A visual stimulus in the courtship of *Drosophila suzukii*

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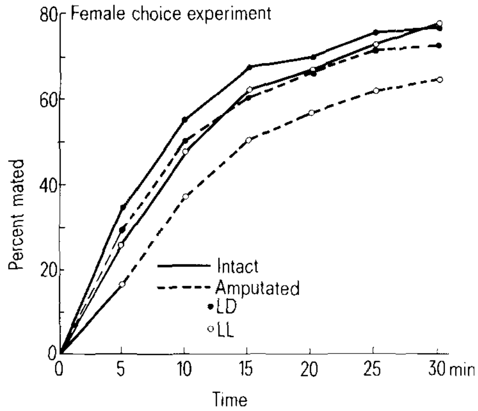
Summary. The males of *Drosophila suzukii* have a specific black spot on their wings and this was surgically removed. In this situation, the males were almost normally accepted by the females when they were stored under a diurnal light-dark cycle, but they were somewhat discriminated against by the females previously exposed to continuous light.

Various physical and chemical stimuli are known to be involved in the courtship behavior of *Drosophila*[1]. Nevertheless, with the exception of an acoustic stimulus recently reported[2], there has been no direct evidence as to what stimulus is received by a courtee. Many *Drosophila* species require light for their mating[3], and in those species it was believed that the visual stimuli played an important role for their courtship. The species which showed a remarkable sexual dimorphism seemed as a rule to belong to the light-dependent group. Such a species is *Drosophila suzukii*, where the male fly has a prominent black spot on the tip of its wings, while the female fly lacks the black spot, and they are known never to mate in the dark[4]. The courting male widely extends its wings in front of the female as if it were displaying its black spots[4]. This suggests that the wing spot may provide a visual stimulus which promotes female sexual receptivity, and/or provide a cue signal for mate recognition. This paper reports the mating activity of the males of *D. suzukii* whose wing spots were surgically removed.

Methods. A Tokyo strain, that was derived from several wild caught females, was used in this survey. Flies were reared at 20 °C on corn meal-molasses-agar medium. After eclosion the flies were subjected, unless otherwise stated, to 12:12 h light-dark cycles until the mating experiments started. The distal half of the male wing was clipped on the 2nd day of emergence under light etherization, and the wing spots were completely removed. Mating propensity was observed in a glass vial (3× 11 cm) at 25 °C for 3-7 h after the light was turned on. 10 pairs of 5-6-day-old flies were introduced into each vial without etherization and were allowed to mate for 30 min. When mating occurred, time was recorded and the copulated pairs were aspirated out. In a no-choice experiment, the females were put together with either amputated or intact males. In a female choice experiment, the females were given a choice between 5 amputated males and 5 intact males. For each experiment, the tests were made on 7 or 8 occasions and the data was pooled.

Results and discussion. When the males mated with the females which had been kept under 12 h light: 12 h dark, the spotless males were very slightly handicapped in their mating ability as compared with intact males in either the no-choice contest or the female choice contest (the left side of the table). Therefore, wing amputation per se seems to have little adverse effects as shown in *D. melanogaster*[5], suggesting that the acoustic stimulus produced by the wing vibration is not important in the courtship of this species. This agrees with the observation that *D. suzukii* male does not vibrate his wings during the course of courtship. It also shows that the visual stimulus made by the wing spot is unlikely to be involved in the sexual isolating mechanisms, since the females almost equally accepted the males lacking it.

It was thus postulated that the wing spots may increase the receptivity of the female when she is sexually less excited. Non-virgin females were tested but no conclusive results were found, because they were quite reluctant to remate even in a week after the 1st mating. Another possible



Mating speed of the wing-amputated and intact males of *Drosophila suzukii* that were mated under female choice condition with the females stored under either the diurnal light/dark cycle or the continuous light. Mating was observed for 30 min in a glass vial (3× 11 cm) into which 10 virgin females, 5 amputated males, and 5 intact males were introduced. For each experiment, the data of 20-24 replicate tests were pooled.

The effect of wing-amputation on the mating propensity of *Drosophila suzukii* male

	Females stored under LD		Females stored under LL	
	Intact	Amputated	Intact	Amputated
No-choice experiment				
No. of males tested	200	200	210	210
No. of males mated within 30 min	131	127	150	130
Percent mated	65.5	63.5	71.4	61.9
	$\chi^2=0.17$		$\chi^2=4.29^*$	
Average time in min to copulation ± SE	10.00 ± 0.63	10.43 ± 0.64	9.80 ± 0.61	10.36 ± 0.66
Female choice experiment				
No. of males tested	200	200	240	240
No. of males mated within 30 min	154	145	187	155
Percent mated	77.0	72.5	77.9	64.6
	$\chi^2=1.07$		$\chi^2=10.41^{**}$	
Average time in min to copulation ± SE	8.31 ± 0.54	8.74 ± 0.53	10.41 ± 0.55	10.93 ± 0.57

* $p < 0.05$, ** $p < 0.01$.

condition which may reduce the sexual receptivity of females is to subject them to continuous light[6]. Such females showed a slight discrimination against the spotless males in the no-choice experiment ($p < 0.05$), though there were no significant differences in the average time of copulation. More significant reduction in the mating success of the amputated males as compared with the intact males was found with the female choice experiment ($p < 0.01$). Again, the spotless males mated as quickly as normal males did. The time course of the female choice experiment is graphically presented in the figure.

These results indicate that the visual stimulus produced by the male wing spots of *D. suzukii* interact with many other stimuli to enhance the sexual receptivity of females, which was generally shown in the courtship stimuli of *Drosophila*[7]. The effect of deprivation of this particular stimulus could be detected only when the female's threshold of receptivity was high. It may also be suggested that the male's specific character of the black spot has been evolved and maintained for their sexual selection.

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Gibberellins and the break of bud dormancy in virus-infected stem cuttings of *Euphorbia pulcherrima*

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Summary. Break in bud dormancy in virus-infected stem cuttings of *Euphorbia pulcherrima* occurs because of the higher quantity of gibberellins present in them than in healthy cuttings in the dormant period of the plant.

During our studies of rooting stem cuttings of virus-infected *E. pulcherrima*, we found that the buds on these cuttings (when planted in sand in earthen pots and periodically irrigated with water) grew into leafy shoots during winter while the buds on identically treated healthy cuttings either remained dormant or grew into cyathia[1]. The virus thus breaks the dormancy of buds in infected stem cuttings. Induction and break in bud dormancy in plants is controlled by an interaction of growth promotors, particularly gibberellins, and the growth inhibitors abscisic acid and phenols[2-5]. The role of growth inhibitors in breaking bud dormancy in virus-infected *E. pulcherrima* has already been reported[5], while the role of gibberellins is reported here.

The conclusion that gibberellins break the dormancy of many woody plants is based on the estimation of endogenous gibberellins at different periods of the year[2,6-8]. Such estimations show that the quantity of gibberellins in plants decreases during their dormant period[2,4,7]. Estimation of endogenous gibberellins was done twice a year to see if gibberellins play some part in breaking bud dormany in *E. pulcherrima.*

Material and method. Gibberellins were extracted from the 150 g of bark of stem cuttings by the method of West and Phinney[9] and MacMillan et al.[10]. The residue so obtained was dissolved in 10 ml distilled water which was then employed for the dwarf pea seedling bioassay according to the method of Radley[11] and Radley and Dear[12]. The increase in shoot length of 20 pea seedlings was measured from the cotyledonary node to the uppermost node after seven days of treatment. The gibberellin activity is expressed in percentage increase in shoot length of pea seedlings over the control (untreated pea seedlings) and is given in the table.

Results and discussion. All the water-treated diseased cuttings showed bud sprouting while buds on water-treated healthy cuttings either remained dormant or rarely grew into cyathia.

Dwarf pea seedling bioassay for the estimation of gibberellin-like activity in the purified extracts of healthy and diseased stem tissues of *E. pulcherrima*

Month	Treatment	Percentage average shoot length (in cm) of the pea seedlings after 7 days	Percentage increase in shoot length as compared to control (water)	Percentage increase (+) or decrease (−) in shoot length of pea seedlings on application of diseased extract with respect to healthy extract
	Control (H_2O)	5.42 ± 0.1559*	-	-
May (growing period)	H-gibberellin extract	11.36 ± 0.3000*	109.59	-
	D-gibberellin extract	8.84 ± 0.1163*	63.09	22.18 (−)
December (dormant period)	H-gibberellin extract	5.72 ± 0.2234*	5.53	-
	D-gibberellin extract	6.50 ± 0.1225*	19.92	13.63 (+)
	Standards			
	0.01 ppm GA_3	5.50 ± 0.177*	1.47	-
	0.1 ppm GA_3	6.72 ± 0.214*	23.98	-
	1 ppm GA_3	7.50 ± 0.066*	38.37	-
	10 ppm GA_3	11.50 ± 0.177*	112.17	-

H = The gibberellin extract of healthy stem tissue. D = The gibberellin extract of diseased stem tissue. * = SE.