

暗飼育下におけるドロソフィラ・メラノガスターの交尾行動変化

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**Mating behaviour stability in strains of *Drosophila melanogaster*
which have been kept under constant darkness
for about 27 years¹⁾**

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ABSTRACT

In October 1954, Mori and his collaborators isolated a number of wild-type strains of *Drosophila melanogaster* and placed them in constant darkness. We have investigated the mate recognition system of individuals from these strains by comparing them with control strains. Mating activity rhythms for each strain were determined by measuring their mating propensity. Deviation in mate recognition was measured at times when "eagerness" of the "dark" lines and their controls were comparable. Our results show that despite such a long period under unusual environmental conditions the mate recognition system of individuals from "dark" strains had not significantly changed from their controls.

1. INTRODUCTION

In sexually reproducing species fertilization is facilitated via a male-female communication system, the Specific-Mate Recognition System (SMRS) (Pater-son, 1978, 1980, 1981). Individuals of a species, according to this view, share a common SMRS. The stability of this system must hence be of great interest to those concerned with speciation.

Environmental effects on the development of the mate recognition system are of fundamental importance, since some species which occupy different geographic regions also exhibit considerable stability in the SMRS. For example, Henderson and Lambert (1982) have shown that individuals from populations of *Drosophila melanogaster* from a large number of geographically widely separated localities exhibited considerable stability in their mate recognition systems. Likewise, Millar and Lambert (1985) have shown a similar result in crosses between individuals of *Drosophila pseudoobscura* from North America and New Zealand.

It has been suggested (Lambert, 1984; Lambert and Hughes, 1984) that the nature of the structural relationship between signals and receivers accounts for the spatial stability of this system.

It was decided then to investigate strains of *Drosophila melanogaster* which

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had been maintained under constant darkness for about 27 years, in order to ascertain if there had been any changes to the mate recognition system. It seemed particularly important to do this in the light of the findings of Kiliias *et al.* (1980) who obtained a positive result when analysing for divergence between isolated lines of *Drosophila melanogaster* which had been maintained under different environmental regimes.

Origin and Laboratory history of Drosophila melanogaster lines.

The lines had previously been subjected to long-term culturing under constant darkness for approximately 700 generations. This was done for "the purpose of investigating what kind of alteration can occur in the dark environment." (Imaizumi 1979: 55). Control lines under natural light conditions were also maintained to check for any genetic changes. Two wild strains were originally used, Oregon-RS and Tokyo, both derived from the stocks which had been cultured in the Laboratory of Animal Genetics of Kyoto University, Japan. A number of lines were set up from these strains, of which three "dark" and control lines of Oregon-RS, Tokyo-p, Tokyo-c were supplied for investigations carried out in this study.

One "dark" line (Oregon-RS) exhibited elongation of sensory bristles compared with those of its control. This character was still retained after returning the "dark" cultured flies to the light condition. "Sensitivity in photic reactions" has increased in flies of the "dark" lines Oregon-RS and an increase also occurred in viability and fecundity (Imaizumi 1979: 66).

The objective of these experiments was to test for divergence in SMRS's between dark lines and their controls by multiple choice methods which check for positive assortative mating. One of the "dark" lines exhibited stable morphological divergence from its light control (Imaizumi 1979).

Previous Crossing Experiments between "Light" and "Dark" Lines.

Kiliias *et al.* (1980) reported that changed environmental conditions had resulted in a stable "sexual isolation" between cage populations of *Drosophila melanogaster*. These differences were established from multiple choice tests and calculation of isolation indices. The populations had been maintained under different environmental conditions for about five years. One of the environmental variables was total exclusion of light and the populations under this regime exhibited a high degree of divergence from control lines in the light. Tests between these populations revealed that the non-random mating preferences were stable (the Isolation index, $I=0.388\pm 0.108$). For these cross-mating experiments there is a difficulty in conducting multiple choice tests in an unbiased environment. This apparent isolation may have been due to non-synchronisation between the mating phases of two populations. Photo-periodically entrained copulatory rhythms are common in Dipterns (Beck

1980). In particular, for *Drosophila* species, Grossfield (1964) reported *D. auraria* to be bimodal in its diurnal activity for copulations. Mayr (1946) mentioned sexual activity in *D. pseudoobscura* and *D. persimilis* is higher in the mornings and evenings. A daily rhythm of mating activity in *D. robusta* was shown by Roberts (1956), such activity being highest in the hours prior to the onset of darkness. Grossfield (1964) studied mating systems of *Drosophila* species under constant darkness and showed activity becomes arrhythmic. Further, the rhythm can be entrained relative to the onset of darkness in a light-dark daily schedule. Shitamoto and Ikeda (1981) compared two strains of *D. melanogaster* for mating activity in red light and light (200 lux). Mating indices were considerably larger in the light condition.

In the experiments conducted by Kiliyas *et al.* (1980), it was not attempted to establish if mating rhythms were comparable between the two populations before conducting multiple choice tests. Mating rhythms in "dark" populations which have been returned to "light" conditions for experimentation, may not be in line with the light control. Preliminary work, therefore, was aimed to eliminate this variable in the multiple choice tests.

2. MATERIALS AND METHODS

Drosophila melanogaster Strains Used in these experiments

The two wild strains, Oregon-RS Tokyo, were derived from wild strain stocks held at Kyoto University, Japan. Oregon-RS had been maintained under darkness since 1954, and the Kyoto strain since 1956. All the control lines had been maintained under natural light conditions for the same period of time. Stocks were supplied by T. K. Watanabe of the National Institute of Genetics Mishima, Japan (S. Mori had previously set up the lines for experimental use). When received, Oregon-RS lines had been cultured for 699 generations, Tokyo-p for 649 generations, and Tokyo-c for 640 generations. Experiments were conducted approximately fifteen generations later.

Culturing of Lines

The "dark" strains were continued to be maintained in the Evolutionary Genetics Laboratory, University of Auckland under conditions of total darkness. Each line was designated the following codes: for "dark" lines Oregon-RS (DORS), Tokyo-p (DTp), Tokyo-c (DTc); for light control lines Oregon-RS (LORS), Tokyo-p (LTp), Tokyo-c (LTc). Cultures were maintained by mass-mating on yeast-agar-cornmeal medium, at 25°C. Light control lines were under a 12:12 cycle.

A red light (700 nm) was constructed using filters, for observing the "dark" flies during culturing, collection and isolation of virgin flies. Bertholf (1933) first investigated the relative spectral stimulation for *D. melanogaster* and

found the visual limits to be 2380 and 6500Å. This was substantiated by Lutz and Grisewood (1934). Thus, the "dark" flies were effectively in continuous darkness.

Experimental Procedures to Determine Any Differences in the Rhythmicity of Mating Activity between "Dark" and "Light" Lines.

All lines were compared with respect to their mating activity, by assessment of mating propensity. The light control lines were cultured, collected as virgins, and aged in the L:D cycle, which was set as follows: a light phase 0900 and 2100 hours, a dark phase 2100 to 0900 hours. For the derived "dark" lines these experimental procedures were conducted in complete darkness. They were then exposed to light at 1800 hours on day two of their ageing to allow entrainment of mating rhythm. This allowed completion of one L:D cycle prior to experimentation. Observations of matings were carried out in the light.

The mating rhythm for each line was determined by measuring their mating propensity (Spiess *et al.* 1966), at two-hourly intervals during the light phase. For each time period, 20 four day old males and 20 four day old females were introduced into a mating chamber similar to that designed by Elens and Wattiaux (1964) under constant conditions at a temperature of 25°C. (The multiple choice tests were also conducted in the same chambers, so constant environmental conditions were maintained). The numbers of matings per five minute interval were scored during a thirty minute observation period. A mating index was calculated by the formula proposed by Spiess *et al.* (1966) on the basis of the data from four to eight runs. Observations were conducted for each two-hourly interval during the light phase.

3. RESULTS

Mating propensity was estimated as an average index of mating speed (Spiess *et al.*, 1966). It has been shown that most matings, whether in fast or slow strains, tend to occur within the first ten minutes (Dow 1976; Spiess and Langer 1964). This index weighs the number of matings per five minute interval by the reciprocal of the average time taken to mate $\times 100$, plus a weight for those not mating after thirty minutes. Such an index is useful since it avoids zeros and gives an approximate comparative basis for the different lines used. Mating propensity results were used to construct a mating activity rhythm for each line. Times at which multiple choice experiments were conducted between each derived line and its control are indicated in Figs. 1, 2 and 3.

Design of Multiple-Choice Tests between Derived Lines and their Controls

From the results of the mating propensity experiments, multiple choice

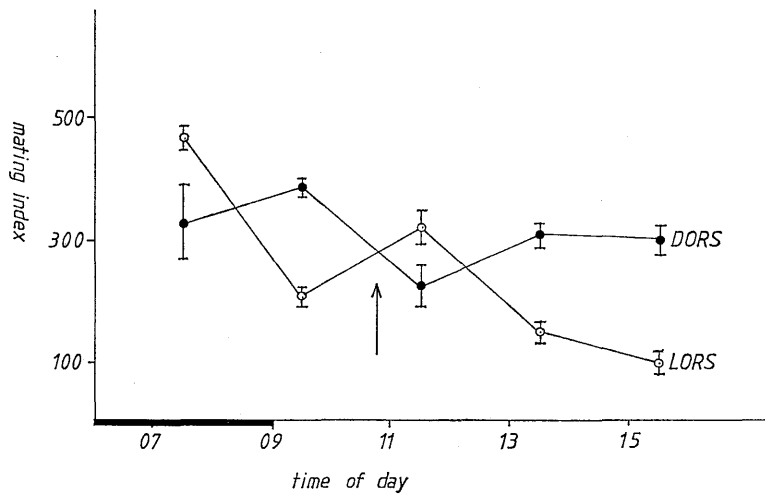


Fig. 1. Mean changes in mating index depending on the time of day at which observations were carried out for derived "dark" lines (DORS) and its "light" control (LORS). Multiple choice experiments were performed at time period indicated. The standard error of each mean is shown.

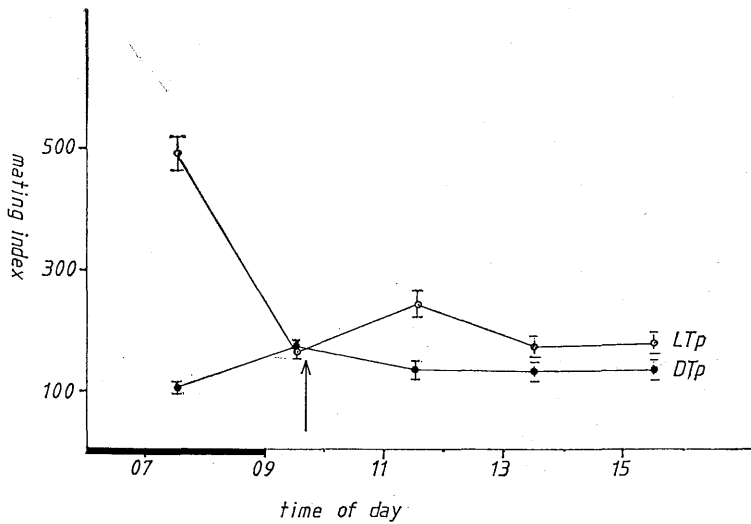


Fig. 2. Mean changes in mating index depending on the time of day at which observations were carried out, for derived "dark" lines (DTP) and its "light" control (LTP). Multiple choice experiments were performed at time period indicated. The standard error of each mean is shown.

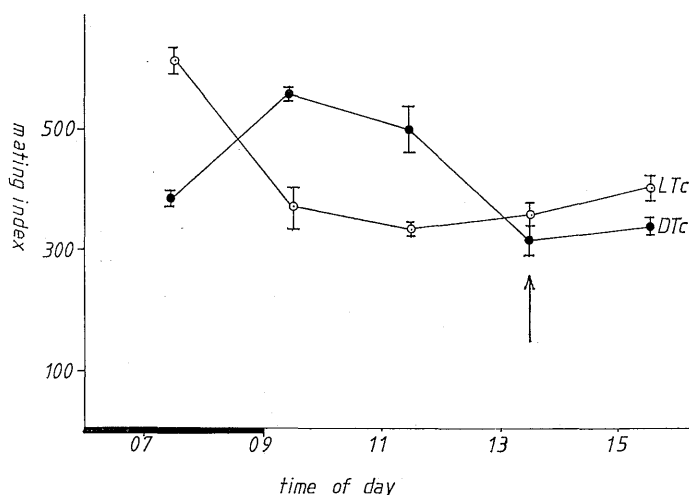


Fig. 3. Mean changes in mating index depending on the time of day at which observations were carried out, for derived "dark" lines (DTc) and its "light" control (LTc). Multiple choice experiments were performed at time period indicated. The standard error of each mean is shown.

tests were able to be conducted at times where "eagerness" to mate between the derived lines and their controls was comparable, thus eliminating propensity to mate as a factor which may influence the tendency to mate assortatively. This problem has been extensively studied by Spiess and Dapples (1981). Multiple choice experiments between each derived line and its control were conducted. Direct observation of individuals involved was enabled by use of the apparatus described by Elens and Wattiaux (1964) originally designed for multiple choice experiments.

A period of one week intervened from calculation of mating propensity to time of direct observation of mating choice experiments. Derived "dark" lines were maintained in the dark and virgins prepared as for mating propensity experiments, i.e. exposed to one L:D cycle prior to testing.

The unetherised flies introduced into the chambers were aged to four days while isolated from individuals of the opposite sex. Alternating strains and sexes were paint-marked on the ventral surface of the thorax for identification. This was easily observable, but was checked with a hand magnifying lens. Twenty possible copulations could be observed per run in an observation chamber. Ten females and ten males of each strain were introduced and observed for a period of one hour. Matings were scored at six minute intervals during this time period, for duration of copulation, position, and types of individuals involved.

Table 1. *Mating preferences in crosses between isolated populations of Drosophila melanogaster. (Isolation index after Malogolowkin-Cohen et al., 1965).*

Cross A × B	LORS × DORS	LTp × DTp	LTc × DTc
A ♀ × A ♂	77	90	73
A ♀ × B ♂	86	94	72
B ♀ × A ♂	65	80	70
B ♀ × B ♂	74	89	85
N	302	353	300
χ^2	1.702	1.186	1.84
I (Isolation Index) ± S.E.	0.019 ± 0.057	0.014 ± 0.053	0.1 ± 0.057

Multiple Choice Tests

For multiple choice tests a joint isolation index and its standard error were calculated according to the formula used by Malogolowkin-Cohen *et al.* (1965).

$$I = X_{11} + X_{22} - X_{12} - X_{21} / N$$

$$S. E. = \sqrt{(1 - I^2) / N}.$$

Isolation between strains was tested for significant deviation from random mating by chi-square. None showed a significant result (Table 1). Therefore, no apparent differences in mating behaviours have yet developed between the "dark" lines and their controls.

4. DISCUSSION

The Environment and the Stability of the SMRS

No significant divergence in the SMRS occurred under environmental change in which light was excluded from three lines of *D. melanogaster* for 700 generations (Table 1).

Signals of a male-female communication system are composed of a number of parameters, one of which may illicit the response. This parameter, which is involved in communication, is called the sign-vehicle (Hailman 1977). Transmission of the sign-vehicle may occur in a visual, auditory, chemical or tactile modality. Hence, these results support the contention that visual sign-vehicles are not involved in mate recognition in *D. melanogaster*. Analysis of auditory systems have revealed a large number of physical parameters (potential sign-vehicles) in any one signal (Busnell 1977). For example in the Indigo Bunting (*Passerina cynea*), analysis of song has revealed a large number of song parameters, only a limited number of which are important in

conveying instructions which result in conspecifics recognising each other (Emlen 1977).

Thus the distinction between the signal and the sign-vehicle is important in determining the parameters involved in communication. As the SMRS in *D. melanogaster* exhibits considerable stability under conditions of complete darkness, the mode of transmission was not disrupted in the new environment. If an artificial selection pressure is to cause a change in the SMRS, it must directly hinder the mode of transmission of sign-vehicles and responses. Therefore, although laboratory environmental changes may cause divergence in other characters, a corresponding divergence in SMRS will not necessarily occur.

SMRS of Drosophila melanogaster

D. melanogaster is considered to be a species essentially unaffected by darkness (Parsons 1973; Ehrman and Parsons 1976; Spieth and Hsu 1950), and has been termed "broad-niched" or cosmopolitan because of this (Grossfield 1971). Grossfield (1971) considers this to be caused by a "greater degree of behavioural flexibility with respect to the degree of lock-in on particular stimuli in courtship." He is presumably referring only to visual components of the courtship sequence, however. The female sexual behaviour of this species has shown they are more responsive to stimuli perceived by their antennae and not to visual aspects of the males courtship (Ehrman and Parsons 1976). For *D. simulans*, a cryptic species of *D. melanogaster*, courtship is however inhibited by darkness. Many consider the behavioural difference between the two cryptic species reflects a divergence in organisation of the courtship and its underlying heritability (Ehrman and Parsons 1976; Grossfield 1971).

Orientation appears to be an important visual stimulus for *D. simulans* (Ehrman and Parsons 1976). *D. melanogaster* uses visual, auditory and chemosensory stimuli in its mate recognition system (Spiess 1970; Spieth 1974). However, the most conspicuous element of the courtship display is the frequently repeated vibration of one wing, usually the one closest to the female's head (von Schilcher 1976). The sound produced by male wing vibrations was shown by Shorey (1962) to be a series of intermittent pulses. The length of pulse song functions were found to be a recognition signal for *D. melanogaster* (Bennet-Clark and Ewing 1969). At 25°C, the pulse interval measured from the beginning of one pulse to the beginning of the next being 34 msec. This has been confirmed by von Schilcher (1976), the pulse-interval acting in a "trigger-like fashion." She noted the signal is 'unambiguous and it takes no time to get the message' (von Schilcher 1976: 624). The variation of this inter-pulse interval (ipi) for courtship song was investigated among strains of *D. melanogaster* (Kawanishi and Watanabe 1980). The variation was small (31.3

-34.8 msec). For *D. simulans*, it was much larger. This emphasises the important role of auditory stimuli in *D. melanogaster* courtship, for allowing the recognition of mates (Ewing 1970). Further, the female's antennal arista serves as a displacement type sound receptor (Manning 1967) and when this has been removed, there has been a corresponding reduction in sexual receptivity (Mayr 1950). Wingless males have been shown to be disadvantaged in courting success when compared to normal males (Sturtevant 1915).

It may be postulated the pulse-interval in sound production is an important stimulus of males in the SMRS. This results in a system which is light-independent, and therefore stable in dark environments, as was indicated by the multiple choice experiments (Table 1). Therefore, in dark conditions, there are strongly coordinated stimuli and responses between males and females. Such components of the SMRS appear to exhibit flexible behaviour patterns. These are "locked into a unique releasing signal by a single sensory system" (Grossfield 1971: 2671). And they can therefore be blocked when no response is given because of the failure to perceive the particular triggering sign-vehicle. For *D. melanogaster*, darkness does not block any stimulus-response component, as the courtship essentially operates in an auditory modality, not visual. It may be argued that initial localisation may be through visual signals, but it has been shown that flies are capable of making physical contact in the dark (Grossfield and Smith 1971). Upon such initial localisation, the auditory components can proceed. For the extremely light-dependent species *D. subobscura*, courtship relies on visual stimulation in both sexes. This behavioural pattern was modified in a strain selected for light-independent mating (Pinsker and Doschek 1980). Males of this strain, when observed in the dark, recognised wild type females by tapping, thus receiving tactile and/or chemical stimuli. They then "raped" them without any preceding courtship. All visual components were eliminated.

For *D. melanogaster*, an important environmental factor affecting auditory modalities may be temperature. Shorey (1962) showed that for the pulsations of sound caused by wing movements, the rate was dependent on temperature, increasing at 1.4 pulses per additional degree Celsius. This may account for Kiliass *et al's* (1980) result in which they had increased the divergence in mate recognition between two isolated lines maintained under different environmental regimes. The variables were light/dark, temperature, and relative humidity. Thus the auditory modalities may have experienced directional selective pressures under these laboratory conditions. However, the situation was uncontrolled in that for each cross, one population had been maintained under natural light conditions, and the other under dark conditions. The workers who simultaneously showed that the pattern of oviposition rhythm differed between light and dark populations, failed to test if mating rhythms were out of phase. In this study, dark lines and their light controls were

shown to have different mating rhythms (Figs. 1, 2 and 3). This is another possible factor in the small degree of divergence as is the change in pulse-interval, due to temperature differences.

Change in mating behaviours which are components of the SMRS do not seem to be correlated to divergence in other traits (Robertson, 1950; Henderson and Lambert, 1982). Thorough testing of this stability will only be achieved by first analysing the signal-response sequence and establishing those parameters of signals which are sign-vehicles and the modalities within which they operate. The sign-vehicle, which is that on which recognition is based in almost all cases is just one of several signal parameters (Johnston, 1976).

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