tests, to be described shortly, were performed in November 1977. Stock R59G4 underwent at least 2 'founder events' and will be considered a derived population whereas stock U28T2, in the lab only 2–3 generations, represents the ancestral population.

Mating preference tests were done in trios following the method described by Kaneshiro[5]. A virgin female from each stock was placed in a 32×98 mm vial containing 4 cm^3 of Wheeler-Clayton medium. One male from one of the stocks was introduced and the trio was observed until one of the females accepted copulation. Observations were made daily between 9.00 and 12.00 h, a period of more intense courtship activity[6]. During the non-observation periods, the vials containing trios were covered with black cloth since neither courtship nor copulation occurs in the dark[8]. Because flies of the 2 stocks did not differ in morphology, it was necessary to mark one of the females in each trio with a small spot of fast-drying enamel paint on the scutellum following the method of Ohta[6]. Only females of U28T2 were marked; Kaneshiro (personal communication) has demonstrated that marking has no effect either on the performance of the fly or on the outcome of the mating preference test.

Ethological isolation was quantified using the Stalker Isolation Index (I)[9] which is calculated as the difference between the frequencies of homogamic and heterogamic matings. I can range from +1 when only homogamic matings occur indicating complete ethological isolation to −1 when only heterogamic matings occur. An I equal to zero indicates random mating or no ethological isolation. Significance of the I values was tested by the proportions method using the formula $c = 2\sqrt{n}\,(p - 0.5)$ where n is the sample size and p is the frequency of homogamic matings.

Results. Results of the mating preference tests on the 2 *D. silvestris* stocks are presented in the table. Both I values are significant. However, isolation is asymmetrical. Females of U28T2 are strongly isolated from males of R59G4 (I = 0.78) whereas females of R29G4 prefer males of U28T2 (I = −0.38). These results can be examined in terms of the Kaneshiro hypothesis. According to this hypothesis, the ancestral-type females of U28T2 would be expected to reject the derived-type males of R59G4. The data show that in only 3 of 27 trials did a U28T2 female accept copulation with an R59G4 male. Furthermore, it would be expected that the derived-type female of R59G4, while accepting copulation with males of their own type, would accept copulation with the ancestral-type males of U28T2 as well. In the experiments R59G4 females accepted U28T2 males in more trials (22/32) than did U28T2 females (10/32).

Discussion. The results of these mating preference tests with the 2 stocks of *D. silvestris* are consistent with the Kaneshiro hypothesis. It appears that the stock which underwent several severe population reductions became partially ethologically isolated from the stock which is representative of the larger native population of *D. silvestris* at Kilauea Forest Reserve.

A similar situation in *D. adiastola* has been investigated by Arita and Kaneshiro[10]. Flies of 1 stock which had been in the laboratory 7 years and had undergone 4 or 5 severe population reductions were shown to be asymmetrically isolated from flies of a 2nd stock which had been in the laboratory less than a year. Females of the ancestral-type stock were strongly isolated from males of the derived-type stock. Females of the derived-type stock accepted copulation with males of both types randomly. These results, too, are consistent with the Kaneshiro hypothesis.

In addition to the original report of Kaneshiro[5] on the plantibia subgroup of species, there are now documentations of asymmetrical isolation between natural wild populations of *D. grimshawi*[6] and between natural wild populations of *D. silvestris* (Kaneshiro, personal communication). Asymmetrical ethological isolation may be one of the 1st steps in genetic divergence leading to speciation. However, the isolation observed is not a direct product of selection. Males and females of a population are attuned to one another in very precise but different ways in order for the stimulus-response chain of courtship to succeed. Episodes of random drift within the derived population during the founder event probably necessitate a reorganization of the genetics of sexual behavior. Ethological isolation is an incidental outcome of this selective reorganization. It seems if we are to understand the speciation process, at least in Hawaiian Drosophila, that attention must be focussed on identifying the courtship behavior pattern components and the genetically based variation in these components upon which drift and selection may operate.

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A study of irrigation fluids for neurosurgery on brain primary cell cultures[1]

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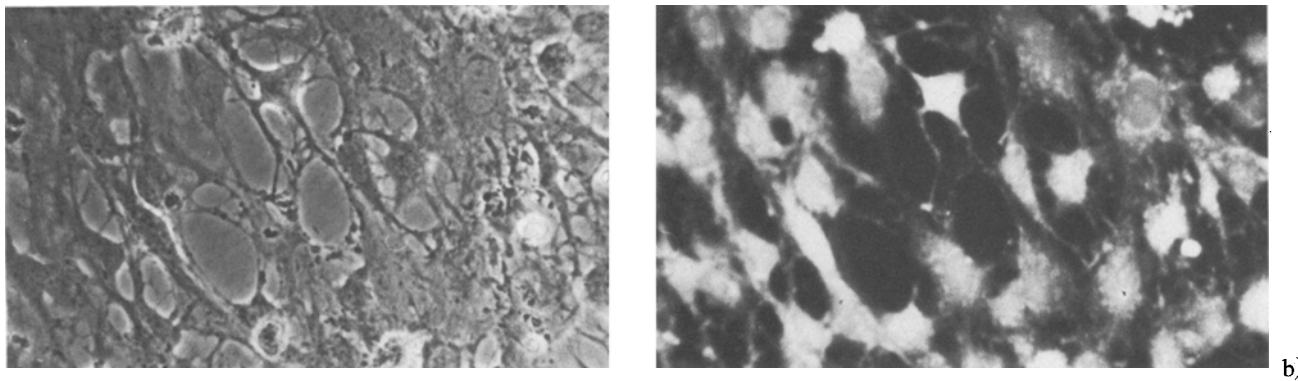
Summary. Primary cell cultures from newborn rat brain hemispheres were exposed to different irrigation fluids used in neurosurgery. The cells died after incubation for 5 min with hydrogen peroxide, and the number of cells was drastically decreased after 10 sec of incubation. They shrank after incubation in Elliott's artificial cerebrospinal fluid for 3 h, but the viability as determined by trypan blue exclusion test was not affected. Physiological sodium chloride, Ringer's solution and the culture medium 199 with Hank's salt had no noticeable effect on the viability or morphology of the cells.

In modern neurosurgery there is an increasing awareness of the importance of non-traumatic operation techniques[2], especially in consideration of spasm problems, production of post-operative duro-leptomeningeal adherences, blood-brain-barrier damage, and neuronal injury. Little attention has been paid to the role of irrigation fluids in this



a) b)

Brain primary cell culture exposed to Ringer's solution for 3 h as described in the text. The cells did not take up trypan blue (a) but gave fluorescence after incubation in fluorescein diacetate (b); these are signs of viability. The morphology resembles that of untreated cultures.

respect[3–5]. The aim of the present investigation has been to study the effect on rat brain primary cell cultures of exposure to some irrigation fluids used in neurosurgery.

Material and methods. The brain hemispheres from newborn rats were prepared as previously described[6]. They were passed through a nylon sieve of 80 µm pore size into Eagle's minimum essential medium, supplied with double concentrations of amino acids (except glutamine), quadruple concentrations of vitamins, 250,000 IU/l penicillin, 2 mM glutamine, 7 mM glucose, 20% (v/v) fetal calf-serum, set at pH 7.3. The cultures were incubated at 37 °C in a humidified atmosphere of 95% oxygen and 5% carbon dioxide. Media were changed every second day. The cultures reached confluency after 2 weeks of cultivation[5,6].

Experimental procedure. 14-day-old cultures were incubated in different irrigation fluids for 3 h: a) 0.9% NaCl, pH 6.3. 280 mosm/l; b) Ringer's solution (147 mM NaCl, 3.4 mM $CaCl_2$, 4.0 mM KCl, pH 6.3). 290 mosm/l (ACO Sweden); c) Elliott's artificial cerebrospinal fluid B, pH 7.3 about 320 mosm/l; d) culture medium 199 with Hank's salt without glutamine, pH 7.3. 280 mosm/l (Flow Laboratories, Scotland); e) Ringer's solution for 2 h 45 min, thereafter in hydrogen peroxide (3%, pH 3.6) 880 mosm/l for time periods from 10 sec to 5 min, and finally with Ringer's solution for a total of 3 h. The cultures were observed in a phase contrast microscope immediately after 3 h. The viability was checked with 0.2% trypan blue and 10^{-6} M fluorescein diacetate after reincubation of the cells in their culture medium. Trypan blue uptake of the cells was recorded as a sign of damage. To the cells was added a solution of fluorescein diacetate, made up in a concentration of about 10^{-6} M in sucrose of suitable tonicity (usually 0.32 M, pH 7.2). Fluorescein diacetate does not fluoresce when free in solution, but entering a living cell it will be metabolized, giving the cell a yellow-green fluorescence if irradiated by UV[8].

Number of cells (in percent of total) taking up trypan-blue or fluorescein diacetate as a sign of viability exposed to the solutions described in the text

	Cells taking up 0.2% trypan blue (%)	Cells taking up 10^{-6} M fluorescein diacetate (%)
0.9% NaCl	0	100
Ringer's solution	0	100
Elliott's solution	0	100
TC 199	0	100
Ringer, H_2O_2, Ringer	100	0

Results and discussion. All cultures at confluency contained predominantly astroglia. However, other cells, such as macrophages, endothelial cells, mesenchymal cells, have also been identified[6]. The ease with which such parameters as viability and cell survival can be studied makes these cultures a good model system for evaluating the cellular effects of irrigation fluids used in neurosurgery. The cells exposed to physiological sodium chloride, Ringer's solution, Elliott's artificial cerebrospinal fluid and TC 199, gave a strong fluorescence with fluorescein diacetate and remained unstained with trypan blue (figure, a and b, table). Cells incubated in Elliott's fluid had a tendency to shrink, probably dependent on the hypertonicity of the fluid.

The cells exposed to hydrogen peroxide took up trypan blue after exposure for only 10 sec, and gave no fluorescence with fluorescein diacetate. The nuclei increased in size about 20%. The number of cells was drastically decreased. After 5 min exposure to hydrogen peroxide the cells died, which was verified by a survival test. Few references exist on the use of irrigation fluids in neurosurgery. Particularly the cell-toxic effects of hydrogen peroxide seem to have been overlooked. The clinical implication of this effect is yet to be evaluated.

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