Results. Figure 1 shows the positions of successful courtships. A χ^2 test on these data (excluding the interspecific matings) shows that the differences are highly significant ($0.01 > p > 0.005$).

Details of the durations of copulations in each species are shown in figure 2. A Mann-Whitney U-test on these data (excluding the interspecific matings) indicates that these interspecific differences are highly significant ($p \ll 0.01$).

Discussion. The biological importance of the differences in mating behavior reported here is not clear. The copulation times of 3 of the 4 interspecific matings are those of the species to which the male belongs. If the male terminated copulation then the mismatings observed in this study may be less successful than intraspecific matings, because the female may have insufficient time to complete a mating sequence. For example, a difference in times of copulation could affect the efficiency of sperm transfer in interspecific matings.

Differences in preferred sites for courtship and mating may play a more direct role in interspecific ethological isolation. The results indicate that *D. heteroneura* and *D. silvestris* prefer different areas for courtship; thus there may be microgeographic isolation between them. All the mismatings occurred in the preferred area of the male involved, suggesting that lack of site discrimination by the female may contribute to interspecific hybridization. Such hybridization has been reported from Kahuku Ranch and morphological studies of hybrid individuals indicate that mismating is occurring in only 1 direction, viz. *silvestris* ♀ × *heteroneura* ♂[7].

Few complete mating sequences have been observed in the field despite intensive observations[8]. However, it is clear that copulation normally does not take place on the lek site where males advertise. On the basis of the observations made in this study it seems possible that mating in *D. heteroneura* commonly occurs on vertical surfaces of the undersides of horizontal structures in elevated positions, whereas mating in *D. silvestris* takes place on or near the ground.

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Evolution of behavioral reproductive isolation in a laboratory stock of *Drosophila silvestris*

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Summary. A laboratory stock of *D. silvestris* has become partially sexually isolated since its origin from 1 natural population of this species. Evolution of reproductive isolation is probably incidental to reorganization of the genome, particularly the genetics of sexual behavior, following random drift in a small population.

Essential to speciation is the origin of reproductive isolating mechanisms. Speciation, as conceptualized by Mayr[2], can occur when a geographical barrier fragments a population of interbreeding organisms. Subsequent to cessation of gene exchange, genetic divergence proceeds in the isolate either in response to selection leading to adaptation to new environmental conditions, or merely due to drift. But without establishment of reproductive barriers the newly derived species could be assimilated into the ancestral population were the geographical barrier to be removed. Indeed, reproductive isolation is an integral part of the biological species concept.

In the Hawaiian Islands speciation of Drosophila has occurred by a series of founder events. Carson[3] has theorized that very few individuals (perhaps but a single gravid female) reach a previously uninhabited island where a new species evolves. It has been established that courtship behavior is of primary importance as an isolating mechanism in Hawaiian picture-winged Drosophila[4-6]. Spieth[7] has observed that courtship of picture-winged Drosophila involves a complex pattern of stimulus-response interactions between male and female. It seems valid to assume the elements of courtship behavior are genetically determined and under strong selection. Furthermore, the genes determining elements of courtship behavior should be subject to genetic drift during the founder event as is the rest of the genome.

Kaneshiro[5] has proposed that 'a few of the elements of the courtship pattern of the ancestral population are changed ('lost') in the genetic revolution which accompanies the founder event in the derived population'. According to his hypothesis, females of the derived species are stimulated to accept copulation by males of the ancestral species but in the converse situation derived males do not perform all the courtship patterns necessary to stimulate ancestral females to copulate. Several serendipitous occurrences in the lab provided an experimental situation in which Kaneshiro's hypothesis could be tested.

Materials and methods. 2 stocks of *Drosophila silvestris*, R59G4 and U28T2, were established from single native females collected at Kilauea Forest Reserve at 1510 m near Hawaii Volcanoes National Park. Female R59G4 was collected in November 1972, and the isoline suffered at least 2 major crashes when the effective population size may have been as low as two, once in 1974 and once in 1975. Female U28T2 was brought to the laboratory in January 1977, and the isoline was maintained at an effective population size of about 100 until mating preference

Mating preference tests between two laboratory stocks of *Drosophila silvestris*

Male[a]	Mating Homogamic	Heterogamic	n	I[b]	c[c]	p
U	10	22	32	−0.38	−2.17	<0.05
R	24	3	27	0.78	4.06	<0.01

[a] U = U28T2, R = R59G4; [b] Stalker Isolation Index; [c] c = t with infinite degrees of freedom; calculated by the method of proportions.

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