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ORIENTAL FRUIT FLY INVESTIGATIONS

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PHYSIOLOGY, Work Project 1-0-6 --- C. C. Roan, Project Leader

SUMMARY

The temperature controls in the room used for the investigation of copulatory stimuli were restored to operation so as to permit the use of temperatures of the order of 15.6° C. (60° F.). These studies have indicated that temperatures below this level tend to inhibit copulatory activity when they occur at the same time as the decreasing light intensity.

The installation and calibration of a Warburg Manometricon made possible the initiation of investigations of the effects of environmental factors on the physiological behavior of adult D. dorsalis. The results of these studies are quite compatible with similar investigations on other insects. The use of CO_2 as an anesthetic in handling these flies prior to respiratory studies gives an erroneous picture of the respiratory quotients at various temperature levels. A preliminary evaluation of the effects of CO_2 anesthesia indicates that the use of this gas may well produce effects other than anoxia. These effects are relatively short-lived.

Line Project 1-c-6-1. Factors Influencing the Mating of the Oriental Fruit Fly.

The previous work on this subject has shown that a decreasing light intensity is adequate to stimulate copulatory activity in D. dorsalis when maintained at temperatures ranging from 20° C. - 30° C. (68° F. - 86° F.). The most recent experiments on this subject have included the factor of temperature as a significant variable.

In the first experiment a cage containing about 200 flies (approximately equal sex ratio) was placed in the experimental area at a temperature of 22° C. (72° F.). As in the previous experiments the light intensities were automatically reduced by 50% at 8 a.m. and returned to normal intensity at 7 p.m. These conditions were maintained for 13 days at which time the temperature was reduced to 18° C. (65° F.). Observations were made daily between 8 a.m. and 10 a.m. to determine the number of pairs in copula. The results of this experiment are presented in Table 1 under Cage 1. A second cage of flies, Cage 2, was placed in the experimental area six days after emergence when the temperature of the area was 16° C. (61° F.). Cage 3, a duplicate of Cage 2, was maintained under normal light and temperature conditions until the eleventh day after emergence. As indicated in Table 1, the temperature of the experimental area was 14° C. (57° F.) at this time.

The activity of these flies, as shown in Table 1, indicates that temperatures below 15.6° C. (60° F.) tend to inhibit copulatory activity. These temperatures also tend to delay the attainment of sexual maturity of D. dorsalis as shown by the activity of Cage 2, which was exposed to temperatures of the order of 15.6° C. (60° F.) from the fifth day after emergence. The flies in this cage did not copulate until 27 days after emergence. The first copula noted in this cage did not occur until they had been exposed to temperatures ranging from 18° C. - 24° C. (65° F. - 75° F.) for a period of six days.

Cage 3, which was a duplicate of Cage 2, was maintained under normal temperatures and lighting for a period of 12 days and then subjected to a reversed artificial lighting rhythm and low temperatures for eight days. The first copulation was observed the second day after the temperature was taken above 18° C. (65° F.).

It was impossible to obtain a better control of the temperature with the equipment available. However, these laboratory studies substantiate field observations and indicate that the occurrence of low temperatures, i.e., below 15.6° C. (60° F.), at or near the time of sunset, may prevent copulation.

The possibilities of a modified behavior occurring after long exposures to gradually decreasing temperatures is a matter which must be considered. Information on this subject may be obtained from the long range studies in the bioclimatic cabinets.

TABLE 1. Copulatory activity of flies under controlled artificial illumination and varying temperatures. Observations made daily between 8 a.m. and 10 a.m. Maximum numbers observed reported here.

Temp. °F.			Cage No. 1		Cage No. 2		Cage No. 3	
2am	8am	2pm	Days after emergence	No. of Prs. observed in copula	Days after emergence	No. of Prs. observed in copula	Days after emergence	No. of Prs. observed in copula
73	72	75	10	0				
70	72	74	11	1				
72	74	73	12	5				
73	72	63	13	13				
64	64	62	14	10				
62	60	58	15	6				
58	58	60	16	0				
61	60	61	17	0				
62	62	62	18	3				
62	61	62	19	3				
62	61	61	20	3				
62	61	60	21	8	6	0		
60	61	62	22	5	7	0		
58	59	58	23	0	8	0		
59	58	57	24	0	9	0		
57	58	59	25	0	10	0		
63	61	57	26	3	11	0		
57	58	57	27	0	12	0	12	0
57	57	58	28	0	13	0	13	0
57	56	58	29	0	14	0	14	0
57	57	58	30	0	15	0	15	0
57	57	58	31	0	16	0	16	0
58	58	57	32	0	17	0	17	0
58	60	58	33	2	18	0	18	0
62	62	58	34	14	19	0	19	0
64	64	65	35	5	20	0	20	0
77	76	75	36	3	21	0	21	3
72	71	72	37	12	22	0	22	1
74	74	74	38	5	23	0	23	3
75	74	63	39	1	24	0	24	4
60	62	60	40	0	25	0	25	0
63	61	70	41	0	26	0	26	0
70	70	71	42	1	27	1	27	1
70	71	71	43	3	28	2	28	1
71	72	71	44	1	29	2	29	1
70	70	69	45	0	30	2	30	3
70	70	71	46	0	31	1	31	3

Cage No. 1—Placed in experimental area 8 days after emergence
Cage No. 2— " " " " 6 " " "
Cage No. 3— " " " " 12 " " "

Line Project 1-c-6-2. Reactions of the Oriental Fruit Fly to Light, Temperature and Chemical Stimuli.

Studies of the effects of temperature on the physiology of the Oriental fruit fly have been continued during this period. The primary objective of these studies was to determine the effects of temperatures ranging from 5° C.-45° C. (41° F.-113° F.) on the rate of oxygen uptake and respiratory quotients.

These investigations were conducted with a circular Warburg Manometricon equipped to maintain constant temperatures (± 0.02° C.) over a range from 5° C.-50° C. (41° F.-122° F.). The Warburg "Direct Method" (Umbriet, et al, 1949) was employed for both oxygen uptake and CO_2 output. Only the modifications of this method as employed in these studies will be described here.

Initially the alkali for CO_2 absorption was placed in the center well. A piece of accordian-folded filter paper was placed in the center well to increase the absorptive surface. A cylinder of brass screen was placed over the filter paper, extending down into the center well, to exclude the flies from the alkali. In the initial experiments all flies were anesthetized with CO_2 and weighed before placing them in the test flasks. The flasks were of approximately 15 ml. capacity, equipped with one side arm which had a vented ground glass stopper. For oxygen uptake flasks with a center well were employed. For CO_2 output flasks with no center well were used.

Only male flies of uniform age were used. At temperatures above 25° C. (77° F.) three flies were used in each flask. Below 25° C. five flies were placed in each flask. The flasks were placed on the manometers and as soon as the flies had recovered from the anesthesia they were placed in the temperature bath and equilibrated for ten minutes. Readings were taken at 15-minute intervals for a period of one hour. In the initial experiments an empty flask was employed as a thermobarometer.

The results of these initial experiments indicated very high metabolic rates and RQ's (respiratory quotients) of the order of 1.5. The high metabolic rates were not improbable, but RQ's significantly in excess of one were very improbable. This phenomenon occurs only under abnormal conditions or in the case of the synthesis of fats from carbohydrates. (Heilbrunn, 1943 and Richardson, 1929). The only case of an RQ of this order for insects discovered in a preliminary search was that of the adult lepidopteron, Agrotis segetum, which gave an RQ of 1.66 just after a carbohydrate meal. (Kozhantschikow, 1938). Analyses reported by this author indicated that this insect was synthesizing fats from carbohydrates.

Since such a synthesis appeared unlikely in the case of D. dorsalis, a careful check was made of all techniques and materials. The results of this check were quite interesting. The brass screens were being oxidized by the alkali at such a rate that their oxygen consumption was significant and the use of CO_2 anesthesia in handling the flies was responsible for the high RQ's.

The effect of CO_2 anesthesia on the respiratory metabolism of this insect made it desirable to include this as a factor in the investigation of the effects of temperature. Techniques were modified so that the alkali and filter paper were placed in the side arm of the appropriate flasks. The flies were excluded from this area by a cone of plastic screen fitted inside the main body of the flask.

This screen did not come in contact with the alkali or filter paper. As a result of the decreased oxygen consumption, four flies were used in each flask at experiments above 15° C. (59° F.), and 7 - 8 flies in each flask at 15° C. (59° F.), and 5° C. (41° F.). Parallel tests with and without CO_2 anesthesia were run at each temperature. Four replicates of each test were run. A flask containing plastic screen, alkali, and filter paper was used as a thermobarometer for the oxygen uptake tests, while a flask containing only the plastic screen was used as a thermobarometer for the CO_2 output tests. The flies were weighed at the end of the test period after sacrificing with chloroform. The flies were weighed in lots from each flask. The data presented in Tables 2 and 3 were obtained by dividing the total volume change in each flask by the total weight of flies in that flask.

It is interesting to note in Table 2 the effects of different temperature increments on the oxygen uptake of the untreated flies. The temperature coefficient, Q_{10}, for these increments shows a considerable variation. Applying the following formula

$$Q_{10} = \left(\frac{K_1}{K_2}\right)^{\frac{10}{t_1 - t_2}}$$

where k_1 and k_2 are the constants at temperatures t_1 and t_2 respectively. The Q_{10} for the region 5° C.-15° C. (41° F.-59° F.) is 1.6; for 15° C.-25° C. (59° F.-77° F.), 3.6; and for the region 25° C.-45° C. (79° F.-113° F.), 1.73. From these data it is apparent that the region 15° C.-25° C. (59° F.-77° F.) has a pronounced effect upon the respiratory metabolism of this insect. It is of interest to note that the lower limit of this region is of the same order as that which inhibits copulatory activity. This temperature, from the data of the Ecology-Biology project, also approaches a developmental minimum.

The oxygen uptake of flies anesthetized with CO_2 presents a similar picture. It is interesting to note that at the lower temperatures the oxygen uptake of the CO_2-treated flies exceeds that of the normal flies, while at the higher temperatures the reverse appears to be true. If anoxia is the primary effect of CO_2 anesthesia, then the situation might be expected to be analogous to immersion in water. During immersion in water grasshoppers accumulated an oxygen debt that was comparable to their normal consumption during a similar period (Bodine, 1928). In the case of the grasshoppers the debt was paid off in a period comparable to the period of immersion. If this applies in the case of CO_2 anesthesia the debt should be paid off during the equilibration period.

It will be noted that in the case of the flies treated with CO_2 the RQ's were always higher than those of the untreated flies and with the exception of the 5° C. (41° F.) level always significantly greater than unity. An inspection of these data in Table 2 could lead to the conclusion that the high RQ's resulting from the anesthesia with CO_2 persisted for at least an hour. Inspection of the data in Table 3 shows that this is not the case. At the 25° C. (77° F.) level the effect persists for the longest period.

These observations may be explained by the following phenomena. At the time of anesthesia the gas pressure around the fly is almost entirely due to CO_2. The body fluids, particularly the fluids in the tracheoles, approach saturation with CO_2. As soon as the fly is placed in the test flask the CO_2 pressure is once again only a partial pressure of the total atmosphere. The dissolved CO_2 will leave the body fluids until the two phases are in equilibrium at that temperature.

If the temperature of the test condition is higher than that of the environment at the time of anesthesia the body fluids will unload at a more rapid rate than if the temperature is lower.

If we accept the above reasoning, then the high RQ's of flies subjected to CO_2 anesthesia are a result of a combination of metabolic CO_2 plus the CO_2 dissolved in the body fluids at the time of anesthesia. The gas is unloaded to the extent permitted by the solubility of the CO_2 in the fluids concerned at any particular temperature and the differences in the pressure of the gas at the time of anesthesia and the time of unloading.

Three factors make it difficult to interpret these data in a quantitative manner. First of all it was impractical to control the temperature at the time of anesthesia and to reduce the time interval between the end of the period of anesthesia and the beginning of the exposure to the experimental temperature. The equilibration period necessary to bring the flask to this temperature introduces a further complication. The last problem, which presents the greatest difficulty, is obtaining a standard metabolic level. The small size of D. dorsalis makes the elimination of random physical activity a very difficult task. Nevertheless, it appears from the data presented in Tables 2 and 3 that the use of CO_2 as an anesthetic involves more than the production of anoxia.

TABLE 2. Effects of Temperature and Carbon Dioxide Anesthesia on the Rates of Oxygen Uptake and Carbon Dioxide Output of Four-Day Old Male D. dorsalis.

Temperature	CO_2 Anesthesia 1/			No Anesthesia		
	cc. O_2/ g./hr.	cc. CO_2/ g./hr.	RQ	cc. O_2/ g./hr.	cc. CO_2/ g./hr.	RQ
5° C. (41° F.)	0.88	0.80	0.91	0.45	0.30	0.66
15° C. (59° F.)	1.11	1.77	1.59	0.73	0.77	1.05
25° C. (77° F.)	2.42	4.40	1.82	2.65	2.52	0.95
30° C. (86° F.)	3.23	5.90	1.83	3.52	3.20	0.91
35° C. (95° F.)	4.43	7.20	1.62	4.95	4.20	0.85
40° C. (104° F.)	5.46	11.4	2.19	6.54	6.35	0.97
45° C. (113° F.)	6.80	8.20	1.21	7.90	7.00	0.89

1/ Anesthetized for five minutes prior to placing in test flasks. Anesthesia carried out at room temperatures, approximately 27° C. (80° F.).

TABLE 3. Effects of Temperature and Carbon Dioxide Anesthesia on Oxygen Uptake and Carbon Dioxide Ouput of Four-Day Old Male D. dorsalis at Various Time Intervals during the Experimental Period.

TEMPERATURE	CO_2 Anesthesia[1] cc.O_2/g	cc.CO_2/g	RQ	No Anesthesia cc.O_2/g	cc.CO_2/g	RQ
45° C. (113° F.)						
1st 15 Minutes	1.9	3.1	1.75	2.35	1.84	0.78
2nd 15 "	2.25	2.23	0.99	2.62	2.26	0.86
3rd 15 "	1.60	1.55	0.97	1.79	1.74	0.97
4th 15 "	1.10	1.05	0.95	0.95	1.01	1.06
25° C. (77° F.)						
1st 15 Minutes	0.85	1.56	1.84	0.80	0.56	0.70
2nd 15 "	0.41	0.69	1.68	0.68	0.67	0.98
3rd 15 "	0.64	0.83	1.29	0.52	0.52	1.00
4th 15 "	0.53	0.50	0.94	0.54	0.37	0.69
5° C. (41° F.)						
1st 15 Minutes	0.66	0.72	1.09	0.25	0.09	0.36
2nd 15 "	0.09	0	0	0.05	0	0
3rd 15 "	0.09	0.11	1.20	0.06	0.04	0.67
4th 15 "	0.04	0	0	0.09	0	0

1/ Anesthetized for five minutes prior to placing in test flasks. Anesthesia carried out at room temperatures, approximately 27° C. (80.6° F.)

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