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Influence of β-Alanine on Mating and Territorialism in *Drosophila melanogaster*

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Effects of β-alanine on mating behavior and aggression were studied in Drosophila melanogaster *using the following competitive pairs: (1) homozygous black* (b/b) *flies, in which β-alanine synthesis is decreased, vs. alanine is blocked vs. wild-type* (e^+/e^+) *flies; (2) dark flies, in which β-alanine incorporation is reduced, owing mainly to chromosome 3, vs. light flies collected from the same population as were the dark flies; (3) homozygous black* (b/b) *flies, in which β-alanine synthesis is decreased, vs. β-alanine-injected* b/b *flies, which are phenocopies of wild-type flies. The behavior of mixed-sex groups was studied in a large, illumination-graded observation chamber containing food and in small uniformly illuminated cells also containing food. The relative competitive mating abilities of these types were measured in both experimental conditions. Uninjected black flies, but not injected ones, showed weak and unsteady gait and weak wing extension. In ebony these abnormalities were more extreme. Dark flies did not show these abnormalities. Accelerated sexual maturation was indicated in males by early onset of courtship and enhanced territorial aggression and in females by earliness of mating. Such acceleration was observed in ebony and dark flies, compared with light flies, and among β-alanine-injected* b/b *flies competing with uninjected black flies. Ebony males, although maturing earlier than wild-type males, were less successful than wild-type males in mating. This difference was even greater when the flies were all allowed to mature before competing. Ebony females outmated wild-type females. Dark flies outmated light flies, and β-alanine-injected* b/b *males outmated uninjected black males, especially in bright light. Ebony flies mated much longer than wild-type flies, and black flies mated slightly longer than injected* b/b *flies. There was some spatial isolation of ebony from wild type, dark from*

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light, and β-alanine-injected from uninjected b/b *flies in the illumination-graded observation chamber. Ebony flies more than wild type concentrated near food. Flies were attracted to the current of moist inlet air. They were also attracted to deposited excrement, and males defended such deposits as a mating area, thus showing rudiments of arena behavior in which a mating area away from the oviposition site is defended. Usually, however, the defended area focused on food.*

KEY WORDS: *Drosophila melanogaster; ebony* mutant; *black* mutant; β-alanine; mating behavior; aggression; phenocopy; dark strains; light strains; territorialism; arena behavior; spatial isolation.

INTRODUCTION

Some populations of *Drosophila melanogaster* show a melanistic polymorphism expressed as a darkened trident pattern on the dorsal thorax with generally darkened sclerature. Light, dark, and intermediate strains can be selected from such populations, with results similar to those of Morgan and Bridges (1919), who attributed the dark phenotype to a mutation, with (with trident pattern). Studies of such strains, as well as of the very dark mutants, ebony and black, show that β-alanine induces tanning while it prevents blackening.

Ebony fails to incorporate β-alanine into developing cuticles (Jacobs, 1966). Consequently, the cuticles fail to tan, elevated levels of β-alanine remain in the hemocoel, and the concentration of β-alanine or its products is higher in developing eggs (Jacobs, 1968). Heterozygotes for ebony (e/e^+) (Jacobs, 1966) as well as true-breeding dark strains (Jacobs, 1974) show β-alanine cuticular incorporation rates intermediate between those of ebony and wild type.

Ebony homozygotes show unsteady gait, weak wing extension, and abnormal courtship and abnormal vision (Jacobs, 1960). When aged 4 days, ebony females mate abnormally quickly but repel males while mating. Ebony males, although above normal in sexual activity, show low mating scores when competing with wild-type males in full light, as they are outmaneuvered by wild-type males. In a large enclosure with food dishes, males localize at and defend the dishes by charging (elevating the wings toward and dashing at male rivals) and tussling (jumping on rivals, clasping their wings and pulling the rival's wings together, and sometimes tumbling about as in wrestling) (Jacobs, 1960; Dow and von Schilcher, 1975). Ebony males, being weak at elevating the wings during charging, emphasize tussling. In dim light, where mating of wild-type males is decreased, ebony males mate about as frequently as wild-type males. In multiple-choice studies (Elens, 1957; Jacobs, 1961) male heterozygotes (e/e^+) show mating advantages over homozygous wild-type males.

The black mutant shows decreased synthesis of β-alanine, and injection of this amino acid induces tanning, protects pupae from ultraviolet light damage, prevents adult blackening, and enhances mating success (Jacobs, 1974). The injection converts the abnormally wide and diffuse cuticular microfibrils of the mutant to normal compaction (Jacobs, 1978).

In addition to its role in cuticle formation, β-alanine may influence metabolism, as it is involved in synthesis of the metabolically important pantothenic acid and coenzyme A molecules. Color variants of *D. melanogaster* may genetically shunt β-alanine from exoskeletal to metabolic uses. Study of such variants may reveal metabolic differences (as reflected in rates of sexual maturation and aggression) or exoskeletal differences (as reflected in posture).

In the present study, observations were made in a large chamber with differently illuminated microhabitats. Flies were placed in the chamber as newly enclosed adults and observed for rates of sexual maturation. Particular attention was given to male vigor as expressed in defense of mating area (herein called territorialism). This form of aggression, in contrast to other forms, such as courtship aggression, reflects male vigor. I also studied microhabitat selection and related mating success, as well as sexual isolation among the phenotypes: ebony vs. wild type, dark vs. light, and black vs. sibs converted to light by β-alanine injection.

MATERIALS AND METHODS

Dark flies were collected each year (1971–1975) from rural areas within a radius of 16.1 km from Goshen College and no closer than 0.7 km. A 3.8-liter wide-mouth jar with a screened lid to prevent entry of large insects was placed in shade. Each day, a tube with food (10 g agar, 50 g sucrose, 20 g white ground cornmeal, 20 g dried active Fleischmann's baker's yeast, and 500 ml water, with live yeast granules added after cooling) was placed in the jar. After 7 days the oldest tube was removed and incubated at 15°C to enhance dark pigmentation of the emerging flies. This was repeated through the collection periods (June–November). Emerging dark flies (classes 4–7 of Morgan and Bridges, 1919) were cultured at 25°C under 10-271 lux continuous daylight illumination. An equal number of emerging light flies were cultured separately.

Each day, 30 males and females emerging from the culture bottles were examined and added to the collected parents upon transfer. Black flies were subcultured from 20 original parents found in the population: Wild-type phenocopies were induced by injection of 0.1 μl of 1.0 M β-alanine into the abdominal hemocoel of newly eclosed *b/b* adults just after wing expansion. Coisogenic ebony and wild-type flies with which they were compared were

cultured by the outcross method of Jacobs (1961) begun with flies collected in 1955 and continued to date. Culture conditions were the same for all flies.

The illumination-graded observation chamber (Fig. 1) had inside dimensions of 43.7 by 17.8 by 6.1 cm. It was made of white enameled wood. The white backgrounds in stations A+C were filter paper. The black backgrounds in stations B+D were photograph album paper. A Silent Giant aquarium pump forced air through water and into the chamber through holes (H) to produce 67% relative humidity, as controlled by screw clamps on the entry tubes. The food (described above) was in 31- by 3-mm aluminum planchets. The chamber was slanted backward from vertical by 21°. Glass covered the front, and the right side and entry were covered additionally by 3.2-mm No. 2423 Hylaeine Plastics red plexiglas (R), which shows a sharp transmission peak at 660 nm, with 20% lowering at 800 nm, and virtually complete absorption below 580 nm. Flies were introduced in vial V. Foam plug P, passing through a hole at the top, was lifted to release

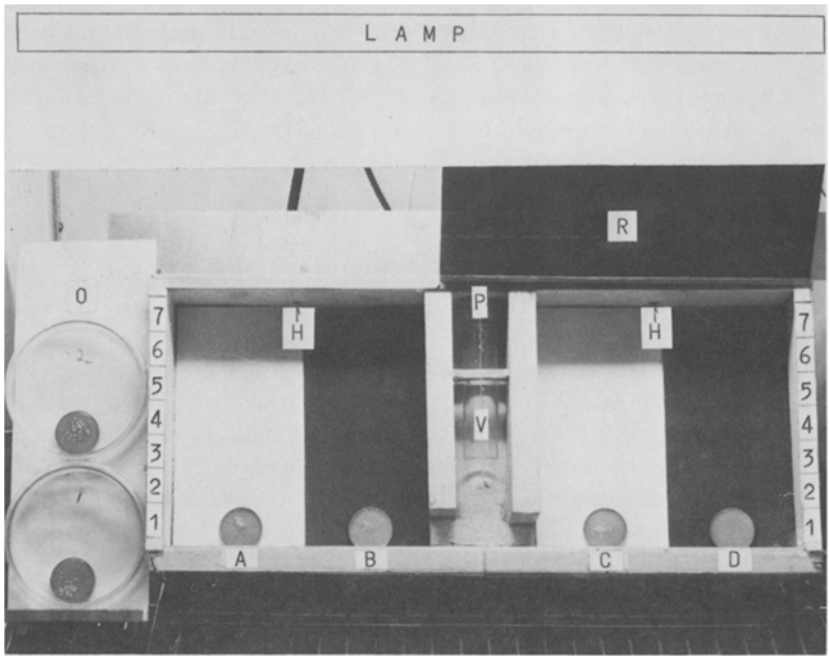

Fig. 1. Illumination-graded observation chamber. Red plexiglas (R) covers the glass over the right side and entry chamber. Humidified air is pumped in through holes (H). Flies placed in the vial (V) need to walk downward to enter stations (A–D). They are released from the vial by lifting the foam plug (P) that passes through a hole in the top wall. Flies are also observed in uniformly illuminated cells (O).

the flies, which had to walk downward to enter the stations (A–D). A 43.5-cm Sylvania F157CW white fluorescent lamp was 22 cm from the top of the chamber. Substations 6 + 7 were shaded by the top wall. Substations 1, being near the illuminated bottom wall, were brightest. Readings of light in lux reflected from the paper in substations 1–7 were as follows: (A) 148, 147, 147, 136, 124, 108, 55; (B) 43, 36, 36, 36, 30, 19, 18; (C) 26, 24, 24, 24, 18, 12, 6; (D) 6, 4, 4, 4, 3, 2, 1. The uniformly illuminated observation cells (O) were 100 by 20-mm petri dishes. The apparatus was in a temperature-controlled incubator (25°C) in an air-conditioned room (25°C).

PROCEDURES

Two weeks were spent using a large (2.4 by 0.2 by 0.1 m) observation chamber and flies individually marked with aluminum enamal patterns on the dorsal thorax. Newly eclosed flies were introduced at staggered periods, so flies of various ages could be compared. Individual case histories were obtained. This was repeated with smaller chambers until the design of Fig. 1 was selected, and the major study (1971–1977) was performed with it, using unmarked flies. Newly eclosed flies were etherized just to the immobile state, and ten females and ten males of each of the two competing phenotypes were placed in the vial, while half this number were placed in each of the two observation cells. After 4 hr the vial plug was lifted. Observations were begun 10 hr later and continued 10 hr, with no longer than 10 min intermissions. All matings were recorded. Courtships were recorded in hours 14–18. Males defending an area 1 hr or more were recorded as territorial males. At the end of hours 20 and 24, frequencies of phenotypes present in various substations were recorded. The apparatus was then wiped with wet cloths before placing new paper, food, and flies in it.

To test whether flies are affected by excrement, a white glossy paper background was placed in the bright side of the chamber containing the usual food dishes and, in addition, a 25-mm square filter paper, 15 mm down from and 30 mm to the right, and another to the left, of the air inlet. Newly eclosed wild-type flies (100 males plus 100 females) were kept 4 hr on food, then placed in a clean vial. After 15 hr the excrement in the vial was stirred into 0.3 ml water and poured onto one square, and clean water was poured onto the other. The flies were released into the chamber and observed 1 hr. This was repeated three times with the excrement to the left and three times with the excrement to the right.

The ebony vs. wild-type study was supplemented with flies aged 2 days as virgins, immobilized at 4°C for 10 min before entry into cells as above. Only the first five matings per cell were recorded. This number per cell is about the average observed in 10 hr with young flies.

RESULTS

General Observations of the Illumination-Graded Chamber

Courtship began a minimum of 15.2 hr after eclosion, and mating a minimum of 16.4 hr. Wing flicking was shown even by newly eclosed flies and appeared to be related to self-defense and possibly to sex recognition. Defense of area began at a minimum of 20.1 hr and was related to strict localization. The area defended was about 8 cm in diameter. The focus was a food dish except in 4% of cases in which the area defended was a spot on the wall in stations A + B at 4 cm from the air inlet. By charging and tussling, the male might eliminate all other male competitors for at least as long as 2.9 hr. In pursuing females, a male wandered the limits of the chamber, then returned directly to his area. In such pursuits, a male stopped at the border of the black, or white, background of his area. The initial attraction to the defended spot on the wall appeared caused by the 2.5 ml/sec flow of moist air from the inlet. Clamping the tube led to immediate dispersal of aggregated flies, and unclamping the tube led to immediate reaggregation. The spot became increasingly attractive as excrement was deposited. In the experiment testing for the effects of excrement, flies were differentially attracted to the excrement-treated paper. Out of 35 instances of feeding on the squares, 29 occurred on the excrement squares, as did 59/64 instances of resting, and 11/13 instances of mating. When a shadow was suddenly cast over the areas, males defending their areas held their places, whereas other flies immediately flew away.

Ebony vs. Wild Type

When placed in the observation cells as newly eclosed adults, ebony males began courtship earlier than wild-type males. In 492 cells, the first courting male was ebony in 63% of the cells, the deviation from 50% being statistically significant ($\chi^2 = 33.3$). (Differences between two proportions were tested using χ^2 tests, with 1 df. Probability values are given only when greater than 0.0001.) The first mating male was ebony in 55% of the cells ($\chi^2 = 5.1$, $p < 0.025$). The first female to mate was ebony in 76% of the cells ($\chi^2 = 133.2$). When the flies were aged 2 days (to sexual maturity) before placement in the cells, ebony females still mated earlier than wild-type females, but ebony males now mated later than wild-type males. In 167 cells with aged flies, the first female to mate was ebony in 60% of the cells ($\chi^2 = 6.5$, $p < 0.011$), but the first male to mate was wild type in 73% of the cells ($\chi^2 = 35.5$). In spite of early sexual maturation of ebony males, as compared with wild type, the overall mating scores of ebony were lower than those of wild type (Table I).

Table I. Mating and Territorial Male Frequencies of Ebony *Drosophila melanogaster* Competing with Coisogenic Wild Type

		Illumination-graded observation chamber stations (mean lux reflected illumination in parentheses)					Uniformly illuminated observation cells	
	Male/female	A(124)	B(31)	C(20)	D(3)	Total	Young flies[a] total	Old flies[b] total
Mating frequencies	Ebony/ebony	223	190	130	86	692	697	140
	Ebony/wt[d]	50	50	48	23	171	507	42
	Wt/ebony	229	256	127	61	673	940	265
	Wt/wt	179	319	71	52	621	545	220
	Total	681	815	376	222	2094	2689	667
	Percent homophenic[c]	59**[e]	62**	53n.s.	62**	60**	46**	54*
	Ebony male	273	240	178	109	800	1204	182
	Wt male	408	575	198	113	1294	1485	485
	Percent ebony male	40**	29**	47n.s.	49n.s.	38**	45**	27**
	Ebony female	452	446	257	147	1302	1637	405
	Wt female	229	369	119	75	792	1052	262
	Percent ebony female	66*	55**	68**	66**	62**	61**	61**
Territorial male frequencies	Ebony male	21	32	10	0	63		
	Wt male	0	7	1	1	9		
	Percent ebony male	100**	82**	91**	0n.s.	88**		

[a] Entered as newly enclosed adults as in chamber, 246 replicates.
[b] Entered as 2-day-old adults, 62 replicates.
[c] Mating between like phenotypes.
[d] Wild type.
[e] One asterisk indicates deviation from 50%, $p < 0.05$. Two asterisks indicate $p < 0.01$; n.s., $p > 0.05$.

In the illumination-graded observation chamber, fewer ebony than wild-type males succeeded in mating. The lowered mating success of ebony males was more evident when the flies were aged before testing. Among *young males* in the cells 45% of the matings involved ebony males, but among *aged males* in the cells only 27% involved ebony males ($\chi^2 = 67.4$) (Table I). Among young flies in the cells, the least frequent matings were wild type ♀ with ebony ♂, and the most frequent was the reciprocal cross. Under all conditions, ebony females were more successful in mating than wild type.

Also in the chamber ebony males mated less frequently than wild type, although ebony showed enhanced territorial aggression (Table I). Among young flies in the cells, only 46% of matings were homophenic (wild type with wild type or ebony with ebony), as compared with 60% in the chamber ($\chi^2 = 86.0$). In the bright stations (A + B) in the chamber, 85% of matings of ebony males but only 54% of those of wild-type males occurred near the food (substations 1 + 2), the remainder being in substations 3–7. The difference between these two percentages is significant ($\chi^2 = 211.0$). The respective percentages for females mating near the food were as follows: ebony 72% and wild type 55% ($\chi^2 = 89.8$). The concentration of matings near food was still more pronounced in the dim stations (C + D), so that wild-type matings were nearly as concentrated close to food as were ebony matings. In the dim stations, the percentages of matings near food were as follows: for males, ebony 87% and wild type 75% ($\chi^2 = 14.2$); for females, ebony 76% and wildtype 80% ($\chi^2 = 1.85$, n.s.).

Dark vs. Light

Of 5672 flies collected from outdoor populations, 6.1% were dark. When dark flies were crossed with *Cy/Pm;D/Sb*, counts of 586 F_2 offspring showed the following percentages of flies showing no darkening of the trident at 25°C: chromosome 2 homozygotes 73%, chromosome 3 homozygotes 24%, and chromosome 2 and 3 homozygotes 13%. Darkening appears caused mainly by chromosome 3, with enhancement by chromosome 2. In 568 cells, the first male to court was dark in 57% of the cells, the deviation from 50% being significant ($\chi^2 = 11.3$, $p < 0.0008$). A dark male was first to mate in 61% of the cells ($\chi^2 = 27.1$). Among females, the first mating involved a dark female in 66% of the cells ($\chi^2 = 58.3$).

Also in the chamber, dark flies were more successful than light flies in mating, and more dark than light males were characterized by territorial aggression (Table II). In the chamber, 54% of matings were homophenic as compared with 51% in the cells ($\chi^2 = 5.8$, $p < 0.017$). Matings were more frequent in the bright than the dim side of the chamber, and in station B

Table II. Mating and Territorial Male Frequencies of Dark *Drosophila melanogaster* Competing with Light Flies Collected from the Same Population

		Illumination-graded observation chamber stations (mean lux reflected illumination in parentheses)					Uniformly illuminated observation cells
	Male/female	A(124)	B(31)	C(20)	D(3)	Total	total
Mating frequencies	Dark/dark	213	393	102	8	716	1159
	Dark/light	111	325	43	17	496	971
	Light/dark	120	265	122	26	533	858
	Light/light	137	253	77	42	509	756
	Total	581	1236	344	93	2254	3744
	Percent homophenic[a]	60**[b]	52n.s.	52n.s.	54n.s.	54**	51n.s.
	Dark male	324	718	145	25	1212	2130
	Light male	257	518	199	68	1024	1614
	Percent dark male	56	58**	42**	27**	54**	57**
	Dark female	333	658	224	34	1249	2017
	Light female	248	578	120	59	1005	1727
	Percent dark female	57**	53*	65**	37**	55**	54**
Territorial male frequencies	Dark male	98	127	1	1	227	
	Light male	17	93	2	1	113	
	Percent dark male	85**	58*	33n.s.	50n.s.	67**	

[a] Mating between like phenotypes.

[b] One asterisk indicates deviation from 50%, $p < 0.05$. Two asterisks indicate $p < 0.01$; n.s., $p > 0.05$.

more than A. The attractiveness of B over A was caused by the black background and by the nearness to the entry port of B. When B was given a white background, and A a black, counts of 401 matings in the bright side lowered the original percentage (68%) of matings in B to 59% ($\chi^2 = 22.3$). Females, but not males, showed a tendency to migrate from the bright to the dim side as time progressed. At hour 20, 28% of the females inhabited the dim side, but at hour 24, 54% did so ($\chi^2 = 893.9$). The respective figures for males were 28% and 24% ($\chi^2 = 23.6$).

Black vs. Wild-Type Phenocopies

Black males converted to wild-type phenocopies by β-alanine injection began courting earlier than their uninjected sibs. In 594 cells, an injected male was first to court in 60% of the cells, the deviation from 50% being significant ($\chi^2 = 23.4$). The first mating involved an injected male in 58% of the cells ($\chi^2 = 15.5$). Among females, the first mating involved an injected female in 54% of the cells ($\chi^2 = 3.9, p < 0.05$).

In the chamber also, injected males outmated black males and more injected than black males were characterized by territorial aggression, particularly in the bright side of the chamber (Table III). Among matings in the chamber, 55% were homophenic (injected with injected or black with black) compared with 52% in the cells ($\chi^2 = 3.0$, n.s.). Although no visual deficiencies of black flies were observed, they showed behavioral abnormalities similar to, but less extreme than, those of ebony (Jacobs, 1960). Among these abnormalities were unsteady gait and weak wing extension with incomplete closure after extension. Weakness of wing flicking by black males appeared to invite aggressive attack by other males. Black males, being weak at snapping the wings forward in charging, accentuated the tussling phase of aggression. About 8% of the black flies wandered about the chamber, occasionally flying as if seeking escape. Although they walked over the food, they often grew thin and failed to mate. All these abnormalities were corrected by β-alanine injection. If 2.0 M β-alanine, instead of the usual 1.0 M, was injected thoracically into newly eclosed males, the wings were permanently dilated, thus making flicking impossible. Such males were charged particularly consistently by other males and were therefore dispersed by them. These overinjected males appeared highly motivated sexually, continuously courting and chasing females.

Unsettled Matings Involving Ebony and Black

When mating, ebony females showed repelling actions, pushing with the legs and bending down the abdomen to prevent wing clasping and settl-

Table III. Mating and Territorial Male Frequencies of *Drosophila melanogaster* Homozygous for the Gene Black Competing with β-Alanine-Injected Sibs

		Illumination-graded observation chamber stations (mean lux reflected illumination in parentheses)					Uniformly illuminated observation cells total
	Male/female	A(124)	B(31)	C(20)	D(3)	Total	
Mating frequencies	Injected/injected	187	453	54	16	710	1292
	Injected/black	110	394	57	22	583	1143
	Black/injected	93	207	81	30	411	938
	Black/black	122	231	89	44	486	994
	Total	512	1285	281	112	2190	4367
	Percent homophenic[a]	60**[b]	53*	51n.s.	51n.s.	55**	52**
	Injected male	297	847	111	38	1293	2435
	Black male	215	438	170	74	897	1932
	Percent injected male	58**	66**	40**	34**	59**	56**
	Injected female	280	660	135	46	1121	2230
	Black female	232	625	146	66	1069	2131
	Percent injected female	55n.s.	51n.s.	48n.s.	41n.s.	51n.s.	51n.s.
Territorial male frequencies	Injected male	36	191	2	1	230	
	Black male	22	80	5	9	116	
	Percent injected male	62n.s.	70**	29n.s.	10*	66*	

[a] Mating between like phenotypes.

[b] One asterisk indicates deviation from 50%, $p < 0.05$. Two asterisks indicate $p < 0.01$; n.s., $p > 0.05$.

ing by the males. The frequency of such unsettled pairs was higher when ebony males were involved (92%) than when wild-type males were involved (74%; $\chi^2 = 31.7$; Table IV). Wild-type females also showed some repelling action when mating with ebony males, the frequency of unsettled pairs being 65% with ebony males but only 9% with wild-type males ($\chi^2 = 45.2$). Matings between uninjected black flies also showed a higher frequency of unsettled pairs (38%) than homophenic injected matings (19%; $\chi^2 = 16.5$).

Durations of Matings of Ebony and Black

Five matings lasted less than 5 min. Since no offspring resulted, these matings were not included in the calculations of Table IV. In the experiment with ebony, means of the four groups varied significantly ($F = 121.61$, $df = 3, 878$, $p < 0.001$), and all pairwise mean differences were statistically significant ($p < 0.05$) According to Scheffé's (1958) method of multiple comparisons. Furthermore, ebony males mated longer than wild-type males ($p < 0.001$), and ebony females mated longer than wild-type females ($p < 0.001$), although the male phenotype had a more pronounced effect than the female phenotype.

There was also significant variation among the means in the experiment with black flies ($F = 7.886$, $df = 3, 535$, $p < 0.001$). However, only one pairwise comparison of means, that between injected ♀ × injected ♂ and

Table IV. Duration of Copulation of *Drosophila melanogaster* in Two Experiments

Male/female	Mating duration (min)		Number timed	Percent unsettled[b]
	Mean[a]	SE		
Wild type/wild type	17.4 (a)	0.24	211	9
Wild type/ebony	18.9 (b)	0.28	259	74
Ebony/wild type	23.1 (c)	0.60	124	65
Ebony/ebony	25.4 (d)	0.36	288	92
Injected/injected[c]	18.0 (a)	0.32	190	19
Injected/black	19.2 (a,b)	0.48	73	35
Black/injected	18.5 (a,b)	0.41	88	31
Black/black	19.9 (b)	0.24	188	38

[a] Means followed by the same letter in parentheses are not significantly different, and means followed by different letters are significantly different ($p < 0.05$), by Scheffé's (1958) procedure for *a posteriori* multiple comparisons.

[b] Pairs in which the female bends down the abdomen to prevent male wing clasping.

[c] Black injected with β-alanine.

black ♀ × *black* ♂, was statistically significant ($p < 0.001$) (Table IV). Uninjected males did not mate significantly longer than injected males, but uninjected females mated longer than injected females ($p < 0.01$).

BEHAVIORAL OBSERVATIONS AND DISCUSSION

Accelerated development of courtship and territorial aggression in males and accelerated sexual receptivity of females were observed in ebony and dark flies, which shunt β-alanine from cuticular to possible matabolic uses, and in flies injected with β-alanine. Normal posture and muscular coordination, as observed in wild-type (light) flies and black flies converted to wild-type by β-alanine injection, may be due to exoskeletal stiffening induced by β-alanine. The microfibrils of β-alanine-deficient ebony and black cuticles lack the compaction of cuticles of wild-type flies and black flies converted to wild-type by β-alanine injection (Jacobs, 1978). Parts of the cuticle involved in flexions, such as the male terminal abdominal tergites, sharply flexed during mating, are untanned (and therefore black). Black portions of the cuticle, such as the trident spot of dark flies, are β-alanine deficient (Jacobs, 1976). Weakness in gait and wing extension and also prolonged mating observed among black and especially ebony flies may be caused by exoskeletal dificiencies. Such deficiencies may result in incompatibilities of genital structures.

The impeded mating success of black and especially ebony males may be caused by exoskeletal deficiencies, leading to abnormal behavior. Conversely, with ebony females, exoskeletal deficiencies may enhance mating success by reducing ability of females to escape from courting males. The persistence of the ebony allele in population cages, in spite of negative selection resulting from competition with normal flies (L'Heritier et al., 1937), may be explained in part by enhanced mating success of ebony females. Part of this success may be a result of female weakness rather than vigor. In males, vigor appears to play a role in mating success when related to defense of mating area. Such defense would be virtually impossible in crowded population cages. It is optimally exhibited when only a few flies are used, and where there is ample space, as in the present study.

Wild-type males chasing females in bright light, especially on the white background (station A), were able to follow females more easily than they could under dim light. They showed increased mobility when seeking females in dim than in bright light. In dim light they wandered aimlessly in a zigzag fashion about the chamber in the manner of the visually deficient ebony males. But in bright light they sought females in a deliberate fashion as if directed by vision. They courted their images on the outside wall of aluminum planchets or the mercury bulb of the thermometer. The lessened

comspicuousness of flies on a black background may explain why station B was more used than A. The black background in B may have served as a resting place for females chased by males. Sexual isolation in the bright side of the chamber resulted from the tendency of ebony, more than wild type, to concentrate mating near food. Lacking visual aids, flies appeared to increase use of chemical (food) attractants in meeting mates. In the small observation cells there is lessened opportunity for spatial isolation, hence the lessened proportion of homophenic matings in the cells as compared with the large chamber. However, even in the cells some isolation was observed, as ebony concentrated more than wild type on the food and wild type more than ebony perched on the top outer surface of the tilted aluminum planchets. Spatial isolation in the chamber was also observed among dark vs. light flies. In the bright side over half of the matings involved dark flies, but in the dim side less than half did so. Also, in the bright side, over half of the matings of injected vs. uninjected black flies involved injected flies.

Chemoreception appeared to play a dominant role in mating. Males and females would rest quietly in groups for hours; then suddenly a female would become highly attractive to males, which would immediately court her wherever she went. Females would sometimes become unattractive after mating, even without wing flicking or visible genital extrusion. Males seemed able to recognize sex even under dim light and without visible wing vibration on the part of male or female. Jacobs (1960) found that males frequently courted female heads, especially females 1 day after eclosion. Male heads were also courted, but much less frequently, and only in bright light, not dim light. In the present study, when no food (only water) was present, the mating system was disorganized, with males chasing females throughout the chamber without mating. No attraction of females to males was apparent, but in wing vibration during courtship the wings of the male were positioned in a downward slant posteriorly, causing air to be driven over the body of the male and toward the female. However, wing vibration of neither sex appeared essential for induction of mating. Averhoff and Richardson (1976) have demonstrated a male airborne substance influencing females of this species.

Territorial behavior in *Drosophila melanogaster* is relatively evanescent and brief, although proportionate to longevity less brief than appears. However, all essential elements of territorial behavior are represented. Noble (1939) has defined "territory" as any defended area. If defined as defense of mating area, territorialism covers the cases of genetic interest. Although with *melanogaster* food appears essential for stimulating territorial aggression, excrement away from the food may also serve. Experiments are in progress here to determine whether the excrement is conditioned

by the food eaten, in such a way as differentially to excite males reared on that same food. Mating away from the place where the eggs are laid and young are reared may prevent extra male molestation of ovipositing females. The presence of numerous males on the oviposition site leads to mass chases of females away from the sites, as observed also among dragonflies (Jacobs, 1955). In certain Hawaiian *Drosophila*, in which the females are not well equipped to repel males, mating away from the oviposition site is accentuated, to lead to strict arena behavior. The males of some species mark their defended areas with exudate (Spieth, 1968). It would be of interest to know whether the exudate is chemically similar to the food of the species. The tendency of *melanogaster* to defend excrement may represent rudiments of arena behavior.

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