SPERM COMPETITION AND SPERM LOSS IN
DROSOPHILA MELANOGASTER FEMALES
FERTILIZED BY $Y^S X \cdot Y^L / 0$ MALES

G. OLIVIERI, G. AVALLONE AND L. PICA

Istituto di Genetica, Città Universitaria, Rome, Italy

Received May 16, 1969

IT is generally assumed that in animal species all the sperm from a heterozygote, though genetically different, have the same functional properties; consequently they cannot compete in fertilization, and no selection is possible. This has been interpreted as meaning that genes stop functioning after the first meiotic division (MULLER and SETTLES 1927). An exception to this principle has been described in the mouse where some alleles at the *T* locus are recovered with higher frequency. This observation is in line with the hypothesis that such a differential recovery might be due to different functional properties which alleles confer on the gametes carrying them (BRADEN 1958). Later experiments (YANAGISAWA, DUNN and BENNETT 1961) seem to have confirmed this hypothesis. Recently, McCLOSKEY (1966) has extended MULLER and SETTLES' experiments in *Drosophila*. This author has shown that no competition exists between sperm having two right arms or two left ones of the same autosome and the other arm completely missing.

During the last few years we have been examining the question of whether, in *Drosophila*, sperm carrying both *X* and *Y* chromosomes (attached-*XY* or *XY* sperm) and sperm without either (0 sperm) have the same physiological properties. The results are in line with the idea that there are functional differences between the two kinds of sperm. A part of this work has already been published in summary form (OLIVIERI, PICA and OLIVIERI 1964).

Since this manuscript was submitted for publication, it was pointed out that similar results were reported in an unpublished thesis for the Master of Arts degree at The University of Oregon by the late J. D. McCLOSKEY. He used several types of *X*·*Y* chromosome in one of which he found results similar to those reported here. In another *X*·*Y* type as well as in the male with the normal chromosome arrangement he found no change in the sex ratio with age.

MATERIALS AND METHODS

Males from two different stocks were used: *In(1) EN·Y^S car f v γ·Y^L* males for Experiments 1, 2, 3, 4 and *In(1) EN·Y^S v cv γ·Y^Lγ⁺* males for Experiments 5, 6, 7. Both these males produce two kinds of sperm—sperm with two heterochromosomes (*XY* sperm) and sperm without heterochromosomes (0 sperm). The cultures from which the experimental flies were derived were kept at $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$, at which temperature the two kinds of sperm have been recovered in the same proportion (ZIMMERING 1962). In the various experiments the males were isolated soon after

eclosion and aged at $20 \pm 1^\circ\text{C}$ for 3–7 days. They were then individually mated with an Oregon-R, virgin female, 3–5 days old, and kept together either until mating was observed (Experiments 1,2,3,4) or for a whole night (Experiments 5,6,7). The males were then discarded. The females were kept at $25 \pm 1^\circ\text{C}$ and transferred into fresh medium, every 2 or 3 days, until all the sperm stored were used. In all experiments except Experiment number 2, the mating scheme used permitted the scoring of the progeny of individual females. In Experiment 2, the progeny were derived from 5–6 females ovipositing in the same culture. Since the progeny consisted of sterile XO males and XXY females, a sex ratio (proportion of males) higher than 0.50 indicates that a higher number of 0 sperm than XY ones were used; the opposite is true for a sex ratio lower than 0.50.

In Experiments 5, 6, and 7, soon after insemination a sample of females was stored in vials containing a glucose-agar medium to prevent oviposition for the desired length of time —eg., in the AG 5 series, females were kept in glucose-agar medium for 5 days.

The statistical analysis of the data was carried out using linear regression between deposition time (x) and the increase of sex ratio noticed in single females (y). The sex ratio in the first period of deposition was set equal to 100 and those in the succeeding periods were estimated in relation to it. Sex ratio was calculated on a progeny size of at least 20. If one brood from a female had a progeny of less than 20, two successive broods were summed and considered as a single deposition period.

RESULTS

The results of the first four experiments are summarized in Table 1. It can easily be seen that the sex ratio is consistently higher in the later sperm-sampling periods than it is in the first one. The results of a regression analysis of the data (Tables 2 and 3, Figure 1) clearly indicate that such a trend is significant—pointing to the conclusion that the female did not use both kinds of sperm in the same proportion throughout the deposition periods.

TABLE 1

Sex ratios in successive broods produced from Oregon-R females following single matings with In (1) EN·Y^S car f v y · Y^L males

Expt. No.	Number females tested	Sex ratio of progeny produced in days following fertilization						Totals
1	12	Days	(1–3)	(4–6)	(7–11)			
		Males	399	458	326			1183
		Females	530	354	268			1152
		Sex ratio	0.43	0.56	0.55			0.51
2	30	Days	(1)	(2)	(3)	(4)	(5–6)	(7–12)
		Males	246	649	482	675	523	140
		Females	236	575	401	493	385	98
		Sex ratio	0.51	0.54	0.55	0.58	0.58	0.59
3	14	Days	(1–2)	(3–4)	(5–7)	(8–12)		
		Males	284	472	638	460		1854
		Females	326	542	459	270		1597
		Sex ratio	0.47	0.47	0.58	0.63		0.54
4	18	Days	(1–2)	(3–4)	(5–6)	(7–12)		
		Males	319	113	400	282		1114
		Females	391	174	322	229		1106
		Sex ratio	0.45	0.39	0.55	0.55		0.55

TABLE 2

Regression of percent increment in sex ratio among progeny of single females on days following fertilization

Expt. No.	Regression line	Student's t	df	P
1	$y = 100.194 + 5.843 x \pm 2.214^*$	2.639	32	< 0.025
2	$y = 103.388 + 1.712 x \pm 0.527^*$	3.248	4	< 0.05
3	$y = 92.905 + 4.668 x \pm 0.912^*$	5.118	48	< 0.01
4	$y = 91.091 + 6.066 x \pm 2.162^*$	2.806	52	< 0.01

* $\pm$ Standard deviation of regression coefficient.

As Figure 1 shows, after the third or fourth day following deposition, almost all the y values are above 100; the phenomenon is general in nearly all the females examined. In Figure 1, although the linear regression faithfully described the phenomena observed, the increase of sex ratio in the last broods of Experiments 1 and 4 is not so high as expected or there is no increase at all. It is considered likely that the increase of sex ratio is not striking in the last days of deposition because there are too few sperm in the spermathecae of the females and the number of 0 sperm is proportionally too high. In fact in this condition, competition between the two kinds of sperm would be less likely.

Two hypotheses can be put forward to explain the increase of sex ratio as a function of deposition time: a) XY sperm are more fit to fertilize eggs, so that first the female uses more XY sperm, then more 0 sperm; b) some time after fertilization a certain number of XY sperm is lost and the progeny produced by 0 sperm gradually increase in proportion.

To test these hypotheses experiments were carried out in which the female, after fertilization, was prevented from laying for some days. If hypothesis (a) is correct, then as soon as the females start ovipositing again, there should be a

TABLE 3

Analysis of variance of y

Expt. No.	Source of variation	df	Sum of squares	Mean square	F	P
1	Regression	1	9135.027	9135.027	6.968	< 0.025
	Deviations from regression	32	41951.914	1310.997		
	Deviations from mean	33	51086.941			
2	Regression	1	135.837	135.837	10.551	< 0.05
	Deviations from regression	4	51.496	12.874		
	Deviations from mean	5	187.333			
3	Regression	1	9882.710	9882.710	26.777	< 0.01
	Deviations from regression	48	17715.299	369.068		
	Deviation from mean	49	27598.000			
4	Regression	1	16776.905	16776.905	7.874	< 0.01
	Deviations from regression	52	110791.044	2130.596		
	Deviations from mean	53	127567.949

FIGURE 1.—Regression of percent increment of sex ratio in the progeny of single females as a function of the number of days following fertilization. Experiments number 1, 3 & 4.

noticeable increase of sex ratio equal to that of the early deposition controls. According to hypothesis (b), however, no increase, or a slight increase of sex ratio similar to that of the last control broods should be observed.

The results of these experiments are reported in Table 4; Table 5 shows the equations of linear regression and Table 6 shows the analysis of their significance. The regression lines are presented in Figures 2 and 3. The three controls (no deposition delay) showed an increase of sex ratio with time. In the delayed deposition (AG) series, the linear regression is significant in three experiments

TABLE 4

*Sex ratios in successive broods produced from Oregon-R females following single mating with
In(1)EN · Y^S v cv y · Y^L y⁺ males*

Expt. No.	Series	Number females tested	Sex ratio of progeny produced in days following fertilization					Totals
5	Control	16	Days	(1-2)	(3-4)	(5-9)	(10-12)	(13-16)
			Males	218	181	103	107	238
			Females	218	125	69	78	181
			Sex ratio	0.50	0.59	0.60	0.58	0.57
	AG 4	17	Days	(5-6)	(7-9)	(10-13)	(14-17)	
			Males	178	95	220	289	782
			Females	184	75	132	187	578
			Sex ratio	0.49	0.56	0.63	0.61	0.58
	AG 6	17	Days	(7-8)	(9-11)	(12-15)	(16-19)	
			Males	297	111	142	133	683
			Females	218	47	85	119	469
			Sex ratio	0.58	0.70	0.63	0.53	0.59
6	Control	31	Days	(1-2)	(3-5)	(6-8)	(9-15)	
			Males	599	563	712	324	2198
			Females	576	411	449	208	1644
			Sex ratio	0.51	0.58	0.61	0.61	0.57
	AG 6	22	Days	(7-8)	(9-12)	(13-15)	(16-18)	
			Males	187	311	483	83	1064
			Females	162	200	275	49	686
			Sex ratio	0.54	0.61	0.64	0.63	0.61
	Control	33	Days	(1-2)	(3-5)	(6-7)	(8-13)	
			Males	858	987	639	482	2966
			Females	832	654	421	267	2174
			Sex ratio	0.51	0.60	0.60	0.64	0.58
7	AG 5	46	Days	(6-7)	(8-10)	(11-13)	(14-17)	(18-20)
			Males	213	1260	1303	905	129
			Females	160	933	804	537	94
			Sex ratio	0.57	0.57	0.62	0.63	0.58
	AG 10	35	Days	(11-13)	(14-17)			
			Males	769	820			1589
			Females	492	513			1005
			Sex ratio	0.61	0.61			0.61

and was not significant in one (AG 6 Experiment number 5). In the AG 10 series of Experiment 7, the use of a linear regression analysis is not meaningful since only two broods are available; there is no apparent difference between the two broods of the AG 10 series. Therefore, the results of the delayed-deposition experiments are not incompatible with either one of the above two hypotheses. Both hypotheses could be partly true.

Attention is drawn to the observation (Table 7) that mean number of progeny

TABLE 5

Regression of percent increment in sex ratio among progeny of single females on days following fertilization

Expt. No.	Series	Regression line	Student's t	df	P
5	Control	$y = 107.642 + 2.728 x \pm 1.212^*$	2.245	44	<0.05
	AG 4	$y = 91.375 + 4.498 x \pm 1.701^*$	2.644	39	<0.02
	AG 6	$y = 106.516 - 0.988 x \pm 0.935^*$	1.057	38	<0.20
6	Control	$y = 100.590 + 4.512 x \pm 1.195^*$	3.776	92	<0.01
	AG 6	$y = 97.610 + 2.623 x \pm 1.238^*$	2.119	44	<0.05
7	Control	$y = 98.790 + 4.617 x \pm 0.938^*$	4.954	102	<0.01
	AG 5	$y = 94.927 + 1.821 x \pm 0.663^*$	2.747	122	<0.01

* $\pm$ Standard deviation of regression coefficient.

per fertile female in the AG series is smaller than in the controls: the longer the period of no deposition, the smaller the mean number of progeny per female. It therefore follows, provided that there is no predeposition egg loss, that those females who were prevented from depositing lose a certain number of sperm.

TABLE 6

Analysis of variance of γ

Expt. No.	Series	Source of variation	df	Sum of square	Mean squares	F	P
	Control	Regression	1	7372.767	7372.767	5.062	<0.05
		Deviations from regression	44	64084.972	1456.477		
		Deviations from mean	45	71457.739			
5	AG 4	Regression	1	10992.294	10992.294	6.990	<0.025
		Deviations from regression	39	61325.511	1572.449		
		Deviations from mean	40	72317.805			
	AG 6	Regression	1	440.760	440.760	1.116	<0.25
		Deviations from regression	38	15005.215	394.874		
		Deviations from mean	39	15445.975			
	Control	Regression	1	21131.367	21131.367	14.230	<0.01
		Deviations from regression	92	136617.239	1484.969		
		Deviations from mean	93	157748.606			
6	AG 6	Regression	1	2184.968	2184.968	4.659	<0.05
		Deviations from regression	44	24346.184	533.322		
		Deviations from man	45	26831.152			
	Control	Regression	1	20062.469	20062.469	24.527	<0.01
		Deviations from regression	102	83433.290	817.973		
		Deviations from mean	103	103495.759			
7	AG 5	Regression	1	4782.617	4782.617	7.534	<0.01
		Deviations from regression	122	77537.988	635.557		
		Deviations from mean	123	82320.605

FIGURE 2.—Regression of percent increment of sex ratio in the progeny of single females as a function of the number of days following fertilization. Experiment number 5.

The number of sperm lost can be calculated, assuming that the AG females received the same number of sperm as the controls (Table 7). Two hypotheses can be proposed to explain the loss of sperm: Either only XY sperm are lost (hypothesis 1) or the sperm are lost in the same proportion as used by control females for fertilization (hypothesis 2). For example, in the controls of experiments 5, the first 436 sperm were used according to a 0.50 sex ratio (Table 4); hence, following hypothesis 2, the 252 sperm lost in the AG 4 series and the 460 lost in the AG 6 series must be added to the recovered progeny of the two series in accordance with this ratio. On comparing the sex ratio calculated according to hypotheses 1 and 2 with that of the controls, hypothesis 2 is perhaps more likely to be correct. That is, in the AG series, during the period of no deposition, both

TABLE 7
A comparison of numbers of offspring produced by females prevented from laying eggs immediately after insemination (Series AG) or not (Control)

Expt. No.	Series	Number females tested	Number of offspring produced		Mean number of progeny per female tested	Number of missing progeny	No. of expected progeny according to hypothesis					
			Males	Females			1		2			
							Males	Females	χ^2	P	Males	Females
5	Control	16	847	671	1518	94.875						
	AG 4	17	782	578	1360	80.0	252.9	830.9	16.72	0.09	704.4	0.09
									<0.001	<0.8		<0.8
6	AG 6	17	683	469	1152	67.8	460.9	929.9	56.60	0.22	699.4	0.22
									<0.001	<0.7		<0.7
6	Control	31	2198	1644	3842	123.935						
	AG 6	22	1064	686	1750	79.5	976.6	1662.6	210.97	0.003	1561.8	0.003
									<0.001	<0.95		<0.95
7	Control	33	2966	2174	5140	155.76						
	AG 5	46	3810	2538	6348	138.0	816.8	3344.8	23.98	2.22	4224.7	2.22
									<0.001	<0.2		<0.2
AG 10		35	1589	1005	2594	74.1	2857.5	3862.5	880.17	0.001	3145.5	0.001
									<0.001	<0.95		<0.95

FIGURE 3.—Regression of percent increment of sex ratio in the progeny of single females as a function of the number of days following fertilization. Experiments number 6 & 7.

XY and 0 sperm are lost, probably in the same proportion as they would be used to fertilize eggs deposited in that period in standard culture. A third hypothesis we might envisage is the loss of XY and 0 sperm in equal number. It is difficult to exclude this possibility as the amount of sperm lost by the AG females was similar to that used by control females in the early days of deposition when sex ratio is nearer to 0.50. However, the results of Experiment 7 (AG 10 series) suggest that this possibility is not very likely. For example, in this series, had the 2857.5 sperm lost been shared in equal number by males and females, we should have obtained 2431 females and 3015 males, and a total sex ratio of 0.554. On comparing these values with the control ones, with a total sex ratio of 0.58, we obtain $\chi^2 = 5.9$ with $P = 0.02$.

DISCUSSION

Increase of sex ratio as a function of deposition time: The data presented thus

far indicate that sex ratio increases with time. This shows that there are likely to be functional differences between XY and 0 sperm. It is perhaps feasible to examine the nature of these differences. There are at least two possibilities as regards their origin:

1) The secondary spermatocytes do not inherit the same metabolic material produced by primary spermatocytes when in the diploid state. This possibility is supported by the fact that a certain amount of RNA, synthesized in the primary spermatocyte, is "inherited" by the spermatids (OLIVIERI and OLIVIERI 1965). Morphological differences have been noticed in the two poles of the primary spermatocyte (PEACOCK and ERICKSON 1964). Lastly, an hypothesis has been advanced (BEERMANN 1965; HESS and MEYER 1968) that in spermatogenesis a part of DNA may operate as a structural substrate on which RNA molecules or other products, synthesized during the diploid state of spermatogenesis, get stuck and are kept stored. These products will be used later for spermatogenesis when the haploid cells are left with an inactive and incomplete DNA. In the present study it is considered possible that the DNA of the nucleolar organizer may have some importance, as it is present in a double dose in one-half of the spermatids, while it is missing in the other half. The nucleolar organizer region is further implicated by the fact that in other experiments, the results of which will be published in another connection, there was no difference observed between the behavior of XY - vs. Y - or X - vs. Y -bearing sperm. This suggests that the region responsible for the difference between XY - and 0 -bearing sperm is common to the X and Y chromosomes. The nucleolar organizer region is such, but of course, any other heterochromatic component would also be a possibility.

2) DNA is not completely inactive in postmeiotic products. This hypothesis is perhaps less likely. Firstly it has been demonstrated, at least in an autoradiographic analysis, that there is no RNA synthesis in the postmeiotic products of spermatogenesis (OLIVIERI and OLIVIERI 1965). It should also be considered that if DNA were active in postmeiotic products, we would observe many irregularities in gene frequencies since selective competition at the sperm level would result in very evident effects (DUNN 1964).

In conclusion, then, it is believed that there are functional differences between the two kinds of sperm produced by a $Y^s X \cdot Y^L/0$ male, and that this event is not necessarily related to DNA activity in postmeiotic stages. In other words, the principle stated by MULLER and SETTLES in their well known paper is still valid. (cf. LINDSLEY and GRELL 1969.)

What then is the nature of the functional differences between XY and 0 sperm? As has already been stated earlier, two hypotheses can be proposed. Either XY sperm were more fit for fertilization or they are lost some time after fertilization. According to the results of the present study it might be inferred that both hypotheses are partly true. Most probably XY sperm are indeed better fit for fertilization. In fact, in some experiments sex ratio is significantly lower than 0.50 in the first brood. Furthermore, when females are prevented from depositing and the sperm stored in them are left aging, sex ratio still increases with time. The fact that this did not happen in the AG 6 series of Experiment 5 and that

the AG 10 series of Experiment 7 shows no increase of sex ratio in the second of the two broods may be explained taking into account that in the control series the increase of sex ratio was not very remarkable or there was none at all in the last broods. If the AG females lose enough sperm to be in same condition as the last broods of the control females, then no further increase could be detected and none was observed.

It is realized that an increase of sex ratio as a function of deposition time cannot be explained only by hypothesizing that *XY*-sperm were more fit for fertilization. In this case, there should be a negative correlation between sex ratio in the first and the last broods of each female and this was not found. The AG series showed that the female may lose part of the sperm stored, and the *XY* and 0 sperm to different extents. This datum can partly explain the increase of sex ratio as a function of deposition time, as will now be discussed.

Loss of sperm in females prevented from depositing: The present experiments showed that if immediately after fertilization, the female was prevented from laying, the number of her progeny decreased. It can, therefore, be assumed that during the no-deposition period, the female loses a certain number of sperm. It was also noticed that both sperm types were presumably lost in the same proportion as they would be used in standard cultures. How does this "loss" of sperm arise? LEFEVRE and JONSSON (1962) have noticed that sperm are incessantly moving in the seminal receptacle. Therefore, they suppose, "some sperm will be approaching the basal end of the receptacle and perhaps even partially emerging from it into the uterus. Should no egg be present, the sperm could reverse direction and travel back through the receptacle."

It is reasonable to suppose that when a sperm has reached the uterus, there are few chances for it to travel back; either the egg is present or not and if not, the sperm gets lost. If this is the mechanism of the loss of sperm, the loss can always occur, but it would become more evident when deposition is suspended. In other words the sperm the female receives from the male are partly used for fertilization and partly lost, according to the process described above.

The loss of sperm might interfere with both the increase of sex ratio as a function of deposition time and interfere with the total sex ratio resulting from $Y^s X \cdot Y^L/0$ males. It can be supposed that some metabolic properties allowed the *XY* sperm to move faster than 0 sperm; this would enable them to fertilize more eggs, but at the same time would enable them to more frequently get lost because of their greater mobility. In other words, if the sperm are partly used for fertilization and are partly lost, it can be supposed that *XY* sperm are positively selected in both these processes; these two mechanisms will explain the recovery of *XY* and 0 sperm in different amounts, as a function of time.

As regards total sex ratio of *XY/0* males, it is well known that in the progeny of these males more 0 sperm than *XY* sperm are recovered (SANDLER and BRAVER 1954). PEACOCK (1965) and YANDERS *et al.* (1967) have interpreted this phenomenon as due to a preferential migration of *XY* sex chromosomes to a nonfunctional pole of the primary spermatocyte; but if the female loses preferentially *XY* sperm, as has been argued in this paper, ours may be an alternative mechanism.

If the hypothesis presented in this paper is correct, it might also explain the data obtained by PEACOCK and ERICKSON (1965) with wild-type males. These authors have found that only half the sperm stored by the yellow female are later used for fertilization. They have hypothesized that half the sperm received by the marked female will be lost because they are nonfunctional. If the wild-type female loses a certain number of sperm even under standard conditions, as do females prevented from laying, there is no need to postulate that half the sperm are nonfunctional in order to explain PEACOCK and ERICKSON's data. (cf. ZIMMERING and FOWLER 1968). According to the present hypothesis too, the female does not use all the sperm stored, but the fate of them was not decided at the first meiotic division. Therefore, the ratio between stored sperm and progeny depends mainly on the female's capacity to use sperm. (cf. ZIMMERING and FOWLER 1968.)

ZIMMERING (1962) has noticed an effect of temperature on the total sex ratio of $Y^S X \cdot Y^L/0$ males. This might mean that temperature affects the processes leading to functional differences between XY and 0 sperm; probably these processes occur in the postmeiotic stages of spermatogenesis.

The authors want to thank DR. K. SANKARANARAYANAN for reading the manuscript and for his critical comments. The authors also thank DR. L. TERRENATO for the statistical evaluation of the data.

SUMMARY

Males of the genetic constitution $Y^S X \cdot Y^L/0$ were mated with Oregon-R females. After mating, the males were discarded and the females were transferred to fresh cultures at two- or three-day intervals until all the sperm stored were used. In some experiments, a certain number of females was prevented from laying eggs for a few days immediately after fertilization, by keeping them in a glucose-agar medium. Throughout the deposition period, the females do not make use of $Y^S X \cdot Y^L$ and 0 sperm in the same proportion; in fact, at the beginning of this period the utilization of $Y^S X \cdot Y^L$ sperm is proportionally higher than at the end.—The females which had been prevented from laying for a few days have a lower number of progeny than the controls; this difference is statistically significant and it is interpreted as due to a loss of a certain number of sperm. The sperm lost belong to both classes and are probably lost in the same proportion as they would have been used if the females had not been prevented from depositing immediately after fertilization. It was noticed in these females too, that sex ratio increased as a function of deposition time when the number of sperm lost was not too high.—The two main conclusions that can be drawn from these experiments are that there are functional differences between $Y^S X \cdot Y^L$ and 0 sperm and that there occurs a loss of a certain number of sperm in the storage organs of Oregon-R *Drosophila melanogaster* females.—These conclusions have been discussed in relation to the hypothesis of nonfunctional genes in the postmeiotic products and the hypothesis that half the sperm produced by *Drosophila melanogaster* males are regularly nonfunctional.

LITERATURE CITED

- BEERMANN, W., 1965 Operative Gliederung der Chromosomen. *Naturwissenschaften* **52**: 365-375.
- BRADEN, A. W. H., 1958 Influence of time of mating on the segregation ratio of alleles at the *T* locus in the house mouse. *Nature* **181**: 786-787.
- DUNN, L. C., 1964 Abnormalities associated with a chromosome region in the mouse. *Science* **144**: 260-263.
- HESS, O. and G. F. MEYER, 1968 Genetic activities of the Y chromosome in *Drosophila* during spermatogenesis. *Advan. Genet.* **14**: 171-225.
- LEFEVRE, G. JR. and U. B. JONSSON, 1962 Sperm transfer, storage, displacement, and utilization in *Drosophila melanogaster*. *Genetics* **47**: 1719-1735.
- LINDSLEY, D. L. and E. H. GRELL, 1969 Spermiogenesis without chromosomes in *Drosophila melanogaster*. *Genetics* **61** (Suppl. 1): 69-78.
- LINDSLEY, D. L. and L. SANDLER, 1958 The meiotic behavior of grossly deleted *X* chromosomes in *Drosophila melanogaster*. *Genetics* **43**: 547-563.
- MCCLOSKEY, J. D., 1966 The problem of gene activity in the sperm of *Drosophila melanogaster*. *Am. Naturalist* **100**: 211-218.
- MULLER, H. J. and F. SETTLES, 1927 The nonfunctioning of genes in spermatozoa. *Z. Ind. Abst. Vererbl.* **43**: 285-312.
- OLIVIERI, G., L. PICA and A. OLIVIERI, 1964 Sulla possibilità che la presenza del cromosoma *Y* condizioni in *Drosophila melanogaster* l'attività degli spermatozoi nei processi di fecondazione. *Atti Assoc. Genet. Ital.* **9**: 169-171.
- OLIVIERI, G. and A. OLIVIERI, 1965 Autoradiographic study of nucleic acid synthesis during spermatogenesis in *Drosophila melanogaster*. *Mutation Res.* **2**: 366-380.
- PEACOCK, W. J., 1965 Nonrandom segregation of chromosomes in *Drosophila* males. *Genetics* **51**: 573-583.
- PEACOCK, W. J. and J. ERICKSON, 1964 An indicator of polarity in the spermatocyte? *Drosophila Inform. Serv.* **39**: 107-108.
- PEACOCK, W. J. and J. ERICKSON, 1965 Segregation-distortion and regularly nonfunctional products of spermatogenesis in *Drosophila melanogaster*. *Genetics* **51**: 313-328.
- SANDLER, L. and G. BRAVER, 1954 The meiotic loss of unpaired chromosomes in *Drosophila melanogaster*. *Genetics* **39**: 365-377.
- YANAGISAWA, K., L. C. DUNN and D. BENNETT, 1961 On the mechanism of abnormal transmission ratios at the *T* locus in the house mouse. *Genetics* **46**: 1635-1644.
- YANDERS, A. F., J. G. BREWEN, W. J. PEACOCK and D. J. GOODCHILD, 1927 Meiotic drive and visible polarity in *Drosophila* spermatocytes. *Genetics* **59**: 245-253.
- ZIMMERING, S., 1962 The effect of temperature on meiotic loss of the *Y* chromosome in the male *Drosophila*. *Genetics* **48**: 133-138.
- ZIMMERING, S. and G. L. FOWLER, 1968 Progeny: sperm ratios and nonfunctional sperm in *Drosophila melanogaster*. *Genet. Res.* **12**: 359-363.