B(C?) - inserted in 2L at 21F-22A (Puro, unpublished). Such flies exhibited an exaggerated Pc phenotype with extreme pleiotropic effects of wing position and texture in all flies and an increased penetration of extra sex combs in males.

(Continued from preceding page)

hemolymph and NaCl solution on the spirochetes was observed.

Oishi (1970, 1971) has shown that SR spirochetes have a virus in each spirochete species of D. nebulosa and D. willistoni, and possibly D. equinoxialis (Poulson, 1973). The viruses act as a virulent to the different SR spirochetes. We examined homologous superinjection in each case of SR pseudoobscura with nebulosa and willistoni spirochete. Little effect of the superinjection was shown in the SR pseudoobscura with willistoni spirochetes, but not nebulosa spirochete.

It is interesting that the results may correspond to behavior of temperate phages. In order to clarify the mechanisms of the self-induced interaction, additional experiments are now underway.

References: Oishi, K. and D.F. Poulson 1970, Proc. Nat. Acad. Sci. USA 67:1565-1572; Oishi, K. 1971, Genet. Res. Camb. 18:45-56; Poulson, D.F. and K. Oishi 1973, Genetics 74: s216; Sakaguchi, B., H. Chikushi, D.F. Poulson and K. Oishi 1968, Proc. Int. Cong. Genet. 12th 2:88.