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Low Temperature Tolerance of Seven Different Species of the Drosophila

Walter Leon Smith

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SMITH

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LOW TEMPERATURE TOLERANCE OF SEVEN DIFFERENT SPECIES OF THE DROSOPHILA

By

WALTER LEON SMITH

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DEDICATION

Sincerely dedicated to my wife, Madalynne V. Smith, and my daughter, Madalynne Verdell Smith, whose faith in my ability to succeed has inspired me thus far.

W. S.

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The writer wishes to express his deepest appreciation and gratitude to his instructor and advisor, Dr. Thomas P. Dooley, under whose immediate direction this study was conducted, for his helpful guidance and constructive criticisms.

W. L. S.

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Introduction

The effects of low temperature upon organisms with special emphasis on insects have been an open field for research since the early part of 1800. The opinions of various workers agree that insect freezing has not been studied extensively as plant freezing. The large economic losses from tender plants and the consequent desire to develop hardy varieties have no counterpart in applied entomology with the exception of some work concerning the insecticidal effects of low temperature over given periods of time.

The Drosophila is ideally suited for experimental work and particularly where one is concerned with the relation of temperature tolerance of insects. For many years Genetists claim this insect as being the best specimen for use in the field of Genetics due to its reproductive ability, variation, and response to different conditions. Numerous of these experiments have been concerned with the effects of low and high temperatures on various characteristics of the Drosophila. It is also believed that the Drosophila is ideal for other experimentation.

Stern (23) in his studies on the influence of temperature on wing mutation of the Drosophila melanogaster stated that flies of the constitution j^2/j and homozygous j (which are allelomorphs of

the 2nd chromosome recessive mutant jaunt) show their wings strongly curled up at 25°C - 30°C. At 16°C - 21°C a high percentage of flies had either normal or slightly curled wings. He further stated that while the wings of j flies curved up strongly regardless of temperature, wings of normal flies are not curved up during expansion. At high temperature the duration of the process does not suffice to flatten wings totally. At low temperature the blood pressure acts long enough to produce straight wings.

Bergner (1) in studying the prolongation of each stage of the life cycle on crossing over in the 2nd and 3rd chromosomes of the Drosophila melanogaster, concluded that the duration of the pupal stage was prolonged by low temperature. The adult stage was prolonged by exposing the flies to formalin fumes. By prolongation of the larvae, pupal and adult stages, an increase in crossing over on the 2nd and 3rd chromosomes was noted.

Mickey (16) irradiated adult females at room temperature and others at 4°C. The irradiation at 4°C increased production of translocation and produced much sterility.

The work of King (13) was confined to low temperature and x-rays. He subjected the flies to an 0.5°C for 30 minutes prior to being exposed to 600 - 3600 x-ray units which caused sterility.

From this brief review, it can readily be seen that the purpose of experiments cited was to find the effects of various temperatures generally in connection with other factors on certain

traits or characters of the Drosophila melanogaster.

The studies of Horvath and associates (10) pp. 172 were confined to the survival time of various warm-blooded animals in extreme cold. He found the lowest temperature at which the animal can maintain its body temperature for one hour to be: for the pigeon, -85°C; for the chicken - 50°C; for the rabbit, -45°C; and the white rat -25°C.

Payne (19) working with different species of the Oak borer (synchioa punctata, Dendriades candensis, Elaster and various Cermbycidae), concluded that insects freezing point lowers in the winter and rises by mid-summer to near 0°C. She found that cold hardiness occurs periodically, but can be induced by exposure to certain temperatures and humidities.

The studies of Payne (20) on (1) Aquatics animals (Ischmura, Gonipus and Symetrum, (2) Animals which attack stored-products (Tribolium confusicum and Sitophilus granarius, (3) Oak-borers (Synchioa punctata, Dendraides canadensis and various Cermbycidae) representing a group of insects which was exposed to extreme high and low temperature. She observed that fully hardened insects may survive the 1st but not the 2nd freezings.

Dr. T. P. Dooley and students (4) have made preliminary studies of low temperature tolerance of the Drosophila. He studied primarily the wild type fly (Drosophila melanogaster). The flies were subjected to temperatures ranging from -4°C to 13°C for a period of 5 minutes to 36 hours. He concluded that the Drosophila melanogaster

could withstand a -4°C for 10 minutes but were killed at this temperature when subjected for longer periods. When subjected to 0°C cor one hour they recovered, if the time is extended over a period of 9 hours, none recovered. 4°C for 16 hours, after 16 hours there is no recovery. Flies exposed to 13°C for 36 hours showed no effect of the temperature.

After reviewing the work of Dr. T. P. Dooley, the question arose in the writers mind do all species of the Drosophila have the same tolerance to low temperature.

The following species of Drosophila were used throughout the investigation: D-ana nassae, D-willistoni, D-virilus, D-mulleri, D-repleta, D-hydei, and D-melanogaster (stephenville wild), which were received from Dr. Patterson of the University of Texas to whom I would like to express my appreciation.

Material and Method of Procedure

The flies used in this study were obtained from a collection of lines of Drosophila maintained by Dr. Patterson, Head of Department of Experimental Genetics at the University of Texas for research purposes. The selection of species was based upon their ability to reproduce without difficulty at ordinary laboratory temperature. They were inbred for several generations before they were used. They were kept in small wide mouth culture bottles in an open laboratory at room temperature. An electric refrigerator was used as the chamber in which the flies were placed at the desired temperatures. To determine the temperature, several thermometers were placed in various

areas of the refrigerator in a manner so that the mercury was not touching any part of the refrigerator. The refrigerator was then adjusted in order to secure the desired temperatures. Wide mouth bottles (extra wide mouth), with mouth of bottle remaining unstoppered, were placed inside the refrigerator at various areas in which the temperature differed. Enough cotton to stopper the bottles were placed beside the bottles in refrigerator. The cotton and bottles were allowed to remain in refrigerator for not less than 6 hours so as to allow the bottles and the air in the bottles and the cotton to reach the same temperature as shown by the thermometer for that area. after bottles had been in the refrigerator not less than 6 hours, flies were introduced into the bottle by placing the mouth of the culture bottle into the mouth of bottle to be used as the cold chamber and shaken vigorously to displace the flies therein. This was to keep from taking bottles out of refrigerator and lessen a variation in temperature upon opening. The flies were kept there at the desired temperature for the desired period of time. At the end of the desired period of time, the bottles containing the flies were removed from the refrigerator and the flies were taken out of the bottles and placed either on a piece of filter paper or in a new culture bottle at room temperature and were kept at room temperature for observation. This observation was to note, if any, the effects of the temperature on various structures and function of the parts of the fly and to determine the time needed to recover after being exposed.

An explanation of terms used in the following tables are namely:

(a) Deactivation time - the time at which all flies ceased to move and remain so, unable to cling to wall of bottle, lying motionless on the floor thereof. (b) Recovery time - the space that elapses from the time of deactivation until the animal resumed movement, as indicated by moving the legs, wings, and crawling. (c) Recovery Behavior, the manner in which recovery was resumed. (d) Appearance of Deactivated Flies, the external appearance of body parts of flies that have been rendered inactive.

Observation and Results

D-virilus was killed when exposed to -4°C for 1 hour, however the flies were able to withstand all other temperatures for the time exposed. Deactivation time was immediate for temperatures ranging from - 4°C to 0°C. At higher temperature to which flies were exposed, a large portion of the flies had the ability to cling to the side of the container (see table I). Similar results were observed for D-mulleri (see table II).

D-melanogaster (stephenville wild) could not withstand an exposure -4°C for 30 minutes. Deactivation occured immediately with the exception of 8°C (see table III). Similar observations are valid in D-hydei (see table IV).

D-repleta evidenced results similar to those of D-melanogaster and D-hydei with the exception of 4 °C wherein approximately ½ of flies of this species showed ability to cling to the sides of container. (see table V).

D-ananassae was killed when exposed to -4°C for 30 minutes. At 0°C the organisms were killed only when exposed for 24 hours, the same was true for 4°C. Flies exposed to 8°C for 24 hours recovered

immediately when returned to room temperature. Immediate deactivation took place in all instances with the exception of 8°C where approximately only 3/4 of flies were completely deactivated. (see chart VI).

D-willistoni was killed when exposed to -4°C for 30 minutes. At 0°C the organisms were killed only when exposed for 24 hours, the same is valid at 4°C. The flies withstood 8°C. Deactivation in all instances occured immediately. At 8°C, approximately ½ of flies appeared to be normal with wings being paralell to body (but were completely deactivated). The wings of the others were perpendicular to body, bodies were bent and legs were drawn inward. (see table VII).

TABLE I - DROSOPHILA VIRILIS

Temp.	Deactivation Time	Time Exposed	Recovery Time	Percent Recovered	Number Of Flies	Recovery Behavior	Appearance Of Deactivated Flies
-4°C	Immediate	15 min.	20 min.	68.8	64	Flies would first get on feet and move around slowly unless agitated, wings and body were revolved in a rotary motion	Approximately 1/2 of flies wings were perpendicular to bodies, bodies bent, others appeared normal
-4°C	Immediate	30 min	35 min.	34.5	58	Same as above	Same as above .
-4°C	Immediate	1 hour	None		29		same as above
0°C	Immediate	6 hours	3 min.	100	26	Upon exposure to room temperature flies became active immediately	All flies wings were parallel to body
0°C	Immediate	12 hours	5 min.	100	35	Would move around slowly until agitated then would fly away	Approximately 1/2 of flies wings were perpendicular to bodies, bodies bent others were parallel
0°C	Immediate	24 hours	8 min.	100	29	Same as above	Same as above
+4°C	About 1/2 of flies had ability to cling to side of vessel, others were deactivated immediately	6 hours	2 min.	100	68	Upon exposure to room temperature flies became active immediately	All flies wings were paralell to body
+4°C	same as above	12 hours	3 min.	100	25	Same as above	Same as above
+4°C	Same as above	24 hours	5 min.	100	52	Same as above	Same as above
8°C	All flies had ability to cling to walls, none were entirely deactivated	12 hours	Immed.	100	25		
8°C	same as above	24 hours	Immed.	100	25		

TABLE II- DROSOPHILA MULLERI

Temp.	Deactivation Time	Time Exposed	Recovery Time	Percent Recovery	Number of flies	Recovery Behavior	Appearance Of Deactivated flies
-4°C.	Immediate	5 min.	10 min.	82.2	28	Feet would move first, then animal would stand on feet and move wings in a rotary motion	all wings were parallel to body, fly appeared to be living but deactivated
-4°C.	Immediate	15 min.	15 min.	32.8	61	same as above	same as above
-4°C.	Immediate	30 min.	10 min.	2.3	35	Same as above	Same as above
-4°C.	Immediate	1 hour	None		25		Same as above
0°C.	Immediate	6 hours	6 min.	100	36	Movement rather sluggish unless agitated, then fly would assume flight.	All wings were parallel to body, appeared to be living but completely deactivated
0°C.	Immediate	12 hours	25 min.	100	28	Same as above	Same as above
0°C.	Immediate	24 hours	45 min.	100	32	Same as above	Same as above
+4°C.	Approximately 3/4 cling to vessel	6 hours	Immediate	100	46	Same as above	Same as above
+4°C.	same as above	12 hours	Immediate	100	25	Same as above	Same as above
+4°C.	All flies had ability to cling to side of vessel	24 hours	Immediate	100	67	Same as above	Same as above
+8°C.	same as above	12 hours	Immediate	100	41		
+8°C.	Same as above	24 hours	Immediate	100	25		

TABLE III- DROSOPHILA MELANOGASTER (Stephenville Wild)

Temp.	Deactivation Time	Time Exposed	Percent Recovery	Number Of flies	Recovery Time	Recovery Behavior	Appearance Of Deactivated flies
-4°C.	Immediate	5min.	80.9	73	6 min.	Bodies were first to move, After which they would move around slowly with a little agitation flies would fly away.	Wings were perpendicular to bodies: bodies bent, feet appeared to be drawn inward
-4°C	Immediate	15min.	16.2	37	1 hour	Same as above	Same as above
-4°C.	Immediate	30min.		94	None		Same as above
0°C	Immediate	6hours	92.9	42	10min.	Same as above	Approximately 3/4 of flies wings were parallel to body
0°C.	Immediate	12hours	64.3	28	30min.	Same as above	Same as above
0°C.	Immediate	24hours	22.3	36	45min	Same as above	All wings were perpendicular to bodies, feet appeared to be drawn inward
+4°C.	Immediate	6hours	91.4	46	3min.	Same as above	Approximately 1/2 of flies Wings were parallel to bodies
+4°C.	Immediate	12hours	73.7	38	6min.	Same as above	Same as above
+4°C.	Immediate	24hours	41	61	15min.	Same as above	Same as above
+8°C.	All flies retained ability to cling to side of vessel	12hours	100	42	Immediate	Immediately upon exposure to room temperature, the flies would fly away	Same as above
+8°C.	Same as above	24hours	100	25	Immediate	Same as above	

TABLE IV- DROSOPHILA HYDEI

Temp.	Deactivation Time	Time Exposed	Recovery Time	Percent Recovered	Number of flies	Recovery Behavior	Appearance of Deactivated flies
-4°C.	immediate	5 min.	15 min.	70.6	34	would get on feet and move around very slowly. Flies appeared to roll over and over until agitated, then flight	All wings were parallel to body. Fly appeared to be alive, but completely Deactivated
-4°C.	immediate	15 min.	35 min.	50	42	Same as above	same as above
-4°C.	immediate	30 min.		None	29		same as above
0°C.	immediate	6 hours	10 min.	100	32	Same as above	same as above
0°C.	immediate	12 hours	23 min.	94.9	58	same as above	same as above
0°C.	immediate	24 hours	30 min	76.7	26	Same as above	same as above
+4°C.	immediate	6 hours	5 min.	82	25	Same as above	same as above
+4°C.	immediate	12 hours	10 min	89.9	59	Same as above	same as above
+4°C.	immediate	24 hours	20 min.	65.4	29	Same as above	same as above
+8°C.	All flies had ability to cling to sides of vessel	12 hours	immediate	100	27	Flies would become very active upon exposure to room temperature	
+8°C.	same	24 hours	immediate	100	26	Same as above	

TABLE V - DROSOPHILA REPLETA

Temp.	Deactivation Time	Time Exposed	Percent Recovered	Recovery Time	Number of flies	Recovery Behavior	Appearance Of Deactivated flies
-4°C.	Immediate	5 min.	56.4	3 min.	32	Bodies were first to move, would get on feet and move around slowly, when agitated, flies would roll over and over	wings were perpendicular to bodies, bodies bent, feet drawn inward.
-4°C.	Immediate	15 min.	53.9	25 min.	26	Same as above	Same as above
-4°C.	Immediate	30 min.	None		38		Same as above
0°C.	Immediate	6 hours	84	10 min.	25	Flies would move bodies first, get on feet, walk around very slowly, when agitated, flew away	All wings were parallel to bodies, bodies bent, feet drawn inward.
0°C.	Immediate	12 hours	50	30 min.	32	Same as above	All wings were perpendicular to bodies
0°C.	Immediate	24 hours	3.9	40 min.	26	Same as above	All wings were parallel to bodies Fly appeared normal, deactivated.
+4°C.	1/2 flies had ability to cling to walls of vessel	6 hours	100	3 min.	29	Same as above	All wings parallel to bodies Fly appeared normal, but was completely deactivated
+4°C.	Same	12 hours	68	7 min.	25	Same as above	Same as above
+4°C.	Same	24 hours	21.9	15 min.	32	Same as above	Same as above
+8°C.	All flies had ability to cling to walls of vessel	12 hours	100	immediate	25	upon exposure to room temperature, flies became active immediately	Same as above
+8°C.	Same	24 hours	100	immediate	25	Same as above	Same as above

TABLE VI- DROSOPHILA ANANASSAE

Temp.	Deactivation Time	Time Exposed	Recovery Time	Percent Recovered	Number of flies	Recovery Behavior	Appearance Of Deactivated flies
-4°C.	Immediate	5 min.	3 min.	80	35	Wings were first to move, got on feet and rolled over and over	wings were perpendicular to bodies, feet drawn inward, bodies bent
-4°C.	Immediate	15 min.		None	28		Same as above
0°C.	Immediate	6 hours	15 min.	91.8	48	Bodies were first to move, when agitated, flies would roll over and over	Wings were parallel to bodies, flies seemed normal, but immobile
0°C.	Immediate	12 hours	35 min.	37.9	32	Same as above	Same as above
0°C.	Immediate	24 hours		None	36		wings were perpendicular to bodies, flies appeared normal but were completely deactivated
+4°C.		6 hours	3 min.	84	25	Same as above	Same as above
+4°C.	Immediate	12 hours	5 min.	65.9	38	Same as above	Wings were perpendicular to bodies, feet drawn inward, bodies bent
+4°C.	Immediate	24 hours	2 hours, 40 min.	None	42	3 flies moved their wings and bodies but died when agitated	wings were parallel to body
+8°C.	3/4 flies were deactivated	12 hours	35 min	100	32	would get on feet and walk around very slowly, when agitated, flew away	Same
+8°C.	Same	24 hours	50 min	100		Same	Same

TABLE VII- DROSOPHILA WILLISTONI

Temp.	Deactivation Time	Time Exposed	Recovery Time	Percent Recovered	Number of flies	Recovery Behavior	Appearance of Deactivated flies
-4°C.	immediate	5 Min.	3 min.	82.4	34	Flies rolled over and over, wings moving rapidly.	wings were perpendicular to body, Bodies were bent, feet drawn inward
-4°C.	immediate	15 Min.	10 min.	70.4	27	Same as above	Same as above
-4°C.	immediate	30 Min		None	48		Same as above
0°C.	immediate	6 hours	15 Min.	47.8	67	Same as above	same as above
0°C.	immediate	12 hours	25 Min.	47.8	41	Same as above	same as above
0°C.	immediate	24 hours		None	28	Same as above	Same as above
+4°C.	immediate	6 hours	20 Min	84.7	78	same as above	same as above
+4°C.	immediate	12 hours	35 min	17.7	34		Same as above
+4°C.	immediate	24 hours	immediate	None	59		Same as above
+8°C.	immediate	12 hours	immediate	100	43	Flies would become active immediately upon exposure to room temperature	About 1/2 of wings were perpendicular to bodies, bodies bent, feet drawn inward
+8°C.	immediate	24 hours	immediate	100	64	Same	same

DISCUSSION

Of the seven species of flies used in this experiment: D-mulleri, D-virilus, D-repleta, D-hydei, D-willistoni, D-ananassae, D-melanogaster (stephenville wild) the species D-mulleri has a higher percentage of recovery than other species for the first 5 minutes at $-4^{\circ}C$. (see table II). This however, is not the case when the time of exposure is increased to 15 minutes, the species D-willistoni, D-repleta and D-hydei can be seen to exhibit the greatest amount of recovery. (tables IV, V, and VII).

It is of interest to note that the species D-melanogaster (stephenville wild), D-willistoni, D-hydei, D-repleta, D-ananassae were not able to withstand $-4^{\circ}C$ for 30 minutes. Although D-mulleri was able to withstand a $-4^{\circ}C$ for 30 minutes, the percentage of recovery was 2.3 % which is comparatively a small number.

For temperatures ranging from $0^{\circ}C$ to $4^{\circ}C$, a greater degree of recovery in the species D-virilus and D-mulleri is noted, both showing recovery in all instances (tables I and II). For the species D-hydei, D-repleta and D-melanogaster (stephenville wild), somewhat of a gradient is observed, almost directly proportional to the increase in temperature. This is not the case for D-ananassae and D-willistoni (tables VI and VII), when the time of exposure is increased to 24 hours at $0^{\circ}C$ and $4^{\circ}C$, there is no recovery.

All species recovered after having been exposed to $8^{\circ}C$ for 24 hours or more.

Of the 7 species of Drosophila used in this study they may be divided into 2 groups according to size. D-virilus, D-mulleri, D-hydei,

and D-repleta constitute a group of larger size of flies. The smaller size flies were composed of the following species: D-willistoni, D-ananassae and D-melanogaster (stephenville wild).

The question may immediately arise in the readers mind, are there differences in tolerance according to size.

Horvath in his studies found that in warm blooded animals there is no consistency of tolerance relative to size. He found that in some instances the smaller animal would have a shorter survival time, then in another group a larger animal would have a shorter survival time. This is seen in the case where a mouse of 19 grams had a survival time of .4 hours, whereas on the other hand a rabbit weighing 1, 774 grams could withstand the temperature for 5.0 hours. In contrast to this a pigeon weighing 408 grams, approximately 4 times lighter than the rabbit had a survival time of 48 to 78 hours. Approximate survival time greater than 10 times that of the rabbit. In my observations, the smaller group as a whole is not able to withstand low temperatures as the larger group, but, the species D-melanogaster (stephenville wild) of the smaller group gives evidence of being able to withstand cold temperature comparable to the species D-repleta of the species comprising the larger group (tables III and V). There also appear to be a difference within species to tolerate low temperature. This is seen where all members of a number of exposed flies are not killed when exposed to the same temperature. This is somewhat similar to Horvath when he used a group of Wistar albino rats varying in sizes from 242 grams to 227 grams. The latter, which is 15 grams

lighter showed an ability to withstand lower temperatures excelling that of the former while this information is in no w ay conclusive, it is of primary importance to the reader to note the amount of variance in regard to temperature tolerance in regard to animals within the species. No studies were made within species asto sex, nor age, however, it is possible that these factors may effect the ability of an organism to tolerate different temperatures.

Summary and Conclusion

1. That the D-virilus and D-mulleri can withstand a $-4^{\circ}C$ for not more than one hour. D-hydei, D-willistoni and D-melanogaster (stephenville wild) and D-repleta for not more than 30 minutes. D-ananassae for not more than 15 minutes.

2. D-virilus, D-mulleri, D-hydei and D-melanogaster (stephenville wild) can withstand a $0^{\circ}C$ for 24 hours. D-ananassae and D-willistoni can withstand a $0^{\circ}C$ for not more than 12 hours.

3. D-mulleri, D-hydei, D-virilus, D-repleta, and D-melanogaster (stephenville wild) can withstand a $4^{\circ}C$ for 24 hours. D-ananassae and D-willistoni for not more than 12 hours.

4. All species exposed to an $8^{\circ}C$ for 24 hours or longer showed no effect of the temperature.

5. That there is variation according to species.

6. Variation within species.

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