understand their comparative physiology and genetics, and the evolution of the C_4 pathway[5]. As an attempt towards this approach, the genus *Mollugo* (family: Aizoaceae) which contains both C_4 species *(M. nudicaulis)* and C_3 species *(M. lotoides* and *M. pentaphylla)*[6] was selected for our study. Plant regeneration is an essential step in somatic hybridization by cell fusion[7] and it is beneficial to induce differentiation in one of the parental protoplasts participating in somatic fusion[8].

Protoplasts could be isolated from the leaves of both C_4 and C_3 species of *Mollugo* and we report herein the isolation and culture of the mesophyll protoplasts from *Mollugo nudicaulis,* a C_4 species.

Healthy and mature leaves from 6-week-old plants were surface-sterilized with 5% sodium hypochlorite for 10 min followed by a quick immersion in 70% ethanol. The leaves were then thoroughly washed several times in sterile water. The lower epidermis was easily peeled off and the leaves, after removal of midrib, were cut into small bits. The leaf bits were then incubated in a filter-sterilized enzyme mixture containing 0.5% macerozyme and 2% cellulase dissolved in CPW medium with 0.7 M mannitol, at pH 6.2 and at 27 °C in diffuse light for 4–6 h. At the end of the incubation period, the leaf tissues were gently shaken manually to liberate the protoplasts. The crude protoplast mixture was first passed through a 500-µm nylon net to remove the undigested tissue and then through a 88-µm nylon net. The filtrate was centrifuged in a screw capped tube at $300 \times g$ for 3 min. The pellet was washed 4 times with CPW medium[9] and resuspended in the F_5 nutrient solution[9] supplemented with 10% v/v coconut milk. All the above procedures were carried out under sterile conditions.

The mesophyll protoplasts had an average size of 40 µm (figure 1) and the yield was determined to be 50–60% of the total leaf cells. The protoplasts were cultured in liquid F_5 medium at a density of 2×10^5 protoplasts per ml at 25 °C with 2000 lux of continuous illumination from a bank of warm white fluorescent tubes.

The 1st division of the protoplasts occurred at 72 h (figure 2) and about 50% of the protoplasts were seen dividing at this time. The 2nd division occurred on the 5th day. Subsequent divisions were irregular and on the 10th day, 8–10 celled colonies were noticed (figure 3). These colonies were transferred to F_5 agar (1%) medium in petri plates. Small green calli of about 0.6 mm were noticed by the 3rd week. However, they failed to grow further in this medium. Therefore, they were transferred to Murashige and Skoog's medium with growth regulators. In this medium the calli grew further in size and produced roots in the presence of NAA (0.1 mg/ml) (figure 4). Efforts to induce shoot formation with different combinations of growth regulators did not meet with success. Further experiments to regenerate whole plants from the calli and to induce somatic fusion between the protoplasts of this and other C_3 species of the genus *Mollugo* are in progress. It was found that the protoplasts of C_3 species of this genus did not divide in F_5 medium.

It is desirable that similar attempts be made to induce somatic hybridization between C_3 and C_4 species of economically important food crops, particularly in those where conventional hybridization is not successful.

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Biological studies on *Pseudohypocera kerteszi* (Phoridae, Diptera)[1]

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Summary. 15 couples of *Pseudohypocera kerteszi* were introduced into individual tubes containing culture medium, maintained at 22–28 °C and 45–55% relative humidity. The courtship and the behaviour of males and females before and after the copula are described for the first time; the number of eggs laid by each female during the oviposition period and the sexual rate in offspring were registered in each tube. The relative viability from egg to imago was determined in 8 of the 15 tubes.

The Phoridae are a large family of the Diptera; more than 2500 species have been described, including a certain number of parasitic forms which attack molluscs, arachnida and insects[2]. *Pseudohypocera* was first decribed by Malloch[3]. The species *P. kerteszi* attacks the nests of social bees (*Apis mellifera, Melipona* and *Trigona*) and their larvae eat the pollen stocked by bees in cells and pots[4]. When the population of *P. kerteszi* increases too much the larvae start to eat the pre-pupae and pupae of the bees. Therefore this parasite is one of the most dangerous enemies of social bees, specially *Apis mellifera*[4]. It is possible to rear this parasite in the laboratory and some biological and cytogenetical studies have been developed[2]. The approach of *P. kerteszi* to the colonies of stingless bees, their mating behaviour in front of recently populated beehives and their recovery in traps were recently described[5]. Nevertheless, no one was able to achieve mating in this species, under controlled conditions. The present research contains data on controlled matings, number of eggs laid by females during the oviposition period, relative viability and sexual ratio in *P. kerteszi.*

Females and males of *P. kerteszi* were capture in nature, being next transferred to small bottles containing culture medium (a type of food for fishes). These bottles were maintained at room temperature (22–28 °C) and 45–55% relative humidity during the experiment. 4 females pro-

duced a great number of eggs. The first pupae were carefully transferred to small tubes (10 cm^3) - each tube received only a pupa. The virgin males and females obtained in such a way were then separated in couples, each couple being introduced into an individual bottle with the same culture medium. We obtained mating of *P. kerteszi* under controlled conditions in all the attempts carried out (15 couples). The courtship and also the behaviour of males and females, before and after the copula, were observed for the first time in the laboratory. The male stays in front of the female and moves the wings rapidly from sec to sec many times. Next, he curves the abdomen downwards and stays in this position while the female is in front of him. If the female goes away he repeats the operation from the beginning. When the female accepts the courtship she stands the abdomen up slowly from time to time; occasionally she may also move her wings. After the mating the male cleans the abdomen with his posterior legs and moves his wings again.

We registered the mating duration in only 2 cases; 32 and 90 sec, respectively. The 15 mated females produced from 25 to 93 descendants ($\bar{X} = 57 \pm 21.48$ descendants). In the 15 tubes we registered 432 females and 423 males (approximately 1:1; $\chi^2 = 0.90$, nonsignificant at 5% level). The relative viability for the descendants, from egg to imago, was determined in 8 out of the 15 tubes. The 8 mated females used in this experiment laid 531 eggs; the number of eggs laid by each female varied from 31 to 102 ($\bar{X} = 66.37 \pm 22.11$ eggs). The mean viability from egg to imago, considering the 8 experimental tubes, was around 59%. The minimum mean viability was observed in the tube number 4 (40.32%) and the maximum, in the tube number 3 (76.19%)[1].

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A note on differences in the mating behavior of *Drosophila heteroneura* and *D. silvestris*[1]

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Summary. Differences between *Drosophila heteroneura* and *D. silvestris* in the time taken to copulate and the preferred location for copulation are reported. The potential significance of these differences is discussed.

Watson[3] described differences in the mating display of *Drosophila heteroneura* (Grimshaw) and that of *D. silvestris* (Perkins) which might, in part, be responsible for the high level of premating isolation between these species[4,5]. This note reports 2 additional differences in mating behavior between these closely-related, sympatric drosophilids.

Methods and materials. All observations were made on courtship patterns of individuals from Kahuku Ranch, Southern Hawaii. Virgin females were reared in the laboratory either from laboratory stocks [University of Hawaii, Stock Numbers: U26B52, U26B57 *(heteroneura)* and U26B9 *(silvestris)*], or from larvae collected in the field by Kenneth Y. Kaneshiro, John Tonzetich and the author. All males were wild-caught.

Observations of mating behavior were made during experiments testing female discrimination. In each experiment, a mature virgin female was caged with a male of each species in a clear glass cylinder (26.5 cm diameter, 12.0 cm high) with a removable loose-weave muslin top and a polyethylene base covered with damp sand. A small dish of food and a piece of plant material (or artificial substitute) were placed in each cage. During some experiments absorbent paper was taped to part of the inner wall of each cage to facilitate locomotion of the flies; no significant effect on positions of successful copulations was noted.

All copulations were timed to the nearest 30-sec using a wall clock with sweep second hand, and the positions of successful courtships were noted. For this purpose the cages were considered to consist of 3 sections: the floor, the walls and plant material, and the roof. All statistical procedures follow the methods of Zar[6]. During the experiments 6 cages were observed for approximately 3 h per day (08.00–11.00 h). The results are based on 27 days of observation involving 77 trios (43 with *silvestris* females; 34 with *heteroneura* females); 39 copulations were observed.

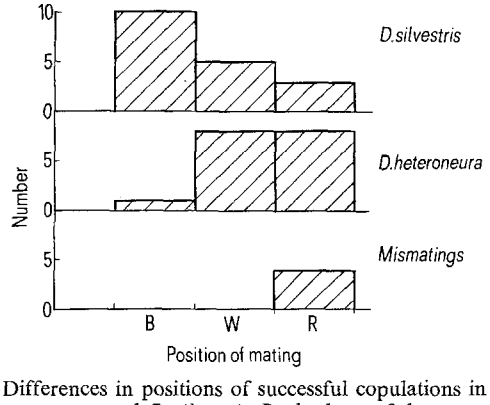

Fig. 1. Differences in positions of successful copulations in *Drosophila heteroneura* and *D. silvestris*. B, the base of the cage; W, the walls of the cage including plant material; R, the roof of the cage.

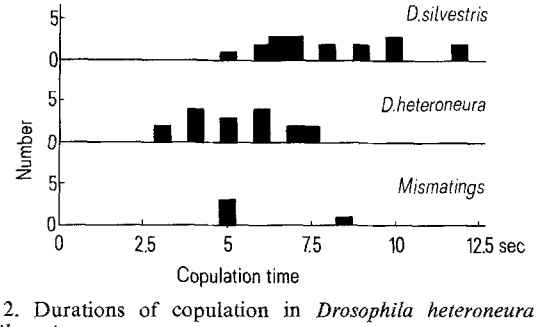

Fig. 2. Durations of copulation in *Drosophila heteroneura* and *D. silvestris*.