Research Notes

Studies of homeostasis of sex ratio in *Drosophila melanogaster*.

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In our recent work (Ivanov, 2002), it was demonstrated that in *Drosophila melanogaster*, when parents of one sex were irradiated, the shift in the sex ratio (**SR**) theoretically expected in their F_1 progeny did not take place, although, since the males and the females received through sex chromosomes different numbers of induced lethals, preferential death rate of one sex had to be expected, which had to be the more considerable the greater the radiation dose was. The great divergence between the theory and the experiment made us study the SR problem more thoroughly. As a result, we observed a regular phenomenon concerning the SR homeostasis. Its mechanism is obscure, but before looking for it one has to get convinced that the very SR homeostasis does exist. It is to this that the present work is dedicated.

SR is always defined uniformly as the ratio of the number of females to that of males. We are going to estimate the SR homeostasis in two ways. 1) We shall compare two SR values in an experiment with irradiation – the factually observed one with the theoretically expected one. In homeostasis, the two SR must be different, i.e. the shift of SR expected at irradiation must be absent. 2) We shall compare SR in an experiment, with irradiation and in the control, without irradiation. In homeostasis, the irradiated variant and the control must have similar SR.

Homeostasis of SR in *D. melanogaster* was discovered in fact by us earlier (Ivanov, 1998a, b; Ivanov, 2002), but it was not correctly interpreted, named and explained. Due to the fact that in the literature opposite views unfavorable for its accepting had been established (Catcheside, Lea, 1945; Hadorn, 1961; et al.), it provoked perplexity and was regarded as a curio until sufficiently numerous data corroborating it were accumulated.

Let, as it was assumed in our previous works, P_1 and F_1 be parents and their direct offspring, respectively; **DLM**, **RLM**, and **VM** be dominant lethal, recessive lethal, and visible mutations, respectively; $A = 0.0214$ and $\tilde{A}(D) = 0.0214 + 0.000201D$ be the mean number of DLM, spontaneous and induced in the genome with the irradiation dose of D Roentgens, respectively; $a_l = 0.00112$ and $\tilde{a}_l(D) = 0.00112 + 0.0000202D$ be the mean number of RLM in the X-chromosome, spontaneous and induced with irradiation in the dose of D R, respectively; $s = 0.19$ be the fraction of X chromosome genes in the whole genome ; $r_1(D)$ and $r_2(D)$ be the SR in F_1 at irradiation of P_1 males and females with the dose of D R, respectively; r_0 be SR in F_1 in the control without irradiation of parents; $n_f = x$, $n_m = y$, and $n = x + y$ be the number of females, males and flies of both sexes, respectively.

The total SR with its standard error both in the experiment and in the control is estimated by the formula:

$$r \pm s = \frac{x}{y} \pm \sqrt{\frac{n^2}{n-1} \cdot \frac{x}{y^3}}\,.$$

The expected SR at irradiation of parents of one sex with the preset dose can be found by the formulae presented in Table 1. We are going to explain on an example how these formulae have been obtained, and to give the necessary instructions.

Let us deduce the formula for the expected SR $r_2(D)$ at irradiation of P_1 females with the dose of D R. Both sexes always obtain via autosomes equal numbers of lethal factors, and therefore the difference in the viability of sexes arises only due to heterosomes (sex chromosomes), and when deducing the formula one has to take into account only heterosomal lethals. Let us designate irradiated heterosomes X and Y with bold letters, and non-irradiated ones with usual letters. At irradiation of females, a cross **XX** × XY produces in F_1 females **X**X and males **X**Y. The number of DLM obtained through irradiated **X** chromosomes is equal in females and males, but RLM induced in **X** chromosomes of the ova in P_1 females go in F_1 males to hemizygous state and act as DLM, so that the death rate of males is higher than that of females, and an increased SR as compared to the control is expected: $r_2(D) > r_0$.

A F_1 female obtains via the irradiated **X** and non-irradiated X chromosomes $(\tilde{A} + A)s$ DLM, so that the probability of her survival, or the probability of her not receiving any DLM in X chromosomes, is $P_0(f) = \exp(-\tilde{A}s - As)$. A F_1 male receives via the irradiated **X** chromosome $\tilde{A}s$ DLM + $\tilde{a}_l$ RLM, so that the probability of his survival, or of his not receiving any of these lethals is $P_0(m) = \exp(-\tilde{A}s - \tilde{a}_l)$. Then the SR at irradiation of P_1 females with a dose D is

$$r_2(D) = r_0 \cdot \frac{P_0(f)}{P_0(m)} = r_0 \cdot \exp[-As + \tilde{a}_l(D)],$$

which, after substitution of necessary numbers and calculation, gives the sought formula:

$$r_2(D) = 0.997 \exp(0.0000202D) \cdot r_0\,.$$

The positive value of the exponent index means that this function is an increasing one. Indeed, as the irradiation increases, so does the number of RLM, and the proportion of surviving **X**Y males must decrease, while SR must increase.

The rest of the formulae of Table 1, which we shall need afterwards, are deduced in a similar way. In each sex in F_1 the number of induced and spontaneous heterosomal DLM, and in males also RLM, is taken into account, and the survival is estimated as the probability of not receiving any such lethal, whereupon the sought expression for SR is found as the ratio of sexes` survivals with a correction factor r_0 on the SR in the control.

Table 1. The sex ratio at irradiation of parents of one sex with the dose of D Roentgens in normal strain and in strain with attached X chromosomes.

Conditions of experiment	Strain	
	Normal XX × XY	Attached X chromosomes XXY × XY
Irradiation of females	$r_2(D) = 0.997 \exp(0.0000202D) \cdot r_0$	$r_2(D) = 0.997 \exp(-0.0000764D) \cdot r_0$
Irradiation of males	$r_1(D) = 0.997 \exp(-0.0000382D) \cdot r_0$	$r_1(D) = 0.997 \exp(0.0000584D) \cdot r_0$
Control (non-irradiated)	r_0	r_0

The case of irradiation of females demonstrated as an example has served as a pretext for the hypothesis of low mutability of X chromosome in ova. Despite the expected high death rate in males, especially when females were irradiated with the dose of 15000 R, the SR was conserved consistently close to unit. The hypothesis that the incidence of RLM and VM in the X chromosome of ova is considerably lower than in X chromosome of spermia could account for the higher proportion of males than expected. A lower yield of mutations in X chromosome of ova than in that of spermia could be observed due to the action of some reparative mechanisms or of some mechanism that discards ova with mutations in the X chromosome.

This hypothesis was checked experimentally and rejected. The results of an experiment on comparison of mutability in the X chromosome of ova and spermia are presented in Table 2. Mutability was estimated by the method Muller-5 (M5 = Basc) in the strain Canton-S. Wild type (+) females and males were irradiated with γ-rays in the dose of 12000 R and crossed with the strain M5. In the cross ♀♀(+) irr. × ♂♂M5 in F_1 virgin females were selected and mated with M5 males, since their brothers were of wild type (+), but not M5 with which the final, mutation-revealing crossing was carried out. In the rest, the experiments on estimation of mutability in ova and spermia were quite similar.

The use of statistical criteria shows that the incidence of mutations in X chromosome of ova and spermia is the same, i.e. the difference is not significant. Therefore the data may be pooled, and the incidence of RLM and VM in the X chromosome in gametes of any sex at the dose of 12000 R may be estimated as $u(12000) = (26.01 \pm 4.71)\%$. This result has served to specify the dependence of the mean number of RLM and VM arising in the X chromosome on the irradiation dose *D*. Now, this dependence is

$$\tilde{a}(D) = 0.00123 + 0.0000222D,$$

and its free term and the coefficient with their errors are $a = (12.3 \pm 2.8) \cdot 10^{-4}$ and $k = (22.2 \pm 2.2) \cdot 10^{-6}$. It is just this specified dependence that we used in the deduction of formulae for the expected SR in Table 1, assuming that $\tilde{a}_l(D) = \frac{10}{11}\tilde{a}(D)$.

When studying the mutability of ova and spermia, we investigated also the SR in reciprocal crossings of wild type (+) and M5 strains with irradiation and in similar crossings without irradiation, and found a significant influence of the strain M5 on the SR. This phenomenon seems to contradict the homeostasis, but at the same time one may not think that SR homeostasis is of absolute character and is not violated in some mutant genotypes. The strain M5 carries mutations of genes *scute*, *Bar* and *white* and overlapping inversions in the X chromosomes, shows hybrid dysgenesis in crosses with natural populations, and, possibly, SR homeostasis is violated in this case by hybrid dysgenesis. The problem deserves being studied, and we are going to present some available relevant data.

In Table 3, SR in reciprocal crosses of (+) and M5 strains with irradiation of (+) parents with the dose of 12000 R and without irradiation is presented. In all cases without exception, one can see that SR is increased. In the experiment with irradiation of females, it exceeds considerably the value expected at such an irradiation dose, which (value) is high in itself. In the experiment with irradiation of males, it is also somewhat increased even as compared to the control, although theoretically it must decrease considerably. In the control it significantly exceeds unit in both cases. Crossing with the strain M5 leads to an increase of SR, especially with irradiation, and this completely suppresses all the expected influences on the SR. It was therefore interesting to clear up what was the SR in the strain M5 itself.

Data on SR in the strains M5 and Canton-S (+) are presented in Table 4. In the strain M5, it was studied twice for a short time, the studies gave different results, and the difference in SR in the preliminary and in the main experiment was significant at the doubtfulness level of $\alpha = 0.01$. In the

Table 2. Mutability, or incidence of mutations, induced in X chromosome of ova and spermia at γ-irradiation with the dose of 12000 R.

P_1 gametes and parents	Number of					Total mutability	Mutability in cultures $P_1 \rightarrow F_1$		
	Cultures $P_1 \rightarrow F_1$ m	Cultures $P_2 \rightarrow F_2$ (gametes) N	Mutant gametes n	RLM	VM	$u = \frac{n}{N}$, %	$\sum_{i=1}^{m} u_i$	$\sum_{i=1}^{m} u_i^2$	Mean $\bar{u} \pm \frac{s}{\sqrt{m}}$, %
Ova ♀♀(+) irr. × ♂♂M5	21	161	31	29	2	19.25	4.5266	1.9720	21.56 ± 4.87
Spermia ♀♀5 × ♂♂ (+) irr.	19	72	17	16	1	23.61	5.8762	4.1976	30.93 ± 8.34
All the gametes	40	233	48	45	3	20.60	10.4028	6.1697	26.01 ± 4.71

preliminary experiment, SR was considerably more than unit ($\alpha = 0.025$), and in the main one it was considerably less than unit ($\alpha = 0.01$), so that it was not possible to pool the data on SR in the M5 strain: the samples were taken from different general sets. The strain M5 was surely unstable with respect to SR, and in crosses with Canton-S the theoretically expected shifts in SR at irradiation were not confirmed, either. In order to show more demonstratively the extraordinariness of this property of the strain M5, in Table 5 long-term data on SR in the strain Canton-S without irradiation are presented, which show a high homogeneity of all the samples with respect to SR. The homogeneity criterion value coincides with that expected when the hypothesis of homogeneity of all the samples is true: chi-square is 7.0535, whereas its expected value is equal to the number of degrees of freedom $df = 7$. Therefore the samples may be pooled and one may obtain an SR estimate in the strain Canton-S, which is $r = 1.0191 \pm 0.0074$.

Table 3. Sex ratio (SR) in F_1 progeny of reciprocal crosses of strains Canton-S (+) and M5 (Basc) at irradiation of (+) parents with the dose of 12000 R and without irradiation.

Cross (P_1)	Number of F_1 flies			Total sex ratio		d **
	n_f	n_m	n	Observed $r \pm s$	Expected $r_E \pm s_E$ *	
♀♀(+) irr. × ♂♂M5	218	98	316	2.224 ± 0.271	1.446 ± 0.037	21.0
♀♀(+) × ♂♂M5 non-irradiated	3279	2882	6161	1.138 ± 0.029	-	-
♀♀M5 × ♂♂(+) irr.	81	63	144	1.286 ± 0.217	0.729 ± 0.019	29.3
♀♀M5 × ♂♂(+) non-irradiated	3218	2781	5999	1.157 ± 0.030	-	-

* The expected SR r_E is equal to

$$r_2(D) = 0.997 \exp(0.0000202D) \cdot r_0$$ when females were irradiated,

$$r_1(D) = 0.997 \exp(-0.0000382D) \cdot r_0$$ when males were irradiated,

where r_0 is SR in cross of respective direction without irradiation.

** Criterion $d = \dfrac{r - r_E}{s_E}$ serves for checking the zero hypothesis $H : r \le r_E$ versus alternative $\overline{H} : r > r_E$. In both experiments the hypothesis $H : r \le r_E$ is rejected in favor of $\overline{H} : r > r_E$ at the doubtfulness level $\alpha = 0.0005$.

From the data of Tables 3 and 4, using the homogeneity criterion χ^2, one can deduce the following conclusions. Homogeneity of strains Canton-S and M5 (in the main experiment) with respect to SR is rejected at the doubtfulness level of $\alpha = 0.05$ (Table 4). Homogeneity of reciprocal crosses of these strains with respect to SR without irradiation is not rejected, i.e. the direction of crossing does not influence the SR (Table 3). However, when (+) parents are irradiated with the dose of 12000 R, the hypothesis of homogeneity of the direct and reverse crosses with respect to SR is rejected at $\alpha = 0.025$, i.e. SR does depend on the direction of crossing (Table 3). The increase of SR caused by crossing with the strain M5 is especially manifested at irradiation. Thereby, the influence of interaction of the hereditary type of the M5 strain and of irradiation on the SR is detected.

Let us demonstrate now the presence of SR homeostasis in normal XX × XY strains when mutagens influence the parents of one sex in order to explain it subsequently on the basis of our experimental data.

Table 4. Sex ratio in strains M5 and Canton-S without irradiation.

Strain	n_f	n_m	n	$r \pm s$	Accepted hypothesis	α
M5 in preliminary experiment	352	296	648	1.189 ± 0.094	$r > 1$	0.025
M5 in the main experiment	1815	1955	3770	0.928 ± 0.030	$r < 1$	0.01
2003; Canton-S	2169	2098	4267	1.034 ± 0.032	$r = 1$	-

Table 5. Homogeneity of SR in samples of Canton-S without irradiation.

№	Year	n_f	n_m	n
1	1977	4286	4292	8578
2	1979	10392	10390	20782
3	1982	7199	7119	14318
4	2001	6966	6679	13645
5	2002	567	537	1104
6	2002	2719	2579	5298
7	2002	4023	3907	7930
8	2003	2169	2098	4267
	Total	38321	37601	75922

Table 6 demonstrates SR homeostasis under the influence of mutagens on males when a decreased SR is expected. In fact, the observed SR values are everywhere higher than that expected at the doubtfulness levels of $\alpha \le 0.025$. At the same time, the factual SR at irradiation deviates considerably from the control only in 2 cases out of 5 studied ($\alpha = 0.02$ и 0.05), and practically coincides with the control SR in the other 3 cases.

In Table 7, SR homeostasis is shown when females are irradiated, i.e. when an increased SR is expected. Despite the high irradiation doses and the expectation of a considerable increase of SR due to the death of males from RLM, the factually observed SR is everywhere lower than the theoretically expected one at $\alpha = 0.0005$. At the same time, in no case was there any significant difference between the irradiated and the control variants, and the zero hypothesis $H : r = r_0$ was not rejected.

The independence of the average SR of the irradiation dose of either males or females has been demonstrated also by regression analysis (Ivanov, 2002).

The strain Oregon-R of wild type after irradiation was crossed reciprocally with the strain D/TMSb carrying in chromosome 3 several mutant markers and inversions for prevention of crossing-over. However, unlike in crosses with the strain M5, here there were no excesses with SR, whence it follows that not all mutations or chromosome rearrangements disturb SR homeostasis.

The notion of SR homeostasis would have been wavered if the SR expected according to the formulae of the last column of Table 1 in a strain with attached X chromosomes – $\underline{XX}Y \times XY$ – had been observed at irradiation of parents of one sex. According to Hadorn (1961), Barth (1929) fertilized ova of a strain with attached X chromosomes with spermia of an irradiated male. In this strain, X spermia determined males, and Y spermia determined females. That is why here, unlike the normal strain, induced DLM and RLM had to eliminate more zygotes of males **X**Y than those of females $\underline{XX}$**Y**, and Barth found a confirmation thereof. Since Hadorn did not present the quantitative data of this experiment, one should check the result, and by the way study SR in the F_1 offspring of irradiated females $\underline{\mathbf{XX}}$**Y** where a considerable excess of X**Y** males over $\underline{\mathbf{XX}}$Y females is expected. A glimpse of Table 1 is sufficient to get convinced that in the formulae of expected SR for the strain $\underline{XX}Y \times XY$, the absolute value for coefficients in the exponent index is larger than in the formulae

for the normal strain XX × XY. This means that the exponents go here more steeply and move away from the level of $r = 1$ more rapidly as the dose increases, and therefore the influence of irradiation on SR in the strain XXY × XY must be more pronounced than in the normal one.

An experiment was carried out with the strain XXY × XY where females had the wild phenotype (+) and males were marked by the mutation *forked*. The history of the strain is as follows. When estimating the mutability in the X chromosome of ova of the Canton-S strain at the irradiation dose of 12000 R, as a result of non-disjunction of X chromosomes in ovogenesis, there appeared in F_1 a female with attached X chromosomes. From her, a strain with M5 males was produced, and from the latter a strain with males *forked* was bred especially for our experiment. The advantages of the latter strain are obvious. The marker abnormality "forked bristles" used in it has a weak expression, but a complete penetrance. It was isolated from the natural population of Alma-Ata and found to be identical to the laboratory mutation of the gene *forked*. No doubt, it decreases less the viability of males than the combination of mutations of chromosome M5 does, and, which is the most important, the latter, as it was found earlier, shifted SR and for this reason was not desirable for our experiment.

Its results are presented in Table 8. The shift of SR at irradiation of XXY females went in the direction of decrease as expected theoretically, but considerably more so, which already did not correspond to the theory. The difference between the factual and theoretical SR was significant at $\alpha = 0.05$. This SR deviated still more from the control ($\alpha = 0.001$). Such a shift clearly contradicted the homeostasis of SR whose violation was possibly associated with the origin of our strain XXY × XY from the strain M5 to which it is tightly related and seems to have conserved hybrid dysgenesis. In any case, such a considerable shift of SR is an extraordinary phenomenon, which is observed in our experiments only for the second time and seems to be caused by an abnormality brought into the genotypes and the cytoplasm by the marking of the strain M5.

The SR shift at irradiation of males XY (forked) was in the direction of increase, which was expected theoretically, but it was very small and non-significant: the experiment did not differ from the control, and the homeostasis of SR was confirmed here. The difference between the observed and expected SR was large, and the zero hypothesis $H : r = r_E$ rejected at the doubtfulness level of $\alpha = 0.001$.

In this way, the expectations of the theory that does not take into account the SR homeostasis are not confirmed on the strain XXY × XY just as on the normal strain, and the violation of SR homeostasis in the case of irradiation of XXY females seems to be caused by the artifact of instability of the strain M5 with respect to SR. If the homeostasis of SR exists, then so do the disturbances of SR against which it is intended, and they must in some way or other be revealed whenever, the homeostasis is disturbed by some or other factor.

Although much mystery remains about the SR problem, something important has already become clear. The experiment on estimation of mutability in the ova and spermia at irradiation (Table 2), which seems not to have justified our hopes, has turned out to be decisive for the explanation of the SR homeostasis in all the cases. It has become clear that when P_1 females are irradiated, F_1 males receive in full measure via the ovum the lethals induced in its X chromosome and for this reason, due to hemizygosity with respect to RLM, die more than females do. Since their increased mortality, which after the experiment is already undoubted, is not accompanied by a decrease of their proportion and increase of SR, this means that a respective additional death rate of females takes place, which is caused already not by mutations, but by the necessity of maintaining SR at the normal level.

Table 6. Sex ratio (SR) homeostasis in F_1 offspring of mutagen (EMS and various doses of γ-rays) treated males

Experiment and γ-ray dose in Roentgens	Total sex ratio			d_E **	α_E **	d_0 ***	α_0 *
	Observed $r \pm s$	Expected $r_E \pm s_E$ *	Control $r_0 \pm s_0$				
1977; Canton-S EMS ~ 19350 R	0.939 ± 0.056	0.475 ± 0.010	0.999 ± 0.022	46.4	0.0005	-0.997	-
1979; Canton-S 1500	0.983 ± 0.015	0.941 ± 0.013	1.000 ± 0.014	3.23	0.001	-0.829	-
1982; Canton-S 2500	0.949 ± 0.019	0.915 ± 0.015	1.011 ± 0.017	2.27	0.025	-2.432	0.0
2001; Oregon-R 7000	1.126 ± 0.047	0.762 ± 0.047 ****	-	7.74	0.0005	-	-
2001; Canton-S 7000	0.977 ± 0.022	0.795 ± 0.014	1.043 ± 0.018	13.0	0.0005	-2.322	0.0
2002; Canton-S 10000	1.127 ± 0.060	0.718 ± 0.043	1.056 ± 0.064	9.51	0.0005	0.809	-

* $r_E = r_1(D) = 0.997 \exp(-0.0000382D) \cdot r_0$ - theoretically expected SR at irradiation of males with the dose D R.

** Criterion $d_E = \dfrac{r - r_E}{s_E}$ serves for checking the zero hypothesis $H : r \le r_E$ versus alternative $\overline{H} : r > r_E$; α_E - doubtfulness level at which the hypothesis $H : r \le r_E$ is rejected in favor of $\overline{H} : r > r_E$.

*** Criterion $d_0 = \dfrac{r - r_0}{\sqrt{s^2 + s_0^2}}$ serves for checking the zero hypothesis $H : r = r_0$ versus alternative $\overline{H} : r \quad r_0$; α_0 - doubtfulness level at which the hypothesis $H : r = r_0$ is rejected in favor of $\overline{H} : r \quad r_0$.

**** For lack of usual control for the strain Oregon-R, the expected SR was calculated assuming that $r_0 = 1$ and $s_E = s = 0.047$.

Table 7. Sex ratio (SR) homeostasis F_1 offspring of females irradiated with various doses of γ-rays

Experiment and γ-ray dose in Roentgens	Total sex ratio			d_E **	α_E **	d_0 ***
	Observed $r \pm s$	Expected $r_E \pm s_E$ *	Control $r_0 \pm s_0$			
2001; Oregon-R 7000	1.006 ± 0.041	1.148 ± 0.041 ****	-	-3.46	0.0005	-
2002; Canton-S 10000	1.148 ± 0.051	1.286 ± 0.035	1.054 ± 0.029	-3.94	0.0005	1.60
2002; Canton-S 15000	1.027 ± 0.030	1.390 ± 0.031	1.030 ± 0.023	-11.7	0.0005	-0.079

* $r_E = r_2(D) = 0.997 \exp(0.0000202D) \cdot r_0$ - theoretically expected SR at irradiation of females with the dose D R.

** Criterion $d_E = \dfrac{r - r_E}{s_E}$ serves for checking the zero hypothesis $H : r \geq r_E$ versus alternative $\overline{H} : r < r_E$; α_E - doubtfulness level at which the hypothesis $H : r \geq r_E$ is rejected in favor of $\overline{H} : r < r_E$.

*** Criterion $d_0 = \dfrac{r - r_0}{\sqrt{s^2 + s_0^2}}$ serves for checking the zero hypothesis $H : r = r_0$ versus alternative $\overline{H} : r \quad r_0$. The hypothesis $H : r = r_0$ is not rejected anywhere.

**** For lack of usual control for the strain Oregon-R, the expected SR was calculated assuming that $r_0 = 1$ and $s_E = s = 0.041$.

Table 8. Sex ratio (SR) in the strain XXY × XY at irradiation with the dose D=5000 R 1) of only female XXY (+), 2) of only males XY (forked) and 3) without irradiation.

No.	Condition of experiment	Number of F_1 flies			Total sex ratio		d_E**	α_E**	d_0***	α_0***
		n_f	n_m	n	Observed $r \pm s$	Expected $r_E \pm s_E$*				
1	Irradiation of ♀♀XXY	1626	2306	3932	0.705 ± 0.023	0.754 ± 0.021	-2.33	0.05	-10.4	0.001
2	Irradiation of ♂♂XY	1974	1685	3659	1.172 ± 0.039	1.479 ± 0.042	-7.31	0.001	1.28	-
3	Control	2631	2375	5006	1.108 ± 0.031	-	-	-	-	-

* Theoretically expected SR r_E is equal to

$$r_2(D) = 0.997 \exp(-0.0000764 D) \cdot r_0 \text{ at irradiation of females XXY,}$$

$$r_1(D) = 0.997 \exp(0.0000584 D) \cdot r_0 \text{ at irradiation of males XY,}$$

where r_0 is SR in the control.

** Criterion $d_E = \dfrac{r - r_E}{s_E}$ serves for checking the zero hypothesis $H : r = r_E$ versus alternative $\overline{H} : r \quad r_E$; α_E - doubtfulness level at which the hypothesis $H : r = r_E$ is rejected in favor of $\overline{H} : r \quad r_E$.

*** Criterion $d_0 = \dfrac{r - r_0}{\sqrt{s^2 + s_0^2}}$ serves for checking the zero hypothesis $H : r = r_0$ versus alternative $\overline{H} : r \quad r_0$; α_0 - doubtfulness level at which the hypothesis $H : r = r_0$ is rejected in favor of $\overline{H} : r \quad r_0$.

Let us consider the cross of females XXY with irradiated males **XY** in which an SR close to control was observed (Table 8, crossing 2). The increased death rate of males **X**Y as compared to females XX**Y** here is beyond any doubt, because in crosses of such irradiated males with M5 females the mutability in X spermia is high and it cannot decrease due to taking XXY females instead of M5 females. Therefore, in F_1 the mortality of males is really higher than that of females. In order to explain why in this case the proportion of males remains high like in the control, one has to assume an additional death of females above that which is caused by irradiation, i.e. death caused not by irradiation but by some regulatory reasons, death for the sake of conserving a normal SR. Something like apoptosis at the organismal level seems to take place, the difference consisting in the fact that it is not tissue cells, but females` zygotes that are self-eliminated, possibly still at the one-cell stage, before the beginning of cell division.

In the normal strain, when males are irradiated, i.e. when a prevalence of death of X**X** females over that of X**Y** males and a decrease of SR take place, its normalization goes by means of self-elimination of males. If one supposes here sex re-determination in a part of males and their transformation into females, impossibility thereof follows from the fact that the sex is determined by the heterosomal content of the zygote.

There arises a wish to mitigate the strangeness and purely teleological character of this explanation, which resorts to the ultimate intention (design) as the cause of the phenomenon, and to find or at least to hypothesize its motive cause, i.e. mechanism. What signals impel the superfluous, the less radiation-affected sex to a partial self-extermination in numbers adequate to restoration of normal SR? How can such an abstract characteristic as SR or the proportion of sexes in the progeny, signalize the progeny, selectively with respect to sex, the necessity of allocentric death? Despite the fact that feed-back in SR regulation is a complete mystery, homeostasis of SR in irradiation may be considered as an established phenomenon and its conception may be formulated as follows.

If one of sexes dies more than the other due to obtaining more irradiation-induced lethal mutations, then the opposite sex also dies off to the level at which the normal (for the given conditions) SR is conserved. This accounts for the SR homeostasis in F_1 observed when either P_1 females or P_1 males are irradiated.

Here, one cannot suppose a sex re-determination in a part of individuals of the excessive sex, because the chromosomal structure of the zygote cannot change. If environment-dependent sex re-determination without any change of heterosomal composition had been possible, then among *D. melanogaster* there would have been XY females and XX males. One cannot completely rule out the possibility of a different, no less satisfactory, hypothesis being found; however, so far no other hypothesis than that of self-elimination of the excessive sex can account for all the phenomena of SR resistance to irradiation.

Probably, the SR characteristic of the species under the given conditions is very important and has a certain concealed meaning, or else there would have been no SR homeostasis. The meaning thereof, as it usually is in cases enigmatic from the viewpoint of individual adaptation, is associated with regulation of population numbers or, ultimately, of the ecosystem. As an example, let us refer to the role of mutagenesis and of non-adaptive genome structure in the limitation of *D. melanogaster* population numbers (Ivanov, Ivannikov, 1997; Ivanov, 1999). The bisexuality (dioecism) of species, i.e. the existence of sexes, also contradicts the individual adaptation, since it increases the dependence on the environment conditions. The reproduction of the species becomes dependent on the availability of 1) two individuals 2) of different sex 3) ready for reproduction, and since this readiness, given the high numbers, depends on the population density as a decreasing function, the dioecism serves as a regulator of the species numbers. When limitations of the growth of numbers are required, the dioecism limits the reproduction by means of decrease of nuptiality (mating frequency).

In man, the population numbers are rather efficiently limited by the increase of nuptial age, and the possibility thereof is ensured just by the bisexuality. In all cases, dioecism creates the possibility of preventive misogamy or external obstacles to mating and limits thereby the reproduction of the species long before the latter reaches numbers devastating to the ecosystem. Therefore, dioecism is not individual adaptation, but an allocentric property of the species and confirms the basic principle of organization of life formulated by us earlier as allocentrism (Ivanov, Ivannikov, 1997).

The SR in *D. melanogaster* close to unit is not optimal in the sense of Darwinian fitness. One male can fertilize a considerable number of females, and a female usually does not mate more than once or twice in her life. That is why, more advantageous would be an SR exceeding unit by several times, i.e. a multiple excess of females over males. Therefore in reality there is a strong excess of males, which, from the point of view of maximization of fitness, is a great wastefulness. This excess of males is evidently an allocentric property of the species and should be studied just from this point of view. Although the importance of SR in the regulation of the ecosystem is not clear, it is confirmed by the fact that at high numbers the proportion of females decreases and at low ones it increases (Grechany, Pogodaeva, 1996). In this case, the reproduction potential of the population decreases at high numbers and increases at low ones, as it is required for stabilization of the species numbers.

It seems that SR homeostasis acts also when the SR is violated by other factors, except for radiation mutagenesis, e.g. in chemical mutagenesis, which one can study experimentally, or in the selective death of sexes from some other environment conditions, abiotic or ecological. So, the notorious demographic phenomenon that takes place during wars – the increase of the proportion of males among the newborn – seems to be an SR homeostasis phenomenon in human populations.

The seasonal changes of SR in *D. melanogaster* populations associated with changes of population numbers do not at all contradict the SR homeostasis conception, although they have a considerable amplitude in samples: $0.992 \le r \le 1.740$ (Grechany, Pogodaeva, 1996). The SR homeostasis is undoubtedly conserved, it is only its stationary point that changes. As any law, which is fulfilled only when certain conditions are available, the SR homeostasis, as we have seen, is sometimes disturbed, but this does not at all mean that it does not exist. It is that we so far do not know the conditions under which it is manifested, its mechanisms, regulatory forces and possibilities.

From the SR homeostasis, an important consequence for calculation of zygotes survival at a given irradiation dose follows. The mortality from induced lethals in F_1 is set by the mortality of the sex that has suffered most from them, because the other sex is self-eliminated in order that the SR is conserved. Then the survival of F_1 zygotes is calculated as the smaller of values for survival of sexes. To correct our former views (Ivanov, 2002), let us present formulae for calculation of zygotes` survival at irradiation of parents taking into account the SR homeostasis:

$$P_0(D|m) = 0.958\exp(-0.000201D) \text{ at irradiation of males;}$$

$$P_0(D|f) = 0.958\exp(-0.000201D) \text{ at irradiation of females with a dose } D \le 146 \text{ R;}$$

$$P_0(D|f) = 0.961\exp(-0.000221D) \text{ at irradiation of females with a dose } D > 146 \text{ R;}$$

$$P_0(D) = 0.958\exp(-0.000402D) \text{ at irradiation of both sexes.}$$

If, as it seems almost obvious, a basic impossibility of finding the driving forces of the adequate self-elimination of the excessive sex becomes clear, then we must assume that here we face a fundamental law of nature which has to be accepted as a fact, without any explanation, just like the law of gravitation or interaction of magnetic or electric charges. Otherwise one would have to account for the SR homeostasis by a supernatural, transcendental interference of the Creator, or by a

miracle similar to that of creation. But does not a constantly reiterated miracle usually become considered as a natural law? It remains only to get accustomed to it.

Let us illustrate the situation with the well-known example. The Kepler`s laws of planet motion are not basic laws of nature, since they are deduced as a consequence of the law of gravitation. The gravitation is for them the moving cause (*causa efficiens*), but it has no moving cause of its own, but only the ultimate goal (*causa finalis*). For this reason, it is considered as belonging to basic laws of nature. Up to now, the supporters of the mechanistic world outlook try not to regard gravitation as a basic law, but to find its moving causes. They believe that the understanding of the universe can be reduced to a few, or even one basic principle, while the experience of natural sciences, especially that of wave mechanics, demonstrates that there are a lot of basic principles (Peierls, 1957), so that a complete clarity is unattainable in principle.

The gnoseological meaning of SR homeostasis consists in the fact that a clearly demonstrable phenomenon has been found in it, which is determined not by genetic, but by external, environmental, ecological factors and happens despite the genetic determination of SR. Here, we have still another confirmation of the fact that the primary role in the studies of the phenomenon of life is played by ecology, but not genetics as it is often believed of late. Life is based on biotic circle, and the latter does not depend on the genetic structure of individuals and species, but is preset by the properties of the planet as a whole. The species are transient, while the circle is continuously renewed and supported by the homeostasis of CO_2 (Ronov, 1978) and O_2 (Bgatov, 1982) concentrations in the atmosphere by means of effusive vulcanism.

Let us summarize the work.

When parents of one sex are irradiated, the sex ratio (SR) in their direct offspring does not change or changes considerably less than it is expected in accordance with the difference between sexes with respect to the numbers of lethal mutations received via sex chromosomes. The phenomenon of SR stability is referred to as SR homeostasis.

SR homeostasis is attained here by the fact that the sex that has suffered less from the lethals induced by irradiation of parents, i.e. that available in excess, is self-eliminated to a level at which the SR normal for the given conditions is restored.

The ultimate cause, or goal, of the additional death of the excessive sex is maintenance of SR at the normal level. Its direct, or motive, causes are unknown and require investigation. If it turns out that they do not exist at all, then the SR homeostasis claims to be considered as a fundamental law of nature.

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