

BEHAVIORAL RESPONSES OF *Drosophila melanogaster* AND *Drosophila simulans* ADULTS TO CONSPECIFICS

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ABSTRACT

Adults of *Drosophila melanogaster* and *D. simulans* transmit and receive signals at some distance from their conspecifics. The behavioral responses differ according to species, sex and physiological state of the flies. Non-virgin *D. melanogaster* females increase their locomotor activity when male and female signals are received, without discriminating between the sexes. Non-virgin males of *D. melanogaster* do not increase dispersal in response to signals emitted by conspecifics, but they may avoid non-virgin females. Virgin and non-virgin flies of *D. melanogaster* move towards conspecifics when they detected flies of the sibling species *D. simulans*. However, virgin and non-virgin males and females of *D. simulans* are unable to discriminate the presence of physically distant conspecifics. It is argued that the substances referred to as sex pheromones may also serve to recognize physically distant conspecifics of *Drosophila*.

INTRODUCTION

Drosophila species show a diversity of courtship behaviors (Spieth and Ringo, 1983). In *Drosophila melanogaster*, wing vibration by the male is an important element in stimulating the female. Females receive and discriminate these signals via their antennae (Burnet and Connolly, 1974). It is now also known that chemosignals are of importance for courtship in *D. melanogaster*, and it is believed that the *dxc* locus

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regulate genes that control sex pheromone synthesis produced by the female (Jallon, 1984; Tompkins, 1984; Jallon *et al.*, 1988). Non-sexual communication between conspecifics of *Drosophila* species is less well known (see Grossfield, 1978 for a review). Existence of a male-produced pheromone which acts as a catalyst, increasing the response to food in both males and females of *D. simulans* and *D. melanogaster* has been also reported (Bartelt *et al.*, 1985; Schaner *et al.*, 1987). It is important to investigate the nature and function of sensory cues perceived by adult *Drosophila* in a non-sexual context. Such information would relate to, for example, feeding strategies and selection of oviposition sites as well as search for conspecifics.

A knowledge of non-sexual communication in *Drosophila* could improve our understanding of behavioral adaptations to local environmental heterogeneity. For instance, the mobility of adult *Drosophila* flies in response to specific signals might lead to formation of groups of conspecifics thus limiting direct adult interspecific competition for food. Non-sexual communication in some *Drosophila* species could also have consequences for the distribution and abundance of the sexes in populations (Parsons and Hoffmann, 1985). Variations in sex ratios may modify the mating conditions differentially from one group of individuals in a population to another and this might have consequences for the evolution of a *Drosophila* species.

Navarro and del Solar (1975) found that among *D. melanogaster* adults aggregation increased when the temperature decreased and males and females aggregated together less than each sex alone. Sexton and Stalker (1961) showed that the regularity of spacing patterns of *D. paramelanica* females increased at higher densities. Adult *D. persimilis* and *D. pseudoobscura* flies may return to their area of origin when moved away (Taylor and Powell, 1983). Atkinson and Miller (1980) have reported that *D. subobscura* flies tend to return to the bait on which they were first collected. The behavioral mechanisms and the genetic and environmental factors involved in these aggregations are poorly understood. Males of *D. melanogaster* and *D. simulans* are attracted to trace odours from homotypic females (Parsons and Hoffman, 1985); Scott (1986) has demonstrated an inhibitory pheromone associated with *D. melanogaster* males and mated females.

While carrying out investigations in an orchard south of Santiago (Chile), we observed several species of *Drosophila* resting, feeding, courting and copulating on over-ripe fruits. Adult flies of *D. melanogaster*, *D. immigrans*, *D. hydei* and *D. pavani* seemed to aggregate in groups of various sizes on the fruit. These aggregations could reflect microecological differences between the different sites on the same fruit. However, such aggregations might also be related to some kind of signalling system associated with a search for conspecifics.

This is the second of a series of papers devoted to the behavioral mechanisms and the type of signalling employed by *Drosophila* species to aggregate in the wild

(see Godoy-Herrera and Fenner, 1984). This paper demonstrates aggregation of virgin and non-virgin *D. melanogaster* and *D. simulans* flies by comparing dispersal in the presence and in the absence of conspecifics. We have chosen this behavior because it is likely to have consequences for the distribution of groups of flies for feeding, oviposition and the utilization of space.

MATERIAL AND METHODS

Stocks

The *D. melanogaster* stock used was Oregon R-C (wild type). The Quilicura strain of *D. simulans* was also used. This latter strain was formed from adults captured by Budnik and Brncic in Quilicura near Santiago (1983). Flies were maintained by mass culture in half-pint bottles containing about 50 cc of Burdick's medium (1954), at $24 \pm 1^\circ\text{C}$ with a constant light/dark cycle.

Virgin and non-virgin males and females were collected and aged 4-5 days before experiments were performed. Flies were etherised within 4 hours of eclosion and males separated from females. In other bottles males and females were not separated until 72 h after eclosion; these were presumed to be non-virgin. Groups of about 25 individuals of each sex were kept in vials containing culture medium at 24°C up to the time of the experiment.

Apparatus

The apparatus consisted of a one liter conical flask fitted around the base with eight regularly spaced tubes; an 18 cm long Y-shaped tube was connected to each of these. The distal end of each Y-tube was fitted with a cell screened with fine muslin which prevented flies from escaping from the cells, but allowed the passage of air between the cells and the Y-tubes. The mouth of the flask was stoppered with a cotton wool plug to avoid air draughts (see Godoy-Herrera and Fenner, 1984, for further details). The apparatus was washed and dried before each experiment. All experiments were carried out at 24°C under homogeneous lighting conditions.

Dispersal experiments

Table I shows the experimental design used to describe dispersal activities of non-virgin *D. melanogaster* adult flies. In experiment 1, 100 non-virgin males, or females, were introduced into the flask when all the cells were empty and their be-

havior was observed. In experiment 2, 25 non-virgin females (or males) were placed in each of eight cells on one side, while the corresponding cells on the other side were left empty. In experiment 3, groups of 25 non-virgin females were each placed in eight cells on one side, and groups of 25 non-virgin males were each placed in the corresponding cells of the opposite side. Again, 100 non-virgin males, or females, were introduced into the flask. One hour later, when the flies had moved throughout the apparatus, the distribution of individuals in the Y-tubes was recorded.

Table I - Experimental design for the study of the activity of *D. melanogaster* adults in the presence of distant conspecifics.

Experiment	Flies tested				Experimental condition
	Flask containing males		Flask containing females		
1. No fly control	-	-	-	-	Dispersal in the absence of distant conspecifics
2. Choice of the opposite sex or the same sex versus empty cells	-	F (M)	-	F (M)	Dispersal in the presence of distant females (or males)
3. Choice between two sex	M	F	M	F	Dispersal in the presence of distant conspecifics of both sexes

-- = Empty cells; - F (M) = Empty cells on one side, cells with females (or males) on the opposite side; M F = Cells with males on one side, cells with females on the opposite side.

Dispersal of virgin and non-virgin D. melanogaster and D. simulans

A further set of experiments was carried out to investigate dispersal of virgin and non-virgin *D. melanogaster* and *D. simulans* adults in the presence of physically distant conspecifics and flies of the other sibling species. The distal ends of each Y-tube on one side of the apparatus were connected with cells each of which held 25 virgin *D. melanogaster* females and the corresponding cells on the opposite side each

contained 25 virgin *D. simulans* females. One hundred virgin *D. melanogaster* males were placed into the flask. One hour later the number of males in the Y-tubes was recorded. The experiment was repeated by placing 100 virgin *D. simulans* males into the flask. In the second condition, 8 cells on one side each held 25 virgin *D. melanogaster* males and the corresponding cells on the opposite side each contained 25 virgin *D. simulans* males. Again 100 virgin *D. melanogaster* females (or *D. simulans*) were introduced into the flask. After one hour the number of females in the Y-tubes was recorded. This set of experiments was repeated by putting non-virgin males and females of these two sibling species into the flask and in the cells. All experiments were repeated six times.

Statistical procedures

One-way analysis of variance was applied to the data to test for differences between replicates of each of the experiments in Table I. In the absence of significant heterogeneity between replicates they were grouped as shown in Tables II and V. We used the *t*s test for comparing percentages of dispersal (Table II to V; Sokal and Rohlf, 1969). The χ^2 -test of independence was also used to test the goodness of fit of distribution in the Y-tubes of males and females of *D. melanogaster* and *D. simulans* (Tables II to V).

RESULTS

An analysis of variance for homogeneity between replicates of the experiments in Table I showed F (females) = 4.23; $P > 0.05$, df 3;28; F (males) = 4.02; $P > 0.05$, df 3;28. Thus, it seems appropriate to group replicate experiments as shown in Tables II to V.

The activity of non-virgin *D. melanogaster* females under the various conditions is summarized in Table II. The presence of conspecifics in the cells enhanced female activity as reflected by the percentage of females found in the Y-tubes. Female dispersal is greater when the cells contain non-virgin males.

Table II also shows the distribution of the non-virgin females in the Y-tubes. Experiment I shows that when the cells were empty, females were distributed at random in the tubes. Females confronted with a choice between: (i) cells with flies of the same sex versus the opposite sex; (ii) the same sex versus empty cells; (iii) the opposite sex versus empty vials; were distributed at random among the tubes. The χ^2 -values for distribution in the Y-tubes ranged from 0.03 (experiment 1) to 2.81 (experiment 4); $P > 0.05$, $df = 1$.

Table III summarizes, the dispersal activities of non-virgin males of *D. melanogaster* under several environmental conditions. It can be seen that the presence of conspecifics in the cells does not increase the percentage of males in the Y-tubes. Random distribution was also obtained in the Y-tubes when the cells were empty or when the non-virgin males made a choice between empty cells and cells containing flies of the same sex (experiments 1 and 2). In contrast, when the non-virgin males made a choice between empty cells and cells containing non-virgin females, or between cells with males and cells with females they moved preferentially away from the cells with non-virgin females (χ^2 -values ranged between 4.53 and 7.58; $P < 0.05$, $df = 1$).

Table II - Number and percentage of non-virgin *D. melanogaster* females (the Oregon R-C strain) in the flask and Y-tubes, and their distribution in the Y-tubes in each experiment. Recorded females stayed in the apparatus for 1 h (see Materials and Methods).

Experiment	Replicates	N females remaining	%	N females in the Y-tubes	%	χ^2 -test for distribution in the Y-tubes ($df = 1$)
Flies Cells tested		Y-tubes		on each side		
1 F -	4	31	16.95	15	48.40	0.03 ns
-				16	51.60	
2 F M	4	84	27.28*	42	50.60	0.00 ns
-				42	50.60	
3 F F	4	58	22.38*	20	35.70	0.70 ns
-				36	64.30	
4 F M	4	81	26.78*	33	59.30	2.81 ns
F				48	40.70	

* = ts (for comparison of two percentages) refers to the empty cells experiment, $P < 0.05$; F = Flask with females; -- = Empty cells; - M = Empty cells on one side, cells with males on the opposite side; - F = Empty cells on one side, cells with females on the opposite side; M F = Cells with males on one side, cells with females on the opposite side.

Table III - Number and percentage of *D. melanogaster* males (the Oregon R-C strain) in the flask and Y-tubes, and their distribution in the apparatus for 1 h (see Materials and Methods).

Experiment	Replicates	N males remaining in the flask	%	N females found in the Y-tubes	%	N males in the Y-tubes on each side	%	χ^2 -test for distribution in the Y-tubes (df = 1)	
1 M	-	5	436	69.09	64	20.96	31	18.40	0.04
	-						33	51.60	
2 M	M	5	414	65.50	86	24.50*	41	47.70	0.18
	-						45	52.30	
3 M	F	4	356	70.63	44	19.37*	31	29.50	7.58**
	-						13	70.50	
4 M	M	4	356	70.63	44	19.37*	15	65.90	4.53**
	F						29	34.10	

* = Non-significant differences with respect to the experiment with empty cells (*t*s test for comparison of two percentages was used); ** = $P < 0.05$; M = Flask with males; - - = Empty cells; - M = Empty cells on one side, cells with males on the opposite side; - F = Empty cells on one side, with females on the opposite side; M F = Cells with males on one side, cells with females on the opposite side.

Table IV shows the percentage of females (virgin and non-virgin) of *D. melanogaster* and *D. simulans* found in the Y-tubes when offered a choice between cells which held conspecifics of the opposite sex and cells with flies of the opposite sex but from the sibling species. Table IV indicates that virgin and non-virgin females of *D. melanogaster* moved preferentially towards their conspecifics of the opposite sex. In contrast, virgin and non-virgin females of *D. simulans* were distributed at random under the same experimental conditions. Table IV also show that the presence of conspecifics of the opposite sex in the cells increase the percentage of *D. melanogaster* females in the Y-tubes. This is in agreement with Table II. The presence in the cells of conspecifics of the opposite sex does not increase dispersal of *D. simulans* females.

Table IV - Number and percentage of virgin and non-virgin *D. melanogaster* and *D. simulans* females in the flask and Y-tubes and their distribution in the Y-tubes in each experiment (see Materials and Methods).

Experiment and species tested	Cells	Replicates	N females remaining in the flask	% N females found in the Y-tubes	N females in the Y-tubes on each side	%	χ^2 -test for distribution in the Y-tubes (df = 1)
1. virgin females							
<i>D. melanogaster</i>							
--	--	6	516	86.00	84	14.00	47.62 0.19 ns
--	--				44		52.38
D.m. (M)		6	448	74.67*	152	25.33	61.84 5.03 P < 0.05
D.s. (M)					58		38.16
<i>D. simulans</i>							
--	--	6	487	81.17	113	18.83	48.60 0.08 ns
--	--				58		51.33
D.m (M)		6	480	80.00	120	20.00	43.33 2.13 ns
D.s (M)					68		56.67
2. non-virgin females							
<i>D. melanogaster</i>							
--	--	6	473	78.83	127	21.17	52.76 0.38 ns
--	--				60		47.24
D.m (M) #		6	427	71.17*	173	28.76	78.61 56.65 P < 0.05
D.s (M) #					37		21.40
<i>D. simulans</i>							
--	--	6	506	84.33	94	15.67	46.81 0.38 ns
--	--				50		53.19
D.m (M) #		6	489	81.50	111	18.50	46.85 0.44 ns
D.s (M) #					59		53.15

* is (for comparison of two percentages) refers to the empty cell experiment; -- = empty cells; D.m (M) D.s (M) = cells with virgin *D. simulans* males on one side, cells with virgin *D. simulans* males in the opposite side; D.m (M) # = idem, but cells containing non-virgin males.

Table V - Number and percentage of virgin and non-virgin *D. melanogaster* and *D. simulans* males in the flask and Y-tubes and their distribution in the Y-tubes in each experiment (see Materials and Methods).

Experiment and species tested	Cells	Replicates	N males remaining in the flask	%	N males found in the Y-tubes	%	N females in the Y-tubes on each side	%	χ^2 -test for distribution in the Y-tubes (df = 1)
1. virgin males									
<i>D. melanogaster</i>									
--	--	6	499	83.17	101	16.83	50	49.50	0.00 ns
--	--						51	50.50	
	D.m (F)	6	505	84.17	95	15.83	65	68.42	12.89 P < 0.5
	D.s (F)						30	31.58	
<i>D. simulans</i>									
--	--	6	501	83.50	99	16.50	47	47.47	0.25 ns
--	--						52	52.52	
	D.m (F)	6	497	82.83	103	17.17	54	52.43	0.24 ns
	D.s (F)						49	49.57	
2. non-virgin males									
<i>D. melanogaster</i>									
--	--	6	488	84.50	112	18.67	53	47.32	0.32 ns
--	--						59	52.68	
	D.m (F) #	6	462	77.00	138	23.00	110	79.71	48.72 P < 0.05
	D.s (F) #						28	20.29	
<i>D. simulans</i>									
--	--	6	495	75.00	105	17.50	47	44.76	1.15 ns
--	--						58	55.24	
	D.m (F) #	6	488	81.33	112	18.67	57	50.89	0.03 ns
	D.s (F) #						55	49.10	
							59	53.15	

-- = Empty cells; D.m (F) D.s (F) = cells with virgin *D. melanogaster* females on one side, cells with virgin *D. melanogaster* females in the opposite side; D.m (M) # D.s (M) # = idem, but cells containing non-virgin females.

Table V shows the percentage of males (virgin and non-virgin) of *D. melanogaster* and *D. simulans* found in the Y-tubes when the cells contain females of these two sibling species. As the females (see Table IV), *D. melanogaster* males (virgin and non-virgin) prefer to move towards their conspecifics of the opposite sex. *D. simulans* males (virgin and non-virgin) are distributed at random in the Y-tubes when offered a choice between *D. melanogaster* females and *D. simulans* females.

DISCUSSION

Our observations reveal that, under laboratory conditions, the activity of non-virgin *D. melanogaster* females is enhanced by signals from physically distant conspecifics. It is interesting to note that the dispersal response of females to males was more intense than to conspecifics of the same sex. However, the females were distributed at random in the Y-tubes when offered a choice between females and males. In contrast, non-virgin *D. melanogaster* males did not increase their dispersal when receiving signals from physically distant conspecifics. However, they moved away from non-virgin females when confronted with a choice between empty cells and cells which held females. Thus, the behavioral response to physically distant conspecifics differs depending on sex. These findings suggest that, in *D. melanogaster*, males may detect physically distant non-virgin females avoiding their presence, and that non-virgin females may also detect physically distant conspecifics not discriminating between the sexes. These results contrast with those reported for *D. pavani*, an endemic Chilean *Drosophila* species (Brncic, 1987). Godoy-Herrera and Fenner (1984) found that non-virgin males and females of *D. pavani* increase their activity when male signals are received, but only males respond to signals emitted by distant females. However, both males and females of *D. pavani*, when offered a choice between males and females, tend to choose males. These results and those reported in this paper suggest that the behavioral mechanisms for recognising physically distant conspecifics have taken different evolutionary pathways in such endemic and cosmopolitan *Drosophila* species.

The behavioral responses of adult *D. melanogaster* flies changes when non-virgin and virgin males and females detect physically distant flies of other related *Drosophila* species. That is, *D. melanogaster* flies, virgin and non-virgin, may discriminate between conspecifics and flies of other *Drosophila* species which are at some distance from them. In contrast, *D. simulans* adult flies were unable to discriminate the presence of flies of *D. melanogaster*. Virgin and non-virgin males and females of *D. simulans* were distributed at random between the Y-tubes when confronted with a choice between Y-tubes connected with conspecifics and Y-tubes joined to cells with *D. melanogaster* flies. These findings suggest that *D. simulans* adult

flies have a limited ability to discriminate signals emitted by physically distant conspecifics.

It is now well established that *D. melanogaster* female has cuticular components which play an important role in the courtship (Jallon, 1984; Tompkins, 1984). It is also believed that the sexes can produce different compounds and that there are differences between the cuticular pheromones of *D. melanogaster* and *D. simulans* (Jallon *op. cit.*). Scott (1986) has reported that during mating, *D. melanogaster* males transfer to the female's cuticle a pheromone which is absent from virgin females. This pheromone has antiaphrodisiac properties acting as an aversive cue to *D. melanogaster* males (Scott *op. cit.*). If this substance is involved in the behavior of *D. melanogaster* here described it could explain why the non-virgin males tend to avoid non-virgin females, when choosing between cells with females and empty cells or cells with males.

D. melanogaster females produce 7,11 heptacosadiene which induces male courtship behavior. Such compound is lacking in *D. melanogaster* males and both sexes of *D. simulans* (Jallon and David, 1987). In this last species a monoene (7-tricosene) is found in both sexes, and it elicits male courtship (Jallon, 1984). This substance is present on *D. melanogaster* males (Bartelt *et al.*, 1985; Antony *et al.*, 1985; Schaner *et al.*, 1987). Again if 7,11 heptacosadiene and 7-tricosene are involved in the behaviors here reported, the sexual dimorphism to the production of them could explain why *melanogaster* males and females move towards opposite-sex conspecifics, whereas adult *simulans* maintain a random distribution when choosing between cells with conspecifics of the opposite sex and cells holding the other sibling species. However, it should be pointed out that these substances are not very volatile, suggesting that they could be detected by contact rather than olfaction (Antony and Jallon, 1982). On the other hand, Bartelt *et al.* (1985) have found that pentane extracts of mature *D. melanogaster* males increase the attractiveness of food odors to both sexes, suggesting that interactions between these two substances may elicit aggregation responses in *D. melanogaster*. However, these findings though prevent an easy explanation to our results do not discard a polyvalent nature for the sex pheromones described in *Drosophila*. They could also act as non-sexual signals in the recognition of physically distant conspecifics (see also to Spence *et al.*, 1984 and Jallon and David, 1987).

We found that 15 to 18% of the flies go from the flask to the Y-tubes. Parsons and Hoffmann (1985) and Spence *et al.* (1984) have also reported that the percentages of *D. melanogaster* males responding to residual odors of conspecific females left in a wind-tunnel olfactometer is low. It is possible that our measure of dispersal (number of flies in the Y-tubes) is contaminated by the reactivity of the flies to the apparatus. Reactivity refers to changes in level of activity as a consequence of en-

vironmental stimulation (Connolly, 1967). The flies tested were initially introduced into a relatively large space (the 1 litre conical flask). Then, they had to move out through a series of 8 exits of 3 cm diameter into a comparatively small space (the Y-tubes). The flies tested could show a reluctance to enter small spaces ("claustrophobic" behavior (see Ewing, 1963). In a more reduced space like the Y-tubes physical contact between conspecifics could also be more frequent and this could induce repelling movements between the flies (Sexton and Stalker, 1961; Ewing, *op. cit.*). As a result, most flies should remain in the flask.

Behavioral mechanisms involved in group formation are of importance for understanding the population structure of animal species (Wilson, 1975). Non-sexual signals between physically distant conspecifics could have consequences for the distribution and abundance of *Drosophila* species. These kind of signals could provide information about distance and abundance of conspecifics, serving as social indicators in habitat selection.

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RESUMO

Indivíduos adultos de *Drosophila melanogaster* e *D. simulans* transmitem e recebem sinais a alguma distância de seus conspecíficos. As respostas comportamentais diferem de acordo com espécie, sexo e estado fisiológico das moscas. Fêmeas não-virgens de *D. melanogaster* aumentam sua atividade locomotora quando sinais de machos e fêmeas são recebidos sem discriminação entre os sexos. Machos não-virgens de *D. melanogaster* não aumentam a dispersão em resposta a sinais emitidos por conspecíficos, mas podem evitar as fêmeas não-virgens. Moscas virgens e não-virgens de *D. melanogaster* se deslocam em direção a seus conspecíficos quando detectam moscas da espécie irmã *D. simulans*.

Entretanto machos e fêmeas virgens e não-virgens de *D. simulans* não conseguem discriminar a presença de conspecíficos distantes fisicamente. Pensamos que as substâncias conhecidas como feromonas do sexo também podem servir para reconhecer fisicamente conspecíficos de *Drosophila* à distância.

REFERENCES

- Antony, C. and Jallon, J.M. (1982). The chemical basis for sex recognition in *Drosophila melanogaster*. *J. Insect Physiol.* 28: 873-880.
- Antony, C., Davis, T.L., Carlson, D.A., Pechine, J.M. and Jallon, J.M. (1985). Compared behavioral responses of male *Drosophila melanogaster* (Canton S) to natural and synthetic aphrodisiacs. *J. Chem. Ecol.* 11: 1617-1629.
- Atkinson, W.D. and Miller, J.A. (1980). Lack of habitat choice in a natural population of *Drosophila subobscura*. *Heredity* 44: 793-799.
- Bartelt, R.J., Schaner, A.M. and Jackson, L.L. (1985). Cis-Vaccenyl acetate as an aggregation pheromone in *Drosophila melanogaster*. *J. Chem. Ecol.* 11: 1747-1768.
- Brncic, D. (1987). A review of the genus *Drosophila* Fallen (Diptera: Drosophilidae) in Chile with the description of *Drosophila atacamensis* SP. NOV. *Rev. Chilena Ent.* 15: 37-60.
- Budnik, M. and Brncic, D. (1983). Preadult competition between colonizing populations of *Drosophila subobscura* and established populations of *Drosophila simulans* in Chile. *Oecologia* 58: 137-140.
- Burdick, A.B. (1954). New medium of reproductive quality stable at room temperature. *Drosoph. Inf. Serv.* 28: 170.
- Burnet, B. and Connolly, K.J. (1974). Activity and sexual behaviour in *Drosophila melanogaster*. In: *The Genetics of Behaviour* (Abeelen, J.H.F. van. ed.). North-Holland, Oxford, pp. 201-258.
- Connolly, K.J. (1967). Locomotor activity in *Drosophila*: III. A distinction between activity and reactivity. *Anim. Behav.* 15: 149-152.
- Ewing, A. (1963). Attempts to select for spontaneous activity in *Drosophila melanogaster*. *Anim. Behav.* 11: 319-378.
- Godoy-Herrera, R. and Fenner, H. (1984). Behavioral responses of *Drosophila pavani* adults to other members of the population. *Brazil. J. Genetics* 7: 41-54.
- Grossfield, J. (1978). Non-sexual behavior of *Drosophila*. In: *The Genetics and Biology of Drosophila* (Ashburner, M. and Wright, T.R.F., eds.). Academic Press, New York, vol. 2c, pp. 1-126.
- Jallon, J.M. (1984). A few chemical words exchanged between *Drosophila* during courtship and mating. *Behav. Genet.* 14: 441-478.
- Jallon, J.M. and David, J.R. (1987). Variations in cuticular hydrocarbons among the eight species of the *Drosophila melanogaster* subgroup. *Evolution* 41: 294-302.
- Jallon, J.M., Lauge, G., Orssaud, L. and Antony, C. (1988). Females pheromones in *Drosophila melanogaster* are controlled by the doublesex locus. *Genet. Res.* 51: 17-22.
- Navarro, J. and Del Solar, E. (1975). Pattern of spatial distribution in *Drosophila melanogaster*. *Behav. Genet.* 5: 9-16.
- Parsons, P.A. and Hoffmann, A.A. (1985). Habitat marking: Parallel genetic divergence in two *Drosophila* species. *Heredity* 54: 203-207.
- Schaner, A.M., Bartelt, R.J. and Jackson, L.L. (1987). (2)-11-Octadecenyl acetate, an aggregation pheromone in *Drosophila simulans*. *J. Chem. Ecol.* 13: 1777-1786.

- Scott, D. (1986). Sexual mimicry regulates the attractiveness of mated *Drosophila melanogaster* females. *Proc. Natl. Acad. Sci.* 83: 8429-8433.
- Sexton, O.J. and Stalker, H.D. (1961). Spacing patterns of female *Drosophila paramelamica*. *Anim. Behav.* 9: 77-81.
- Sokal, R.R. and Rohlf, F.J. (1969). *Biometry*. W.H. Freeman and Company, New York.
- Spence, G.E., Hoffmann, A.A. and Parsons, P.A. (1984). Habitat marking: males attracted to residual odors of two *Drosophila* species. *Experientia* 40: 763-765.
- Spieth, H.T. and Ringo, J.M. (1983). Mating behavior and sexual isolation. In: *The Genetics and Biology of Drosophila* (Ashburner, M., Carson, H.L. and Thompson, J.N., eds.). Academic Press, vol. 3c, pp. 223-284.
- Taylor, C.C. and Powell, J.R. (1983). Population structure of *Drosophila* Genetics and Ecology. In: *The Genetics and Biology of Drosophila* (Ashburner, M., Carson, H.L. and Thompson, J.N., eds.). Academic Press, vol. 3d, pp. 29-59.
- Tompkins, L. (1984). Genetics analysis of sex appeal in *Drosophila*. *Behav. Genet.* 14: 411-440.
- Wilson, E.O. (1975). *Sociobiology: The New Synthesis*. Harvard University Press, Cambridge, Mass.

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