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Genetic Analysis of the Light Dependence of Courtship in *Drosophila subobscura*

Rita Aldinger-von Kleist[1] (1985)

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The subject of this investigation is the genetic basis of light independence (lin) in the courtship and mating behavior of Drosophila subobscura. *Lin flies, in contrast to wild flies of this species, do not depend on light for reproduction. Most elements of the typical courtship behavior of* D. subobscura *are omitted. Crosses of lin flies with wild-type flies were performed. By the use of chromosomes marked with different alleles of enzyme loci, the genetic effect of each of the four autosomes was determined. A positive selection success for light independence of mating behavior always proved to be correlated with frequency changes for the alleles of the* Phi *locus on chromosome E and the gene arrangements of the same chromosome. From this it can be concluded that a main genetic factor for light independence (lin factor) of courtship of* D. subobscura *is located on chromosome E.*

KEY WORDS: courtship in *Drosophila*; genetics of courtship behavior; light dependence; *Drosophila subobscura.*

INTRODUCTION

The problem of how complex behavioral traits are determined by genetic factors is one of the basic questions of behavioral genetics. To study this point experimentally, *Drosophila* is a very suitable organism. Among the many behavioral characters investigated so far, courtship behavior seems to be most complex. There are significant differences between species, indicating the evolutionary importance and genetic basis of courtship behavior.

[1] Lehrstuhl für Populationsgenetik, Institut für Biologie II, Universität Tübingen, Auf der Morgenstelle 28, D-7400 Tübingen, FRG.

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Drosophila subobscura, a European species of the *obscura* group, appears especially advantageous for a genetic analysis of mating behavior. The species has been intensively studied with respect to its genetic variability in natural populations [inversion polymorphism (Krimbas and Loukas, 1980; Sperlich and Feuerbach-Mravlag, 1974; Prevosti, 1966); allozyme variation (Pinsker and Sperlich, 1979); quantitative traits (Pfriem and Sperlich, 1982; Pfriem, 1983)]. Observations on its ecology and especially on its courtship behavior were also performed by several investigators: wild-type flies of *D. subobscura* never mate in darkness (Philip *et al.*, 1944; Rendel, 1945; Grossfield, 1972). Despite this, natural populations seem to be variable for genes determining the degree of dependence of mating on light, since it is possible to obtain light-independent strains by selection (Andjelković and Marinković, 1983). Such a strain, called the "lin" strain, was established by Springer (1973). Further studies revealed that the courtship behavior of the lin strain has been changed in an extreme way. Instead of showing the wild-type courtship, composed of a complex reaction chain culminating in a wingdance involving both partners (Brown, 1965), lin males rape the females without a preceding courtship, and lin females accept males without preceding courtship stimulation (Pinsker and Doschek, 1980). The aim of the present investigation was to find the genetic factors responsible for the ability to mate in darkness. In the studies cited above it was shown that the X chromosome is not involved in the genetic determination of light dependence but that there might exist a polygenic system on one, two, three, or all four autosomes. The purpose of this study is to find out whether the responsible polygenes are distributed randomly over all four autosomes or concentrated in one or two components of the chromosome set. Chromosomal inversions and allozyme variants were used as genetic markers for the analysis. These traits have the advantage of having no known direct influence on the behavioral characters, as is frequently the case with visible markers.

MATERIALS AND METHODS

The following strains of *D. subobscura* were used in the experiments (see Table I).

lin: a strain selected for "light independence" (lin) for 14 generations through gradually reduced light intensities (Springer, 1973). The strain is monomorphic for standard gene arrangements in all five chromosomes and for the enzyme alleles Mdh^{96}, Pgm^{101}, and Phi^{100}; variability exists in the enzyme locus *Me* with the two alleles Me^{105} (32.4%) and Me^{106} (67.6%).

Kü: a standard strain from Küsnacht, monomorphic for the standard gene arrangements of all five chromosomes and for all enzyme loci used in this study (Lankinen and Pinsker, 1977).

The following laboratory strains were specifically constructed for the present investigation. They show all "wild," that is, normal light-dependent, mating behavior.

Wild (E): a strain monomorphic for the allele Phi^{108} (on chromosome E) and for the gene arrangements $E_{1+2+9+12}$, I_1, and U_{1+2+8}.

Wild (I): a strain monomorphic for the allele Pgm^{97} (on chromosome I) and for the gene arrangements E_{St}, I_{St}, and U_{St}.

Wild (O): a strain monomorphic for the allele Me^{103} (on chromosome O) and for the gene arrangements E_{St} and U_{St}.

Wild (U): a strain monomorphic for the allele Mdh^{105} (on chromosome U) and for the gene arrangements $E_{1+2+9+4}$, I_1, and U_{1+2+8}.

All strains were kept in light under the usual laboratory conditions, but the lin strain and one group of experimental strains (see below) were always in complete darkness.

Gel Electrophoresis. Horizontal starch gel electrophoresis was used according to Ayala *et al.* (1972). The following enzyme systems were assayed: Mdh (malate dehydrogenase), Me (malic enzyme), Pgm (phosphoglucomutase), and Phi (phosphohexose isomerase). The designation of allozymes corresponds to the system proposed by Saura *et al.* (1973). The biochemical methods applied are those described by Shaw and Prasad (1970).

Cytological Analysis. Chromosomal analysis was carried out on acetoorcein-stained salivary gland chromosomes of F_1 larvae from crosses between the males tested and virgin females from the standard Küsnacht strain. The designation of inversions and gene arrangements corresponds to the system of Kunze-Mühl and Sperlich (1955) and Kunze-Mühl and Müller (1958).

Preceding Investigations. Preceding investigations revealed the following differences between lin and wild-type flies of *D. subobscura*.

(1) Lin flies have a reduced pattern of mating behavior. They do not show the wingdance with all the preceding courtship elements typical of *D. subobscura* (Pinsker and Doschek, 1980; Aldinger-von Kleist, 1982). Most probably the predominance of the sensory stimuli in courtship has changed during the course of selection for light independence; visual signals were replaced by tactile and olfactory susceptabilities (Ripfel and

Becker, 1982). Our own observation is that the main element of courtship behavior used by lin males is exaggerated tapping.

(2) All flies of the lin strain are homozygous for the standard structures of the five chromosomes, in contrast to flies from natural populations (Aldinger-von Kleist, 1982), which normally show a high degree of inversion heterozygosity (Sperlich *et al.*, 1980; Krimbas and Loukas, 1980).

(3) All flies from the lin strain proved homozygous in one allele of the following eight enzyme loci: *Ao*, *Aph*, *αGpdh*, *Hk*, *Mdh*, *Odh*, *Pgm*, and *Phi* (Aldinger-von Kleist, 1982). Three of the eleven loci tested were still polymorphic (i.e., *Adh*, *Lap*, and *Me*). Natural populations usually show a high variation in most of these enzyme systems (Pinsker and Sperlich, 1979).

Experimental Design. In order to localize the genetic factors determining the light independence of the lin strain, crosses between wild-type flies and lin flies were performed. Four hundred lin males were crossed to 400 virgin wild-type females from each of the strains wild (E), wild (I), wild (O), and wild (U) respectively. The four larval F_2 generations were divided into two lines each. One of these lines was then kept in complete darkness, automatically producing a strong selection pressure

Table I. Frequencies (%) of the Various Gene Arrangements and the Enzyme Alleles Present in the lin Strain and the Wild-Type Marker Strains

Chromosome	Gene arrangement	Enzyme allele	lin	Wild (E)	Wild (I)	Wild (O)	Wild (U)
E	E_{St}		100	0	100	100	0
	$E_{1+2+9+12}$		0	100	0	0	0
	$E_{1+2+9+4}$		0	0	0	0	100
		Phi^{100}	100	0	100	100	100
		Phi^{108}	0	100	0	0	0
I	I_{St}		100	0	100	42	0
	I_1		0	100	0	58	100
		Pgm^{97}	0	0	100	0	0
		Pgm^{101}	100	100	0	100	100
O	O_{St}		100	0	0	0	0
	O_6		0	0	50	0	0
	O_{3+4}		0	43	0	0	0
	O_{3+4+2}		0	0	50	53	0
	O_{3+4+6}		0	0	0	47	0
	O_{3+4+8}		0	57	0	0	100
		Me^{103}	0	0	15	100	0
		Me^{105}	32	18	0	0	0
		Me^{106}	68	82	85	0	100
U	U_{St}		100	0	100	100	0
	U_{1+2+8}		0	100	0	0	100
		Mdh^{96}	100	100	100	100	0
		Mdh^{105}	0	0	0	0	100

for mating ability in darkness; the other corresponding lines were kept under normal light conditions. In each of the four crosses [wild (E) × lin, wild (I) × lin, wild (O) × lin, wild (U) × lin], the effect of one of the four autosomes is tested. The whole crossing procedure was repeated after 4 to 6 weeks, again with replicas in darkness and in light. All populations were kept in a discrete generation cycle. Table I summarizes the genetic differences between marked wild-type strains and the lin strain.

In order to determine the allozyme frequencies in the following generations, a sample of 100 flies was taken from generations F_2, F_3, F_5, F_7, and F_{10} of each of the lines and electrophoresed. In generation F_{10} an additional cytological investigation was performed using 50 larvae from those lines that survived (two were unfortunately lost), and the frequencies of the chromosomal structures were determined. A special insemination test was carried out in order to study whether a correlation exists between the actual frequency of a marked "wild" chromosome in the population and the ability to copulate in the dark. A sample of 100 virgin females was taken from each of the F_2 generations (i.e., before the lines were divided into the two lines in light and dark) and the F_7 generations (i.e., five generations of selection for the dark-kept sublines). These virgin flies, aged for 7 days, were then added to the same number of males out of the same line, put into small vials of 15 cm^3 (each containing 10 pairs), and kept together in complete darkness for 7 days. After this period the females were dissected and checked for motile sperm in their seminal receptacles.

RESULTS

The results of the insemination tests performed in the dark (see Table II) show highly significant differences between the dark and the light

Table II. Average Frequencies (%) of Inseminated Females Before Selection Started (F_2) and After Five Generations (F_7) of Selection (i.e., Sublines Kept in Dark) Compared to Those of the Corresponding Unselected Sublines (i.e., Kept in Light) (N = 100; from both replicas)

	Original cross											
	Wild (E) × lin			Wild (I) × lin			Wild (O) × lin			Wild (U) × lin		
Sublines in:	Dark		Light	Dark		Light	Dark		Light	Dark		Light
F_2	3.6		3.6	10.3		10.3	4.4		4.4	4.6		4.6
	***			*		**	***			***		***
F_7	30.9	***	5.2	17.6	***	1.5	21.9	***	4.0	29.2	***	0.0

* Statistically significant at the 0.05 level.
** Statistically significant at the 0.01 level.
*** Statistically significant at the 0.001 level.

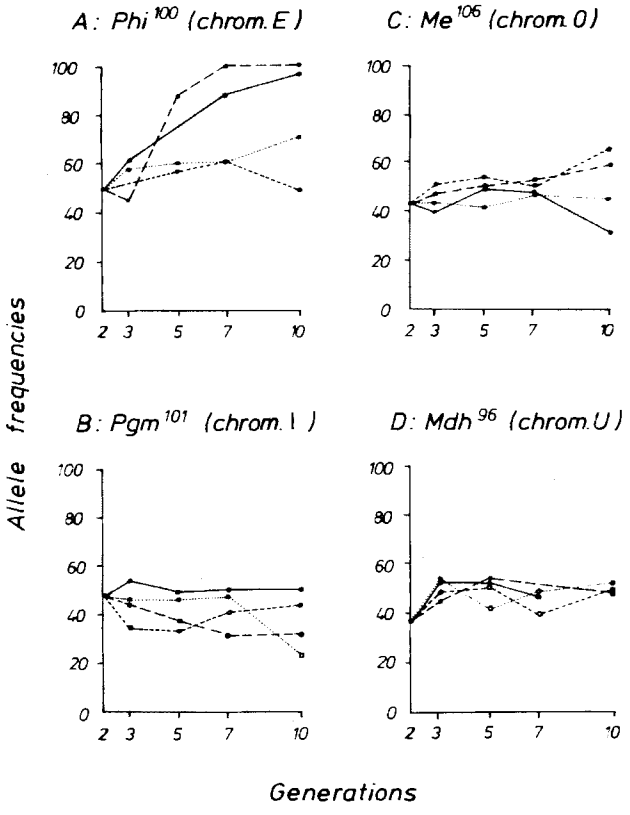

Fig. 1. Frequencies of those enzyme alleles which mark the four autosomes of the lin stain. Data come from the following intercrosses: (A) wild (E) × lin; (B) wild (I) × lin; (C) wild (O) × lin; (D) wild (U) × lin = D. (●——●, ●– – –●) Sublines kept in darkness; (○– – –○, ○·····○) sublines kept in light.

sublines in generation F_7, in all four experiments. There is an average of 24.4% inseminated females in the dark sublines, compared to only 2.6% inseminated females in the light sublines. This indicates a rather strong response to the selection after the short period of five generations.

Figure 1 shows the allele frequency changes of the main allozyme markers of the different autosomes: Phi (on chromosome E), Pgm (on chromosome I), Me (on chromosome O), and Mdh (on chromosome U). The only experiments in which a statistically highly significant difference between the light and the dark sublines appears is that in which the sublines come from the intercross wild (E) × lin. Starting with a frequency of 50% for *Phi*^{100} (on chromosome E of the lin strain), an increase to 100

Table III. Frequencies (%) of the Nonstandard Gene Arrangements in F_{10} in the Various Sublines (L, Light; D, Dark)[a]

Chromosome	D_1	D_2	L_1	L_2
Wild (E) × lin				
E	0.0***	—	41.7	48.0
			***	
I	46.7	—	62.5	88.0***
O	30.0*	—	20.8**	48.0
U	40.0	—	50.0	44.0
N	30		24	25
Wild (I) × lin				
O	26.9*	57.7	57.7	15.4***
N	26	26	26	26
Wild (O) × lin				
I	39.1	35.7	4.2***	26.9
O	17.4**	21.4**	20.8**	38.5
N	23	28	24	26
Wild (U) × lin				
E	—	9.8***	32.1	41.7
			**	
I	—	68.3*	71.4*	70.8*
O	—	12.2***	25.0**	66.7
U	—	61.0	42.9	70.8*
N		41	28	24

[a] The standard gene arrangements come from the lin strain.

* Deviation from 50% frequency or between samples statistically significant at the 0.05 level.

** Deviation from 50% frequency or between samples statistically significant at the 0.01 level.

*** Deviation from 50% frequency or between samples statistically significant at the 0.001 level.

and 96%, respectively, in the replicate sublines kept in dark was observed, whereas in the light sublines only a slight increase in the Phi^{100} frequency, to about 60%, appeared. The difference is statistically highly significant ($\chi^2 = 97.64$ in F_7 and $\chi^2 = 119.8$ in F_{10} at the 0.001 level). This is an indication that chromosome E might carry a major factor for the behavioral trait "light independence" of mating, linked rather tightly to the *Phi* locus. The karyological tests confirm this supposition. Table III gives the frequencies of some of the gene arrangements in the various lines. The frequencies given are those of the nonstandard gene arrangements, coming always from the wild-type strains and hence carrying the alleles for light dependence. The statistical analysis tests either the differences between the samples or the deviation from the frequency 50% at which all

the lines were started. The only exception is in the experiment wild (O) × lin, where the original frequency of the nonstandard gene arrangement of chromosome I was only 29% in the initial generation. In the cross wild (E) × lin, the frequencies of the nonstandard gene arrangement of chromosome E went to zero in the subline kept in darkness, whereas no significant change could be detected in the sublines kept in light. The same phenomenon appeared in the cross wild (U) × lin. In this case, however, the nonstandard chromosome E did not disappear completely in the subline kept in darkness but decreased to a frequency somewhat below 10%.

CONCLUSIONS AND DISCUSSION

The working hypothesis was as follows: the frequencies of the chromosomes derived from the lin strain or from the wild-type strains, respectively, should change during the investigation period in such a way that a chromosome which carries the crucial genes for light independence should increase in frequency in the sublines kept in dark, the same chromosome should not change its frequency in the same direction in the corresponding control sublines kept in light. The sublines kept in complete darkness had been exposed to a strong selection pressure for mating ability in the dark, whereas the sublines kept in light were not exposed to this specific selection. A positive selection response of the dark sublines should become directly visible through an increase in the insemination rate after a few generations. It can be excluded a priori that enzyme alleles or gene arrangements do directly influence the mating ability in darkness. They are considered only as useful markers. The other markers used are chromosomal inversions. They prevent crossing-over when heterozygous in the inverted regions. If they happen to include genes for "light independence," these might be hitchhiking in the dark selected sublines. Yet inversions in *D. subobscura* are frequently heterotic (Sperlich, 1959) and are hence also subject to unspecific (i.e., not connected with light dependence of the mating) natural selection.

The results of the insemination test (Table II) prove that a directional selection for mating ability in the dark was successful in all dark sublines. Previous experiments by Pinsker and Doschek (1980) have shown that F_1 males can copulate at a very low rate (0.8%) in the dark, indicating some dominant effect of some of the lin genes. In another experiment by Andjelković and Marinković (1983) the copulation rate of the F_1 hybrids was 0.5–4.8%. No F_1 test was performed in the present experiment in order to prevent too strong a bottleneck effect. However, it is interesting that the insemination rate of the light lines went down from about 6% in

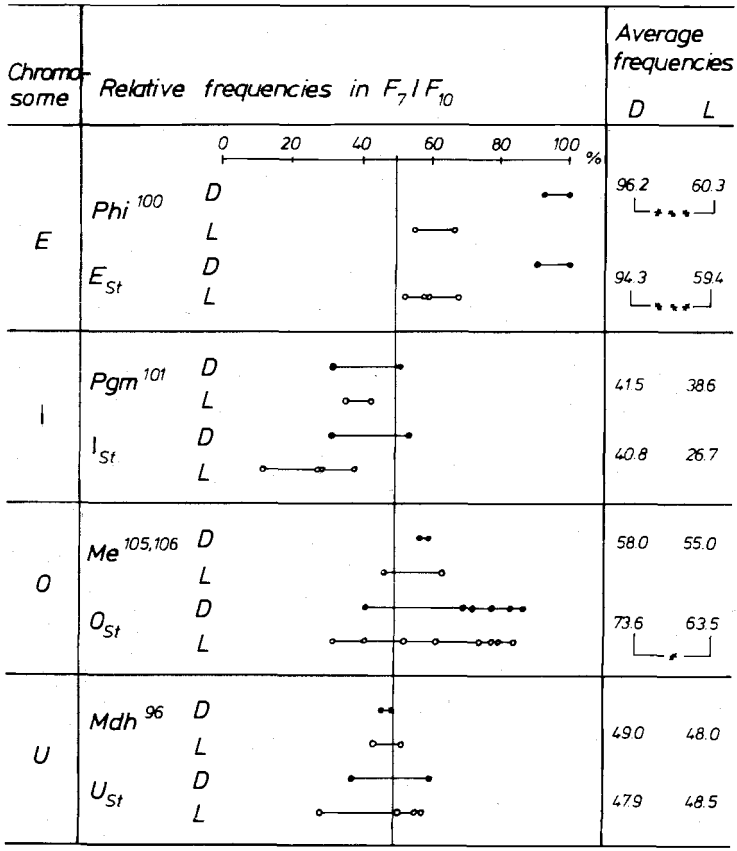

Fig. 2. Average frequencies (percentage) (in generations F_7 and F_{10}) of the "lin alleles" in the sublines in darkness and light, respectively (the frequencies in generation F_2 were 50%), and the frequencies (percentage) of the "lin gene arrangements" (always standard structures) in generation F_{10} in the dark and light sublines. Each point in the figure gives the data from one subline (D) in dark (●) and one subline (∠) in light (○). Since the original frequency of I_{St} was *not* 50% in the cross wild (O) × lin, the corresponding data were omitted from the graph. (*, ***) Statistically significant at the 0.05 and 0.001 levels, respectively.

F_2 to 2%, on the average, in F_7. An interpretation for this decrease could be that there is some natural selection against the alleles for mating ability in the dark. Yet under the dark condition this natural selection is not strong enough to overcome the directional selection pressure.

The results of the allozyme and chromosomal tests are summarized in Fig. 2. The only clear frequency changes appear in chromosome E. The allele *Phi*^{100} from the lin strain increases in frequency significantly

in the dark sublines but only slightly in the light sublines. The difference between the dark and the light sublines is statistically highly significant. The results for the gene arrangements of chromosome E are in general accordance. The standard gene arrangements increase in frequency in the dark subline, but similar to *Phi*^{100}, a much weaker increase appears in the light subline. The difference between the light and the dark conditions is again statistically significant. Both markers indicate that pairing in the dark might depend on the presence of the lin E chromosome, which carries either one major factor for light independence or an important group of polygenes. The latter could appear to act as a single "major" factor since the gene arrangements used as markers in the experiment are inversion complexes ($E_{1+2+9+12}$ and $E_{1+2-9+4}$, respectively) including more than half of the regions of the cytological map of the chromosome. The results for the other autosomes are less clear. An increase in frequency is observed for the lin alleles *Me*^{106} and *Me*^{105} of chromosome O; the gene arrangements from the lin strain behave in like fashion. Yet the changes are very similar in the light and dark sublines. In chromosomes I and U no remarkable frequency changes occurred, either for the enzyme alleles or for the inversions. These findings do not allow final conclusions for chromosome I since the inversion on that chromosome is rather small. In chromosome U, however, the gene arrangements used differ from each other by a rather long inversion complex (U_{St} vs. U_{1+2+8}), covering about two-thirds of the cytological map. The assumption that there are no important polygenes for light independence on chromosome U seems justified.

It is not now certain whether the assumption of Pinsker and Doschek (1980) that the behavioral trait light independence is determined by a polygenic system or whether a single factor on chromosome E is responsible. The latter assumption would be in accordance with results of experiments with other *Drosophila* species. Grossfield (1966, 1970) suggested a single autosomal gene responsible for the light independence of courtship patterns of *D. palustris* and *D. auraria*. The mode of inheritance proved to be autosomal recessive.

Assuming that a single recessive gene (or a supergene) regulates the behavioral trait, one would expect that 25% of F_2 males and females should be homozygous for the lin allele and hence capable of copulation in darkness. As can be seen from Table II, about 4–10% of the females were inseminated in the insemination test using F_2 flies. From previous experiments (Aldinger-von Kleist, 1982) with the same conditions, pure lin flies have an insemination rate of 71% of the females. Taking into account that mating ability in the dark is dependent on male and female

genotype, an insemination rate of 4–10% does not disprove the single-gene hypothesis.

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