Effect of Social Isolation on Behavior of *Drosophila silvestris* from Hawaii[1]

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Disruption of sexual behavior as a result of early social isolation has been reported in numerous species: rhesus monkey, chimpanzee, guinea pig, dog, rat (Duffy and Hendricks 1973). This effect has also been found in birds (Thorpe 1961).

In *Drosophila* some studies show that the mating behavior, sexual preference or sexual discriminatory ability to recognize flies of the same species can be affected by age, experience in previous mating or previous contacts with flies of other species. Mayr and Dobzhansky (1945) found modification of the degree of sexual isolation between *D. pseudoobscura* and *D. persimilis* when the *D. persimilis* male was conditioned with its own female and with *D. pseudoobscura* female. Manning (1959), working with *D. melanogaster* and *D. simulans,* concluded that males of both species court young foreign females better than old foreign ones and that the increasing discrimination against the latter as they age is related to the strengthening of some species-specific scent. Pipkin (1963) in *D. victoria* species group, described some "learned repugnance" in inter-specific crosses. Pruzan and Ehrman (1974) using *D. pseudoobscura* females which differed in age and in previous mating show that frequency-dependent mating is both age and experience dependent.

The present experiment analyzes the effect of social isolation on courtship ability and on rate of mating, involving intraspecific crosses in *D. silvestris* (Perkins). *D. silvestris* is a species endemic to the Island of Hawaii, belonging to the *planitibia* subgroup of the picture-winged flies. This species is referred to as *nigrifacies* in Carson and Stalker 1968, but the latter is a synonym of *D. silvestris* according to Hardy and Kaneshiro (1968). These flies present a complex courtship behavior. Spieth (1966, 1968, 1970 and 1974) notes that "after feeding for a short time, the males leave the food and each of them selects a small territory or lek in the surrounding vegetation." There, the male vigorously defends his territory against intruders and advertises his presence by waving its wings and walking alertly about; field and laboratory observations have shown that sexually receptive females are attracted by advertising males to these leks, where courtship occurs.

These flies have a long period of sexual maturation and preliminary observations by Ahearn (personal communication) revealed the following facts about *D. heteroneura*, a closely related species (Craddock 1974).

[1]Published with the approval of the Director of the Hawaii Agricultural Experiment Station as Journal Series No. 2078.

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Although males show motile sperm after about the 7th day, the ability to engage in courtship starts at approximately 14 days of age and reaches maximum efficiency in courtship only after about 21 days. It is assumed that *D. silvestris* is very similar. Although systematic data on egg maturation and sexual receptivity of females are lacking, preliminary observations indicate that at least two or three weeks is involved.

Normally, in the laboratory the male and female flies are aged in groups of five to eight flies per vial for four weeks. Crosses are then made. Some results using this species in sexual isolation experiments can be found in Ahearn, et al (1974), Craddock (1974) and Kaneshiro (1976). *D. silvestris* shows a low rate of intraspecific crosses in laboratory conditions. Craddock (1974), leaving mature flies for four to ten weeks in pair-matings, found only 68% of the females inseminated. In previous experiments, Sene (unpublished observations) found that when the mature flies, aged separately by sex, five to eight flies per vial, were placed in 61 pairs for 24 hours, only 36% of the females were inseminated.

Normally, in *Drosophila*, laboratory-reared specimens exhibit courtship behavior which is quantitatively and qualitatively similar to that performed under natural field conditions (Spieth 1974). However, the author of this paper found that wild-caught males, placed in the presence of mature virgin females from laboratory stock, showed a different courtship behavior from stock males. The wild-caught males kept more distance from the female during courtship and moved more quickly. In order to determine if this difference was related to inbreeding in the stock, F_1 males from wild-caught females were reared in the laboratory. They were tested and showed the same courtship behavior as that of the stock males. This is evidence that laboratory conditions affect the complex courtship pattern of these flies.

Since it is well known that the maturation period is the principal learning period for many animals, the present laboratory experiment was done to see to what extent the complex courtship pattern of this species could be affected by learning experience in the long maturation period.

Materials and Methods

A strain of *D. silvestris* (R59G4) and a strain of *D. heteroneura* (Q71G12), both from the island of Hawaii were used for the present experiments. Females of *D. silvestris* were separated from the males about 20 hours after eclosion. Then the females and the males were separated into two classes each: "female aged alone" and "female aged together," "male aged alone" and "male aged together." Flies of the "female alone" set were placed individually into an eight dram vial (25mm X 95mm) containing Wheeler-Clayton medium (Wheeler and Clayton 1965) and placed in special trays in which the flies could not see each other. The same thing was done with the "male alone." The "female together" flies were placed in a vial of the same size with a *D. heteroneura* male, of the same age. The "male together" flies were placed with a *D. heteroneura* female of the same age. Under these conditions the flies were aged 30-35 days at a temperature of 16-18° C with a change to fresh food each seven days.

After this conditioning period the following pairs of *D. silvestris* females and males were made: "female alone" X "male alone;" "female alone" X "male together;" "female together" X "male alone;" "female together" X "male together." The flies were then kept as pairs for 24 hours after which the female was dissected and assayed for the presence of sperm by examination of the reproductive tract under a light microscope (100X magnification).

As a control, *D. silvestris* females from the same stock were aged 30-35 days, five in each vial. The same was done with *D. silvestris* males. After the maturation period they were crossed in the same way described before.

Fifty "females together" were dissected after the aging period and five were found to be inseminated by the *D. heteroneura* male. This proportion was taken into consideration in the results of pair-matings where "females together" were involved.

Results

Table 1 gives the results of the crosses and the frequencies of copulations observed. Value of $X^2_{(3)}$ was calculated for the frequencies of all four classes and gave the value of 8.941 (significant at 5% level). Two partitions of this X^2 show that the value of $X^2_{(2)}$ between p_1, p_2, p_3 is 0.102 (not significant at 5% level); p_4 compared against p_1, p_2, p_3 show $X^2_{(1)}$ value of 8.839 which is significant at 1% level.

Table 2 gives the results of the crosses and frequencies of copulations obtained from a control group of flies which were aged together with flies of the same species and sex. One X^2 value was calculated comparing the results of the crosses shown in Table 2 for the results obtained in the crosses involving "female together" X "male together." The value of $X^2_{(1)} = 0.42$ (not significant at 5% level).

The results show that in crosses where flies aged alone were involved the frequency of success in mating decreases significantly. They also show that the *D. silvestris* flies which were aged with *D. heteroneura* flies of the opposite sex have the same mating ability as the *D. silvestris* flies aged with flies of their own species and of the same sex.

Visual observation of the "alone" flies showed an unusual behavior when they were placed with other flies; both male and female exhibited "escape behavior" by jumping in a disorderly manner inside the vials. Observations during the first four hours showed that "alone males" were not able to court effectively and "alone females," when courted by the "together male," interrupted his courtship frequently with her "escape behavior." Tests made with the "alone" flies, both male and female, showed that the "escape behavior" started when one "alone" fly sees another fly even if the other fly is in a different vial lying near it on the laboratory bench.

Discussion

Despite the fact that mating behavior is instinctive in these flies, the data show that they need "training" in order to be able to perform courtship efficiently. Probably, the laboratory conditions do not give the flies all the

prior "conditioning" that they need in order to be successful in mating. This probably explains the low rate of copulation found in this species under laboratory conditions. Without a doubt, the effect of social isolation has a drastic consequence in flies aged alone. Although this situation is probably unrealistic for a natural population, it is sufficient to emphasize the complex nature of the behavior. Effective population size is undoubtedly affected by social interaction during maturation.

According to Spieth (1974), females usually have to be courted several times by a male before they become sexually receptive. The present results show that the training can take place with flies of the opposite sex of a different species or with flies of the same sex of their own species. This emphasizes the idea that prior experience ("training") through social contact is necessary for performing efficient courtship behavioral patterns. Thus, flies aged alone are unable to perform effective sexual behavior, and, in fact, display a kind of "anti-social" (escape) behavior. The interaciton of both these factors results in a low rate of success in pair matings.

Acknowledgments

The author performed this work at the University of Hawaii as a Post-doctoral Fellow of Fundacao de Amparo a Pesquisa do Estado de Sao Paulo, Sao Paulo, Brazil. It was supported by National Science Foundation grants to H. L. Carson, Department of Genetics (GB27586) and D. E. Hardy, Department of Entomology (GB29288).

Drs. H. L. Carson and K. Y. Kaneshiro encouraged this work and suggested the problem. Both read the drafts of the manuscript and provided helpful comments. Dr. G. A. Peres of the University of Sao Paulo gave advice in the statistical analyses and A.C.S. Sene in the technical care of the flies.

Summary

Despite the existence of an innate courtship behavior, imagos of *D. silvestris* (Perkins) need "training" in order to be able to perform a successful courtship. This training can take place either with flies of the opposite sex of a different species, or with flies of the same sex of its own species. It is postulated that effective population size is influenced by the interaction of an inhibition of sexual behavior and a form of anti-social behavior caused by a prolonged period of social isolation.

TABLE 1. *Type of Crosses and Frequencies of Mating in Each Type*

CROSSES	MATING	NO MATING	TOTAL	PERCENT MATING
♀ alone X ♂ alone	6	44	50	12% = p_1
♀ alone X ♂ together	5	45	50	10% = p_2
♀ together X ♂ alone	5	45	50	10% = p_3
♀ together X ♂ together	14	36	50	28% = p_4
TOTAL	30	170	200	15% = p

TABLE 2. *Frequency of mating in 50 pair matings of control group flies over a 24-hour period.*[1]

MATING	NO MATING	TOTAL	PERCENT MATING
17	33	50	34%

[1]Both males and females were aged for 30 days with other individuals of the same sex and species, before crossing.

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