Light dependent mating inhibition in the white-eye mutant of *Drosophila pseudoobscura*

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Summary. White-eye *Drosophila pseudoobscura* males display a deficiency in their mating ability in the light, although they are able to mate readily in the dark. The present data suggest that the mating deficit is due to a neurobehavioral disruption produced by faulty visual input.

Drosophila species exhibit different degrees of light dependency in their mating behavior. Grossfield[1] contends that *Drosophila* species which mate equally well in the light or in the dark are able to adjust their behavior, especially their mating behavior, in order to adapt to varying environments. Both *Drosophila melanogaster* and *Drosophila pseudoobscura* are widely distributed species with such light independent mating behavior.

Mating behavior in the light is disrupted in pigment-deficient *D. melanogaster* mutants. Connolly et al.[2] found that white-eye *D. melanogaster* males exhibit a significant decrease in the mean bout length of wing vibration during courtship under lighted conditions. Previous investigations showed that eye pigment-deficient mutant flies in *D. melanogaster* display decreased abilities in pattern contrast for both movement and form perception[3]. This suggests that the impaired contrast perception of the white-eye males may prevent these males from establishing contact with the females. Indeed, Connolly et al.[2] hypothesized that the female receives less stimulation from the white-eye male so that longer courtship time is needed for copulation to occur.

We found that white-eye *D. pseudoobscura* males also show a decrease in their mating ability under lighted conditions. 3- and 4-day-old virgin white-eye Arrowhead double mutants (*or, pr;* homozygous on the 3rd chromosome for both *orange* and *prune*) were allowed to mate under the following conditions. The mating groups consisted of 20 mutant females exposed to either 20 mutant males or 20 wild-type males, and 20 wild-type females exposed to either 20 mutant males or 20 wild-type males. Two mating conditions were used: 1. total darkness, or 2. light, intensity = 0.48/100 footcandles. All matings ran for 2 h, after the appropriate dark adaptation (12 h) or light adaptation (2 h). Presence of larvae from individual females indicated a mating.

It is clear from table 1 that white-eye Arrowhead males mate at a drastically reduced frequency in the light, although there is no mating disruption in darkness within the 2 h interval. Since the mutant males are as successful in mating in the dark as the wild-type males, visual input is not essential for courtship and copulation in this species. The decreased success of the mutant males in the light suggests that light has a disruptive effect on the behavior of these mutant flies.

Further support for this hypothesis comes from competition studies. When either groups of 20 white-eye females or 20 wild-type females were given a mating choice between 10 white-eye males or 10 wild-type males, both types of females mated exclusively with wild-type males under lighted conditions (table 2). Yet, when both types of females were given the same mating choice in the dark, there was no significant difference in their mating choices. As before, a 2-h interval was employed. Clearly, these white-eye males display a markedly decreased mating success relative to the wild-type only in lighted conditions.

We have found similar results in race A of *Drosophila aurauria.* Males homozygous for the *white saffron* mutation did not mate in the light, even if they were exposed to females for 24 h[4]. The light-inhibited mating behavior of both white-eye Arrowhead *D. pseudoobscura* males and *white saffron D. aurauria* males suggests that some aspect of sensory and/or motor integration necessary for mating performance is affected by the presence of illumination. Our observations of the courtship of white-eye *D. pseudoobscura* males indicates that the orientation component of the mating sequence is inhibited by light. The light may function in either of 2 ways. First, the light may directly inhibit some motor ability that is needed for male orientation. On the other hand, a loss of visual acuity in these white-eye mutants may interfere with the sensory-motor integration necessary for maintaining orientation. In either case, faulty visual information disrupts the male's courtship pattern, although no visual input (darkness) does not. This agrees with Connolly et al.'s[2] conclusion that light may disrupt a feedback mechanism in *D. melanogaster* males.

Neither white-eye Arrowhead *D. pseudoobscura* nor *white saffron D. aurauria* females are inhibited in their mating behavior. Clearly, these pigment-deficient *Drosophila*

Table 1. The median proportion of total matings in *Drosophila pseudoobscura* Arrowhead mating groups

Visual condition	Female	Male	Median	Range Minimum	Maximum
Dark	*or,pr*	*or,pr*	0.85	0.70	1.00
Dark	*or,pr*	+ +	0.87	0.70	0.95
Dark	+ +	*or,pr*	0.83	0.76	0.90
Dark	+ +	+ +	0.88	0.75	0.95
Light	*or,pr*	*or,pr*	0.02	0.00	0.37
Light	*or,pr*	+ +	0.89	0.72	0.96
Light	+ +	*or,pr*	0.26	0.09	0.30
Light	+ +	+ +	0.86	0.71	1.00

2 genotypes, *or,pr* and + +, and two visual conditions, light and dark, were used.

Table 2. The total number of matings in *Drosophila pseudoobscura* Arrowhead under conditions of competition

Visual condition	Female	*or,pr* Male	+ + Male	N	df	χ^2
Dark	*or,pr*	38	31	69	Total 4	2.93
					Pooled 1	0.71
					Heterogeneity 3	2.22
Dark	+ +	32	36	68	Total 4	1.28
					Pooled 1	0.24
					Heterogeneity 3	1.04
Light	*or,pr*	0	64	64	Total 4	64.00**
					Pooled 1	64.00**
					Heterogeneity 3	0.00
Light	+ +	0	58	58	Total 4	58.00**
					Pooled 1	58.00**
					Heterogeneity 3	0.00

$p < 0.05$; ** $p < 0.01$.

females do not experience any sensory or motor deficiency in the light that disrupts their mating behavior. Previous studies in *D. melanogaster* report that both male and female pigment-deficient flies exhibit a deficit in their optomotor response. Therefore, pigment-deficient *Drosophila* females are most likely not affected by light in their mating behavior due to their passive role in courtship activity.[5]
The light dependent disruption in mating behavior of both white-eye *D. pseudoobscura* and *D. aurauria* males is extreme when compared to the light-dependent disruption reported for *D. melanogaster*. Nevertheless, the inhibitory response shown by white-eye Arrowhead *D. pseudoobscura* males in the light may also be relevant in previous studies using pigment-deficient *D. melanogaster* mutants. If so, the contrast perception deficiency exhibited by *D. melanogaster* mutants in the light might be due to a neurobehavioral disruption produced by faulty visual input.

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Insulin can completely replace serum in *Drosophila melanogaster* cell cultures in vitro

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Summary. D225 medium for *Drosophila* cell lines contains some chemically undefined compounds. An attempt was made to replace them with chemically defined substances. It was revealed that insulin can replace foetal calf serum in supporting *D. melanogaster* cell growth.

D225 medium[2] used for *Drosophila* cell lines contains some chemically undefined compounds: lactalbumin hydrolysate, yeast extract and foetal calf serum. Their presence in the medium causes difficulties in many physiological and genetical studies. Therefore efforts have been made to replace them with chemically defined substances.
Lactalbumin hydrolysate supplies amino acids. Wyss and Bachmann[3] found that 2 *D. melanogaster* lines were able to grow in D22 medium in which lactalbumin hydrolysate had been replaced by a synthetic amino acid mixture.
Yeast extract is a source of B-complex vitamins and purines. Replacing this compound with either adenosine or inosine, Marunouchi and Miyake[4] obtained growth of 2 lines of *D. melanogaster*. It is believed that the main role of serum, present in all cell culture media, is to provide hormones. In fact, when a mixture of hormones replaces serum in medium some mammalian cell lines grow at the same rate as in serum-supplemented medium[5,6].

This report describes an attempt to develop a completely synthetic medium, taking advantage of the findings of earlier works. 7 kinds of media were prepared (table 1): media 1,2 and 3 in which yeast extract (YE), lactalbumin hydrolysate (LH) and foetal calf serum (FCS) were replaced respectively by inosine, an amino acid mixture and insulin; media 4, 5 and 6 in which 2 of the 3 chemically undefined substances were replaced and medium 7 which was completely synthetic.
Inosine was used at a concentration of 0.003 g/l. The amino acid mixture was the same as that used by Wyss and Bachmann in ZD medium[3]. Insulin crystalline bovine (BDH, approximately 25 IU/mg, Zn 0.33%, SO_3 1.5%) was used at a concentration of 0.001 g/l.
These media were tested on 4 *D. melanogaster* established cell lines of embryonic origin: 1XII, 0.57, 1.56, C1 82, the last kindly provided by Prof. G. Echalier, Paris. The others were obtained from the wild stock Varese of *D. melanogas-*

Table 1. Culture media tested for their ability to support growth of *D. melanogaster* cell lines in vitro

Medium D225[a]	Medium 1	Medium 2	Medium 3	Medium 4	Medium 5	Medium 6	Medium 7
YE[b] 1.3 g/l	Inosine 0.003 g/l	YE[b] 1.3 g/l	YE[b] 1.3 g/l	Inosine 0.003 g/l	Inosine 0.003 g/l	YE[b] 1.3 g/l	Inosine 0.003 g/l
LH[c] 13 g/l	LH[c] 13 g/l	Aa mixture	LH[c] 13 g/l	Aa mixture	LH[c] 13 g/l	Aa mixture	Aa mixture
FCS[d] 15%	FCS[d] 15%	FCS[d] 15%	Insulin 0.001 g/l	FCS[d] 15%	Insulin 0.001 g/l	Insulin 0.001 g/l	Insulin 0.001 g/l

[a] Standard medium used for *D. melanogaster* cell lines in vitro. [b] yeast extract; [c] lactalbumin hydrolysate; [d] foetal calf serum.

Table 2. Growth of the 4 *D. melanogaster* lines in the different media tested

Cell lines	Medium 1	Medium 2	Medium 3	Medium 4	Medium 5	Medium 6	Medium 7
1XII	Standard	None	Standard	Slow	Slow	Slow	None
1.56	Standard	None	Standard	Slow	Slow	Slow	None
0.57	Standard	None	Standard	Slow	Slow	Slow	None
C182	Standard	None	Standard	Slow	Slow	Slow	None