organs, whether from third instar larvae, prepupae, or pupae, immediately flattened out when placed on such rafts. After 24 hours in culture most organs were so distorted that it was difficult to visualize their original outlines. None of the organs survived as long as their controls in hanging drops, nor was there, with few exceptions, any significant differentiation. Millipore filter rafts had one further disadvantage in that it was extremely difficult to see the explants against the solid white background. Placement of Drosophila organs at an air interface within the culture chamber should therefore be avoided since such an arrangement promotes neither long survival nor extensive differentiation and as such is decidedly inferior to complete submersion of explants in a liquid medium.

 $\frac{\text{Ayala, Francisco J.}}{\text{Development of incipient sexual isolation}}.$  Development of incipient sexual isolation between laboratory populations.

Two strains of  $\underline{D}$ .  $\underline{birchii}$  from Cairns, Australia, and from Popondetta, New Guinea (Ayala, 1965a), were crossed in both directions. Two populations were started with 150 pairs of  $F_1$  hybrids each, one being maintained at  $25^{\circ}$  C and the second at

 $19^{\circ}$  C. The technique of maintaining the populations has been described elsewhere (Ayala, 1965b). After 48 weeks of "natural" selection (about 16 generations at  $25^{\circ}$  and 10 generations at  $19^{\circ}$  C) samples were taken from both populations, and crossed to each of the parental strains in the two possible directions. The test was made by placing  $10^{\circ}$  Q and  $10^{\circ}$  d together in a 1/2 pint bottle for 10 days, and then the females were dissected and examined for presence of sperm. The results are given in table 1. (The control crosses give 90% or more insemination). The two hybrid

Table 1: Sexual preferences between two hybrid experimental populations and their parental strains.

Cross	çç tested	çç inseminated	Per cent inseminated
Hybrid population 25°x Cairns	64	14	22
Hybrid population 25°x Popondetta	57	26	46
Hybrid population 19° x Cairns	55	40	73
Hybrid population 19°x Popondetta	60	31	52

populations show a similar moderate degree of sexual isolation with the Popondetta parental strain. However their behavior with the Cairns parental strain is strikingly different, the  $25^{\circ}$  population showing fairly high isolation and the  $19^{\circ}$  very little. The difference in isolation of the  $25^{\circ}$  and  $19^{\circ}$  populations with Cairns is highly significant ( $\chi^2=30$ , P<.001), and the difference between the behavior with Cairns and with Popondetta is significant at P<.01 for the  $25^{\circ}$  hybrid population and at P<.05 for the  $19^{\circ}$  population. The two populations have, then, evolved different degrees of sexual isolation with respect to the parental strains. Since the populations were kept completely separated, sexual isolation has evidently arisen as a by-product of genetic divergence.

Literature: Ayala, F. J. 1965a. Sibling species of the <u>Drosophila serrata group</u>. Evolution, in press. Ayala, F. J. 1965b. Relative fitness of populations of <u>Drosophila serrata</u> and Drosophila birchii. Genetics, in press.

Ayala, Francisco J. Columbia University. Improvement of fitness in experimental populations.

Two experimental populations, one maintained at  $25^{\circ}$  C and the second at  $19^{\circ}$  C, were started with 150 pairs of founders taken from mass culture stocks of the Popondetta strain of <u>D</u>. <u>serrata</u>. The technique of maintaining the populations has

been described elsewhere (Ayala 1965), the relevant factor being that strong competition exists

both between the adults and during the immature stages of development. The populations reached equilibrium between the 8th and the 15th week, and thereafter oscillated around the same level. After 52 weeks samples were taken from both populations and from the original mass culture stocks, and a new generation was produced at  $25^{\circ}$ C under uncrowded conditions. From this progeny different amounts of flies were placed in 1/2-pint culture bottles, and transferred regularly to fresh bottles (3 times a week at  $25^{\circ}$ C, twice at  $19^{\circ}$ C). A total of 10 transfers were made at  $25^{\circ}$  for each level of parental density, and 6 transfers at  $19^{\circ}$ C. The mean number of flies produced per bottle are presented in Table 1. In all the six cases studied the flies

Table 1: Mean number of flies produced per food unit by two experimental populations and their parental stocks.

	Number of flies produced per bottle							
Number of Parents	25° C			19° C				
	Experimental Population	Control	Diffe- rence	Experimental population	Control	Diffe- rence		
100 300	396 423	363 381	33 42	233	208	25		
1000 1500	513 543	495 497	18 46	477	379	98		

from the experimental populations produced more progeny than the controls. It seems likely that, under the action of strong natural selection, new genotypes have been selected in the experimental populations which improved their fitness, as measured by the number of progeny produced per food unit.

Reference: Ayala, F. J. 1965. Relative fitness of populations of <u>Drosophila serrata</u> and Drosophila birchii. Genetics, in press.

Thompson, Peter E. Iowa State University. The killing and resorption of eggs after injection of <u>Drosophila</u> females with actinomycin-D.

In the course of tests of the effect of actinomycin-D on crossing-over, females were injected with about 0.25 microliter of dissolved material at concentrations of 10 and 50 microgram/milliliter. Injection was via the thorax. Neither concentration had a marked effect on survival

under sterile conditions. While the 10-gamma solution did not reduce fertility, the 50-gamma solution invariably led to a permanent or temporary cessation of egg laying.

In the 50-gamma series, females were inseminated during their first imaginal day and injected at 1 1/2 days. Each female laid 2-5 eggs soon after treatment, after which oviposition ceased for at least 6-7 days. Following this lapse, roughly one-third (37/124) of the females showed a recovery of fertility. The pattern of crossover effect after recovery was comparable in its time scale to effects of the 10-gamma treatment, heat treatments, etc., as if no marked retardation of survivng oocytes were involved. This included the observation of multiple rare exchanges, presumably gonial in origin, in individual females from the tenth day on.

Females examined early in the sterile period show degeneration of all advanced egg stages, and the ovary is distended with decomposition products. At day 6 (about 4 1/2 days after injection) most of this debris has been resorbed and oocytes up to about stage 3 or 4 can be seen. Again, the rate of development of these surviving oocytes must be nearly normal, for their maturation is complete in some females within another two days, at the return of fertility. A more thorough treatment of this aspect is projected, hopefully to establish a time scale of oogenesis similar to that found by Welshons and Russell (PNAS 1957) in their irradiation of Drosophila males. (Research supported by U.S.P.H.S. Grant GM 08912).