THE EFFECT OF TEMPERATURE ON THE MUTATION RATE IN *DROSOPHILA MELANOGASTER*

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Summary

The incidence of sex-linked recessive lethal mutations in *Drosophila melanogaster* after heat shock treatment of both larvae and adult males is reported. There was no increase in the mutation rate after treatment of larvae and the results with adult males were not consistent. Treatment of the latter at 38°C caused an increase in mutation rate, due apparently to the large response of a few sensitive males. Treatment at 40°C caused no increase, and if one sensitive male was excluded, the mutation rate was significantly less than control. These results do not entirely support those of previous workers in the literature and possible reasons for this are discussed.

The mutation rate has also been studied, over a series of successive daily mating periods, of males undergoing development at three different temperatures. There was a significant regression (both linear and quadratic) of mutation rate on age, mutation rate decreasing with age of male, and this age effect did not differ between temperatures.

The linear regression of mutation rate on temperature was significant, mutation rate *decreasing* with increased temperature. Previous results in the literature have supported the opposite conclusion that mutation rate *increases* with increased temperature. It was postulated that the previous results may have been due to confounding with the effect of temperature on storage of mature sperm. The present results indicate that temperature during development has no direct effect on the mutation rate, since the higher rates with lower temperature are probably a function of longer developmental time at the lower temperature.

I. INTRODUCTION

Plough (1941) reviewed the early literature on the influence of temperature on the mutation rate in *Drosophila melanogaster*, the first demonstration of an effect having been given by Muller with the introduction of his methods for the detection of lethal mutations.

The results of Muller (1928), Timofeeff-Ressovsky (1935), and Plough (1939) (the latter as reported in Plough (1941)), all showed that the mutation rate increases with increased temperature during development. Further results of Buchmann and Timofeeff-Ressovsky (1935, 1936) and Plough, Child, and Ives (Plough 1941) showed increased mutation rates after high temperature shocks for short periods both in larval and adult stages. Birkina (1938) and Kerkis (1941) obtained increases in the mutation rate after extremely low temperature shocks. The latter finding was supported by the results of Byers (1954), but Rendel and Sheldon (1956), in attempting to repeat the work of Birkina and Kerkis, obtained completely negative results.

In view of the many results showing an affect of age of adult and storage of sperm (Muller 1946; Lamy 1947; Mossige 1955), and temperature during sperm storage (Byers and Muller 1952; Byers 1954) on mutation rate, it is apparent that

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in many of the earlier experiments the effects due to temperature could have been confounded with the effects of some or all of these other processes. Rendel and Sheldon (1956) made allowances for this difficulty to some extent by ensuring that as far as possible control and treated flies were of about the same age group when mated to a tester stock. The same procedure has been followed in the heat shock experiments reported here, while the design of the present experiment on temperature during development attempts to remove completely any confounding with age of adult or storage of sperm.

Previous results showing an increase in mutation rate with increased temperature during development have generally been explained in terms of increased rates of chemical reactions at the higher temperatures. Results with temperature shocks (high and low) were not interpreted in the same way and a completely different set of phenomena was thought to be involved. The present experiments were planned firstly to repeat and extend the heat shock work of Buchmann and Timofeeff-Ressovsky, and secondly to study the effect of temperature during development, with the confounding effects of age of adult, storage of sperm, and different temperatures during such storage removed. From this detailed study of temperature during development, stages in the life cycle more mutagenically sensitive to such treatments and those most susceptible to temperature shocks may be found.

II. Materials and Methods

The flies used in these experiments came from the same Oregon R-C wild-type stock of *D. melanogaster* as was used by Rendel and Sheldon (1956), and the same control results of spontaneous mutation rate at 25°C have been used here for comparison with results under the different heat shock treatments. Mutation rate in all cases was scored as the percentage of sex-linked recessive lethals, obtained by the standard procedure of mating males to be tested to females of a tester stock (Muller–5). An F_2 culture was scored as a lethal if no red-eyed males occurred out of 30 or more adults. In doubtful cases the test was carried on to an F_3. All cultures were kept at 25°C except during actual treatments.

(*a*) *Heat Shock Treatments*

Heat shock was applied to both larvae and adult males. In the former case adult females were allowed to lay eggs in ordinary culture bottles for a 1-hr period during the afternoon and then removed. Cultures were inspected the following morning when many larvae were present. Heat shock treatment was applied to these cultures exactly 3 days later, so that treated larvae were in their fourth day of life. The cultures were treated in a water-bath at 36·5–38°C for periods of 1, 6, 12, and 24 hr (Buchmann and Timofeeff-Ressovsky (1935, 1936) had treated 3–5-day-old larvae at 35–38°C for 12–24 hr). Of the adults arising from the treated cultures only the males were tested for mutation rate.

For the heat shock treatment of adult males, the flies were placed either in sealed ampoules or empty stoppered 4 by 1-in. specimen tubes in water-baths at 38°C for 15 min, or at 40°C for 15–30 min (Buchmann and Timofeeff-Ressovsky had treated males and females at 36–39°C for 12–24 hr).

Except where otherwise indicated males were a random group up to 4 days old when mated to tester females in a single test-mating for each male. Usually one but

sometimes two tester females were allowed to each male. When adult males were treated they were mated immediately after treatment.

(b) Temperature during Development

Adult females were allowed to lay eggs in a number of culture bottles for a 1-hr period at 25°C, after which they were removed, and several cultures were then placed in constant temperature rooms at each of three different temperatures—20, 25, and 30°C. The first progeny emerged at 13–14, 9–10, and 7 days respectively. The first 30, 24, and 20 males emerging were immediately set up individually with about eight Muller–5 tester females. At 24-hr intervals the males were transferred to fresh batches of tester females, the number of females used varying from eight in the first few days down to five or six at about the fifteenth day. This type of mating

TABLE 1

MUTATION RATES AFTER HEAT SHOCK OF LARVAE

Treatment on Fourth Day		No. of *X*-chromosomes Tested	No. of Recessive Lethals	Lethals (%)	Fiducial Limits (5% level)
Temperature Range (°C)	Time (hr)				
Control		6410	15	0·23	0·13–0·39
36·5–38	1	1489	7	0·47	0·19–0·97
36·5–38	6	1387	3	0·22	0·05–0·63
36·5–38	12	1425	4	0·28	0·08–0·72
36·5–38	24	None survived	—	—	—

procedure was followed to ensure as far as possible that each day a newly matured sample of sperm was being tested (Mossige 1955), and so avoid confounding of the main age and temperature effects by the effects of storage of sperm at different temperatures.

All test matings were carried out at the temperature at which the males developed. In other words, males developing at 30°C would spend the rest of the period of the experiment at that temperature except for the short time each day involved in transferring to fresh batches of females. The latter was all done at room temperature which on most occasions was not greater than 25°C. After removal of the males the testing procedure beginning with fertilized tester females was carried through to the F_2 at 25°C.

The three different temperature treatments were done at different times, because the available laboratory facilities would not allow them to be handled concurrently. For similar reasons in the 25 and 30°C treatments males could be tested only till the fifteenth day of age, whereas in the 20°C treatment they could be tested till the twenty-second day.

III. RESULTS

(a) Heat Shock

The results obtained after heat shock in the larval stage are given in Table 1. Whereas Buchmann and Timofeeff-Ressovsky indicated that they had obtained some results after a shock period of 24 hr, this could not be repeated in the present

experiment, as larvae so treated all died. No attempt was made to get mortality rates for larvae in the other treatment periods, but it was observed that adults were over a day later emerging in the 12-hr treated cultures than in the 1- or 6-hr treatments. In addition, less adults were obtained from the cultures treated for 12 hr. The main feature of the results is that none of the treatments differs significantly from the control. There is an indication of an increase (not significant, $\chi_1^2 = 1{\cdot}6$) for the larvae treated for 1 hr and this will be considered further in Section IV.

Table 2 shows the results obtained after heat shock of adult males. The treatment at 40°C was carried out because it was close to the most extreme shock possible without killing all flies. Mortality under this treatment varied between 20 and 50 per cent., and fertility of survivors was greatly reduced, up to 70 per cent. being completely sterile. After treatment at 38°C for 15 min all males survived and their fertility was not obviously affected.

TABLE 2

MUTATION RATES AFTER HEAT SHOCK OF ADULT MALES

Treatment	No. of *X*-chromosomes Tested	No. of Recessive Lethals	Lethals (%)	Fiducial Limits (5% level)
Control	6410	15	0·23	0·13–0·39
38°C for 15 min				
Replicate 1	2468	28	1·13	0·76–1·64
Replicate 2	1533	7	0·46	0·18–0·94
40°C for 15–30 min				
Replicate 1	918	1	0·11	0·003–0·61
Replicate 2	1809	0	0·00	0·00–0·20
Replicate 3	1922	8	0·42	0·18–0·82

There is a significant difference between the two replicates of the 38°C treatment ($\chi_1^2 = 6{\cdot}1$), the very high value for the first replicate being due to the extremely high mutation rate of two of the males out of the 40 used in this replicate. Thus one of these males had nine recessive lethals out of 35 tested *X*-chromosomes, and the other had 12 lethals out of 77 tested *X*-chromosomes. This marked clumping of lethals did not occur in the second replicate, where only one male out of 55 treated had more than 3 per cent. lethals and then only four lethals out of 40 tested *X*-chromosomes. Replicate 1 is significantly higher than the controls ($\chi_1^2 = 16{\cdot}5$, $P<0{\cdot}001$), but there is no significant difference between replicate 2 and controls ($\chi_1^2 = 1{\cdot}5$).

If the results of the 40°C replicates are pooled, the result (0·19 per cent. lethals) is not significantly different from control ($\chi_1^2 = 0{\cdot}205$). There is, however, a significant difference among the three replicates of this treatment ($\chi_2^2 = 8{\cdot}94$, $P<0{\cdot}02$). Replicates 1 and 3 are not significantly different from control, but replicate 2 is significantly lower than the control at the 0·1 per cent. level ($\chi_1^2 = 14{\cdot}7$).

(*b*) *Temperature during Development*

The results for the three treatments over all ages tested are given in Table 3. The values for the number of *X*-chromosomes tested give some indication of the

TABLE 3

EFFECT OF AGE OF MALE AND TEMPERATURE DURING DEVELOPMENT ON THE MUTATION RATE

Age of Males (days)	20°C			25°C			30°C		
	No. of *X*-chromosomes Tested	No. of Recessive Lethals	Lethals (%)	No. of *X*-chromosomes Tested	No. of Recessive Lethals	Lethals (%)	No. of *X*-chromosomes Tested	No. of Recessive Lethals	Lethals (%)
1	126	1	0·79	615	3	0·49	310	4	1·29
2	482	4	0·83	1010	6	0·59	900	2	0·22
3	773	5	0·65	1036	3	0·29	797	—	—
4	901	1	0·11	939	4	0·43	826	1	0·12
5	1007	1	0·10	849	3	0·35	646	—	—
6	1082	3	0·28	1000	1	0·10	638	2	0·31
7	535	—	—	929	2	0·22	453	—	—
8	435	—	—	821	1	0·12	214	—	—
9	670	1	0·15	756	—	—	172	—	—
10	672	—	—	744	1	0·13	147	—	—
11	533	2	0·38	617	—	—	32	—	—
12	499	1	0·20	613	—	—	46	—	—
13	555	—	—	552	1	0·18	21	—	—
14	440	1	0·23	545	—	—	13	—	—
15	278	—	—	446	—	—	—	—	—
16	292	—	—						
17	282	1	0·35						
18	294	—	—						
19	294	—	—						
20	251	—	—						
21	194	—	—						
22	269	—	—						
To 15th day	8988	20	0·223	11,472	25	0·218	5215	9	0·173

decline in fertility with age in all three treatments, since the experiment was planned to test approximately 1000 chromosomes per day per treatment, granted normal fertility, from 20–30 males per treatment. This drop in number of chromosomes tested was, however, not only a reflection of lower fertility with age under these conditions of mating, but was also due in part to a certain proportion of flies dying or being lost during transfers over the course of the experiment. The much lower number of tested chromosomes in the 30°C treatment was due to an extreme degree of infertility and mortality in the adult stage at this temperature.

TABLE 4

ANALYSIS OF VARIANCE OF SQUARE ROOT TRANSFORMATIONS OF PERCENTAGE LETHAL FREQUENCIES IN TABLE 3

Source of Variation	D.F.	Mean Square	*F*
Between temperatures	(2)		
Linear	1	0·3435	5·90*
Quadratic	1	0·0495	0·85
Between ages	(14)		
Linear	1	1·9421	33·37***
Quadratic	1	0·4230	7·27*
Remainder	12	0·0282	
Interaction			
Linear × linear	1	0·0024	0·04
Linear × quadratic	1	0·0012	0·02
Quadratic × linear	1	0·0114	0·20
Quadratic × quadratic	1	0·0900	1·55
Error	24	0·0582	

*$P<0·05$. ***$P<0·001$.

Analysis of variance of the square root transformations of the percentage lethal figures up to the fifteenth day was carried out using orthogonal polynomials, and the result of this analysis is given in Table 4. The significant linear component of the between-temperatures mean square shows that the *decrease* in mutation rate with *rise* in temperature is significant and linear. The difference between mutation frequencies at different ages is highly significant, and is shown to have a significant quadratic as well as a very highly significant linear component. The quadratic component is due to the initial decrease in mutation rate over the first few days being followed by a rather constant rate, fluctuating around 0·1–0·2 per cent. approximately, depending on the temperature. Finally, the absence of significant interaction terms indicates that (i) the regression of mutation rate on temperature does not vary with age, and (ii) the regression of mutation rate on age does not vary with temperature. Figure 1 shows the fitted regression lines for the regression of mutation rate on age of male for the three different temperatures used in this experiment.

IV. Discussion

(*a*) *Heat Shock*

In Table 1 there is slight increase, though not significant, in the mutation rate for the 1-hr treatment of larvae at 3 days of age. This can readily be explained, even

if the figure were significantly higher than the other two larval treatments, because males arising from this particular treatment were mated in error as a group at 1–2 days of age instead of the usual procedure of 1–4 days old. This could have caused the slight rise in mutation rate in the 1-hr treatment.

The real difference between the 38°C replicates in Table 2 cannot be so easily explained. However, the higher rate in replicate 1 is undoubtedly due to the clumping of lethals in two particular treated males. The reason for this clumping is by no means clear. If males having more than, say, 10 per cent. lethals among their tested chromosomes are arbitrarily removed from the 38°C treatments, then the resulting mutation rates are approximately the same as control. It appears then that only a low proportion of males has sperm of high mutability when exposed to heat shock, and that the chance inclusion of two of these in replicate 1 is responsible

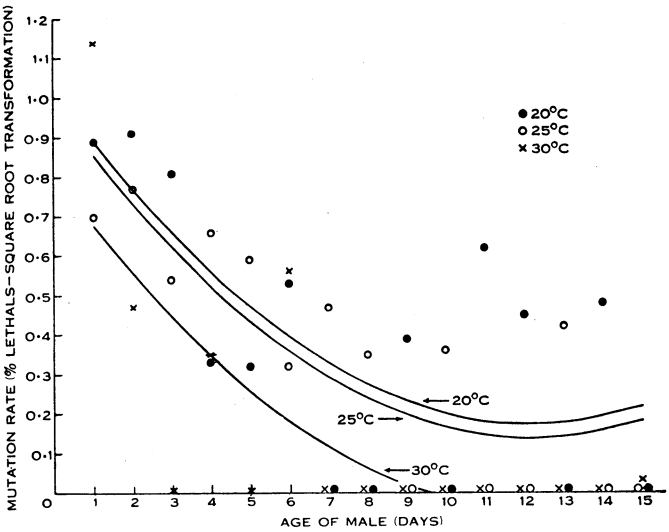

Fig. 1.—Fitted regression lines for the regression of mutation rate on age of male for the three different temperatures used.

for the difference between it and replicate 2. Such clumping of lethals is not a feature of a number of control series for determining spontaneous rates of adult males, at least within the author's experience, and response to heat shock might be simply a function of the number, if any, of sensitive males included in the treated samples.

The latter argument is supported by the results of heat shock at 40°C. Here again the higher figure for replicate 3 of the series is due to a particular male having seven lethals out of 31 tested X-chromosomes. If this male is excluded, then the resulting overall mutation rate at 40°C (0·04 per cent.) is significantly lower than controls ($\chi_1^2 = 9{\cdot}7$, $P < 0{\cdot}01$), as in the second replicate. In other words, shock of adult male sperm at extreme sublethal high temperature causes a drop in mutation rate for the majority of individuals but the usual increase in the 2 or 3 per cent. of sensitive flies. Reasons for such a decrease in mutation rate are not obvious. Two possibilities are that lethal-bearing sperm or even mutable males are killed by the

treatment, but in absence of general supporting evidence this must remain conjecture, especially as the sperm of one male survived the treatment to give 22 per cent. lethals.

It is not clear from Buchmann and Timofeeff-Ressovsky (1935, 1936), or from Plough (1941), whether their increases in mutation rate after heat shock of adult males were similarly due to clumping of lethals in a few sensitive individuals. The results reported here for adult males do not support their general conclusion that heat shock of adults necessarily increases the mutation rate. As indicated above, a number of conditions, including a specific temperature treatment, the presence and survival of highly mutable individuals, and the viability of lethal-bearing sperm apparently need to be fulfilled before an increase in mutation rate is obtained, as in replicate 1 at 38°C in this experiment. The possibility remains that the large number of mutations in the sensitive males might be the result of single or a few mutations occurring in spermatogonial cells. Since, however, males were test-mated immediately after treatment, it is unlikely that mutations occurring in spermatogonial stages in response to the treatment would have been represented so soon in the sperm sampled in this way. It is much more likely that the mutations obtained arose from the treatment of mature sperm, since these were the only treatments in which such clumping of lethals was found.

The present negative results on heat shock of larvae similarly do not support the finding of Buchmann and Timofeeff-Ressovsky. However, if a stage in the life cycle sensitive to heat shock is involved, it is quite possible that these workers, with their slightly wider range of larval ages during treatment covered the sensitive period, while the present experiment did not. A further explanation could be that the difference between the two sets of results is merely a strain difference, since it is generally known that different wild strains differ, at least in spontaneous mutability. Plough (1941) produced some evidence that only certain stocks with a high spontaneous mutation rate did not respond to heat shock. As the stock used here has an average spontaneous mutation rate, it is unlikely that its lack of response to heat in the shock larval stages is peculiar to this particular strain. It is far more likely that a sensitive stage has been missed in the present experiment, or that some other unknown variable was responsible for the increases obtained by the previous workers.

(*b*) *Temperature during Development*

Previous studies had produced evidence to show that the mutation rate increases with increased temperature during development, and the data were interpreted in terms of van't Hoff's rules on the rates of reactions (Muller 1928; Timofeeff-Ressovsky 1935; Plough 1941). Increased mutation rate, in other words, was due to the increased rates of chemical reactions at the higher temperatures. The data reported here, however, in showing that mutation rate *decreases* with increasing temperature during the life cycle, renders the above interpretation untenable, and alternative explanations must be sought for the divergent results.

The present experiment made use of a mating scheme to ensure that, as far as possible, sperm were completely utilized as they matured. The results, therefore, are not confounded with environmental effects after maturation of sperm. Since Muller (1946) has reported that aging of mature sperm leads to an increase in mutation rate, and Byers (1954) has reported that this effect of aging is increased at

higher temperatures, it is possible that the earlier results of Muller, Timofeeff-Ressovsky, and Plough quoted above can be interpreted in terms of these environmental effects after maturation of sperm. The results reported here show that the developmental processes up to maturation of sperm do not respond to increased temperature by increased mutation rates. The significantly lower mutation rate with increased temperature can perhaps best be explained by the longer time period of development at lower temperatures, which would allow longer exposure, perhaps of sensitive stages, to other mutagenic agents. If the latter explanation is correct, then the possibility that increase in temperature is causing some increase in mutation rate cannot be ruled out. It is, however, obvious that such effects, if they exist, are completely over-shadowed by the indirect developmental effects of temperature in the opposite direction, and hence must be small in magnitude. Since it is difficult to postulate reasons for increased temperature *per se* being the cause of lower mutation rate, except perhaps in terms of a temperature-dependent balance between different chemical reactions, the above explanation based on developmental time appears to be a simple and reasonable one.

The results on effect of age in this experiment support the findings of Muller (1946) and other workers that the mutation rate in the sperm of the first few days is much higher than in later sperm, provided of course that sperm are used as they mature. The data also give some indication that the regressions (linear or quadratic components or both) of mutation rate on age vary with temperature, but this is not supported by the analysis of variance.

(*c*) *General*

As indicated in the Introduction, one of the aims of these experiments was to relate responses obtained under heat shock treatments to those obtained when temperature during development was the variable. No obvious relationship can be observed from the data.

Temperature during development was varied only within a fairly narrow range (20–25–30°C) and responses obtained were apparently variations on a fairly random mutation process. No evidence of any highly mutable individuals was obtained, except perhaps for one male arising from the 30°C treatment. This individual scored three lethals out of 18 chromosomes tested, i.e. 17 per cent. lethals, on the first day, but no further lethals out of about 280 chromosomes tested on subsequent days up to the eleventh day. There is some indication, therefore, that this individual was sensitive to higher temperature in some pre-adult stage, but not after emergence, as were the particular males in the heat shock experiment. It must be remembered, however, that the sperm treated by heat shock were an older, and certainly more heterogeneous sample than sperm tested in the temperature-during-development experiment, unless the sperm present at the time of emergence of the adult are a relatively older sample than those newly matured sperm tested on the second and following days. If the latter point is a critical one then the initial response shown by the one sensitive individual at 30°C may bear some relationship to the type of response obtained with heat shock, but further evidence on all these aspects is needed before more definite conclusions can be drawn.

The general conclusion that mutation rate decreases with increased temperature during development, probably as an indirect effect of temperature on the length of the life cycle, follows readily from the data. Nevertheless, the weight of evidence in the literature supports the opposite view and the possible explanations of the difference are not entirely satisfactory. Further experiments on other strains along the lines of the design used here, and at the same time extending the range of temperatures used, should help to clarify the problem.

The experiment on heat shock in the larval stage covered only one well-defined period. Because of the negative correlation obtained between mutation rate and temperature during development, no evidence was obtained on whether any stages in the life cycle are more sensitive to heat shock. Evidence on this point will probably be provided only by heat shock treatments over a large number of short, well-defined periods in the life cycle.

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VI. References

Birkina, B. N. (1938).—The effect of low temperature on the mutation process in *Drosophila melanogaster*. *Biol. Zh., Moscow* **7**: 653–60. (In Russian with English summary.)

Buchmann, W., and Timofeeff-Ressovsky, N. W. (1935).—Über die Wirkung der Temperatur auf den Mutationsprozess bei *Drosophila melanogaster*. II. Behandlung der Männchen mit Temperaturschocks. *Z. indukt. Abstamm.-u. VererbLehre* **70**: 130–7.

Buchmann, W., and Timofeeff-Ressovsky, N. W. (1936).—Über die Wirkung der Temperatur auf den Mutationsprozess bei *Drosophila melanogaster*. III. Behandlung der Weibchen mit Temperaturschocks. *Z. indukt. Abstamm.-u. VererbLehre* **71**: 335–40, 2 figs.

Byers, Helen Louise (1954).—Thermal effects on the spontaneous mutation rate in mature spermatozoa of *D. melanogaster*. Proc. 9th Int. Congr. Genet. Bellagio, 1953. pp. 694–6.

Byers, Helen L., and Muller, H. J. (1952).—Influence of aging at two different temperatures on the spontaneous mutation rate in mature spermatozoa of *D. melanogaster*. *Rec. Genet. Soc. Amer.* **21**: 14–15; *Genetics* **37**: 570–1.

Kerkis, J. (1941).—The effect of low temperature on the mutation frequency in *D. melanogaster* with consideration about the causes of mutation in nature. *Drosophila Inform. Serv.* **15**: 25.

Lamy, Rowena, (1947).—Observed spontaneous mutation rates in relation to experimental technique. *J. Genet.* **48**: 223–35.

Mossige, Jeanne Coyne (1955).—Sperm utilisation and brood patterns in *Drosophila melanogaster*. *Amer. Nat.* **89**: 123–7.

Muller, H. J. (1928).—The measurement of gene mutation rate in *Drosophila*, its high variability, and its dependence upon temperature. *Genetics* **13**: 279–357.

Muller, H. J. (1946).—Physiological effects on "spontaneous" mutation rate in *Drosophila*. *Rec. Genet. Soc. Amer.* **14**: 55; *Genetics* **31**: 225.

Plough, H. H. (1939).—*Collecting Net* (*Woods Hole*) **14**: 1–6.

Plough, H. H. (1941).—Spontaneous mutability in *Drosophila*. *Cold Spr. Harb. Sym. Quant. Biol.* **9**: 127–37.

Rendel, J. M., and Sheldon, B. L. (1956).—The effect of cold treatment on mutation in *Drosophila melanogaster*. *Aust. J. Agric. Res.* **7**: 566–73.

Timofeeff-Ressovsky, N. W. (1935).—Über die Wirkung der Temperatur auf den Mutationsprozess bei *Drosophila melanogaster*. I. Versuche innerhalb normaler Temperaturgrenzen. *Z. indukt. Abstamm.-u. VererbLehre* **70**: 125–9.