Tab. II. Mean number of inversion per individual *D. willistoni* from natural irradiated and control populations.

Irradiated natural population			Control population
N1R + N2R	3.08 ± 0.15	=	Control A
N3R	2.67 ± 0.17	<	3.59 ± 0.15
N4R	3.03 ± 0.16	=	
N5R	2.79 ± 0.09	<	
N6R	2.61 ± 0.09	<	4.14 ± 0.17
3N6R	3.35 ± 0.17	<	
4N6R	2.17 ± 0.08	<	3.26 ± 0.09
5N6R	2.49 ± 0.10	<	3.26 ± 0.10
6N6R	2.53 ± 0.11	<	

(XLI·E1) inverts the block comprising the distal half of section 4 to the end of 6 in the reference map[3]. No other chromosomal aberrations were observed.

In experimental *D. melanogaster* irradiated populations, Paget[4] observed some new inversions in high frequency. Nevertheless, Seecof[5] detected no increase of new rearrangements in *D. ananassae* heavily irradiated natural populations.

The adaptive advantage of heterozygous for inversions in *Drosophila*, demonstrated by the valuable work of Dobzhansky and many others, would, in the *willistoni* irradiated natural populations here studied, represent a protection for lethals and semi-lethals accumulated[1].

The crossing over suppression effect of inversions will favour this 'incorporation'. These trapped and protected lethals or deleterious mutants may correspond to the interesting allelic affinities discovered in the same populations[1,6].

The decrease of inversions frequency in irradiated natural population might be due to their overload with deleterious mutants.

Zusammenfassung. In einer isolierten natürlichen Population von *Drosophila willistoni* wurden innert 7 Monaten viermal je 70000 γ-bestrahlte Fliegen freigelassen. Anschliessend wurde eine beträchtliche Abnahme der mittleren Inversionshäufigkeit pro Individuum festgestellt. Einige Inversionen im 2. Chromosom und IIIC, IIID, IIIE und IIIF waren sogar seltener als in der nicht bestrahlten natürlichen Kontrollpopulation, selbst 14 Monate (= 35 Generationen) nach dem letzten Freilassen. Doch wurden in der bestrahlten Population auch 2 für diese Art neue Inversionen gefunden (eine im X- und eine im 3. Chromosom).

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[5] W. S. Stone, R. Wheeler, W. P. Spencer, F. D. Wilson, J. T. Neuenschwander, T. G. Gregg, R. L. Seecof, and C. L. Ward, Univ. Texas Publ. *5721*, 260 (1957).

[6] Work supported in part by grants of the Rockefeller Foundation, Conselho Nacional de Pesquisas (Brazilian Research Council) and Comissão Nacional de Energia Nuclear (Braz. Atomic Energy Com.).

Genetic Effects of γ-Radiation on Natural Populations of *Drosophila willistoni*[1]

As radiation levels in our planet increase, the importance and urgency of knowing its effects on natural populations, becomes more evident. Several valuable experiments have been done with *Drosophila* population cages by Wallace[2,3] and others, but only recently Stone et al.[4], Stone and Wilson[5,6], started comparative studies on natural populations of *D. ananassae* from Bikini and other less irradiated islands. Amongst *D. willistoni*'s advantages are: the possibility of obtaining homozygous strains (II and III chrom.) by a method similar to ClB using Dobzhansky's marked stocks[7], and consequently, of having exact data on the frequency and allelism of lethal, semi-lethal, sterility and visible mutants; the easy-growth in laboratory and abundance in wild environments; the low dispersion rate[8] that limits its populations to the isolated 'capões' (island of woods in the grassland regions of Rio Grande do Sul State) etc.

A short report is here presented showing the most significant results obtained after a three-year period (1957–1960) of studies on genetic effects of γ-radiation, exhibited by an isolated natural population of *D. willistoni* inhabiting a specially chosen 'capão A' and compared with a control natural population from another 'capão' in the same region[9,10].

To start the experiment 1200 *willistoni* individuals were collected from the 'capão A' and bred in laboratory by daily culture bottle transfers. During one year, six doses, three of approximately 10000 r and three of about 5000 r, were given to each alternating generation of about 15000 individuals each time. These were bred again to be released in the '*capão A*' (about 70000 flies) and to maintain a large laboratory stock. As Ives[11] demonstrated, γ-radiation produces an average increase of 2% lethal mutations per 1000 r in the dosage range of 300 r to 12500 r. The decrease of fecundity or fertility by high doses of Cobalt 60 γ-radiation seems to be less severe than the X-rays.

Each of these releases had about 10 to 3 times the experimentally determined number of adult flies living in the '*capão*'. The last laboratory irradiated 'generation': 6R$_1$ was sampled before the release and genetically analysed. Four samples from 'capão A' were also analysed: the N6R, 3N6R, 4N6R, and 5N6R, respectively: 2, 6, 7, and 10 months after the last release of irradiated flies (6R$_1$).

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[1] Work developed (1957–1960) with grants in aid of the Conselho Nacional de Pesquisas (CNPq: Brazilian Nat. Council of Research) and the Rockefeller Foundation.
We acknowledge the constant interest and suggestions of Dr. A. R. Cordeiro in charge of the general planning of the radiation genetics project.

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Sterility tests were made separately for the homozygous males (479: II and 732: III chromosomes) and the homozygous females (489: II and 811: III chromosomes). The unexpected results of these very carefully performed tests was that no increase of such 'recessive' sterility, induced by radiation, could be detected. The $6R_1$ stock had the same level of the control non-irradiated natural population (II chrom.: males $X^2 = 1.13$ $P < 0.30$; females $X^2 = 0.04$ $P < 0.90$, both: $X^2 = 0.42$ $P < 0.70$; III chrom.: males $X^2 = 2.77$ $P < 0.10$, females $X^2 = 0.34$ $P < 0.70$; both: none). This observation suggests to us that there is only a small number of *loci* that can produce recessive sterility mutants. It is also possible that these mutants are near maximal frequency in natural populations, compatible with a very efficient selection pressure over the heterozygous condition. There is, nevertheless, evidence that significant oscillations in natural populations do occur (males of 5N6R of II chrom. $X^2 = 12.80$ $P < 0.01$).

This class of mutants deserves further study.

The frequency of lethals and semi-lethals (Table I) increased significantly in the laboratory stock $6R_1$ (II chrom. $X^2 = 38.17$ $P < 0.01$ and III chrom. $X^2 = 15.19$ $P < 0.01$). Nevertheless, five generations after the last release, a sample from the irradiated 'capão A' (N6R) showed only slight but no significant difference for the II chromosome, and no increase at all for the III chromosomes (Table I).

Comparable results of such elimination were observed by STONE and WILSON[5,6] for *D. ananassae*, and for a sample of about 10 lethals of *D. willistoni* introduced in natural population by DA CUNHA et al.[12]. According to CORDEIRO[13] and CORDEIRO and DOBZHANSKY[14], even the spontaneous natural population lethals of *D. willistoni* have semidominant effects, depressing, on the average, the viability of the heterozygous bearing individuals. Nevertheless, some combinations of lethal/normal chromosomes were shown to be highly viable. From this it is conceivable that all sorts of semidominant effects will be produced by newly induced 'recessive' lethals if a large sample of such mutations is studied.

However, an important discovery appeared when the allelism tests of intra and inter samples of $6R_1$, N6R, 3N6R, and control were made. The high frequency of allelism intra the 6R, and the N6R samples differing significantly from controls was accompanied by a high frequency of inter 6R × N6R allelism, both for the II and the III chromosomes (Table II and III). This interesting fact shows that 5 generations after the last release, the irradiated natural population, even though having 'normal' frequency of lethals + semi-lethals, incorporated many such new radiation-induced mutants (Table II and III). Up to date, a total of 11674 crosses have been made for the II chromosome (12726 will be possible) and 8102 crosses for the III chrom. (10155 possible). The sample 3N6R, taken 15 generations after the last irradiated release, is significantly different ($P < 0.01$) from $6R_1$ and N6R with regard to the amount of allelism.

The inter-allelic frequency of 3N6R × control is slightly greater than the $6R_1$ × control and N6R × control, but this difference is not significant considering the numbers of crosses obtained. More interesting is the fact that 3N6R has as much alleles with the $6R_1$ and N6R ($6R_1$ × 3N6R) and (N6R × 3N6R) as with the *controls* which greatly differ from the $6R_1$ and N6R. Furthermore, the allelism of ($6R_1$ × 3N6R) is due to different *loci* from those observed among the (control × 3N6R) crosses. These observations indicate that, 15 generations after discontinuing the invasion of the population by irradiated individuals, its effect has left detectable vestiges.

Tab. I. Frequencies, in %, of lethals + semi-lethals among II or III homozygous chromosome strains from natural population of *Drosophila willistoni* irradiated by a Co 60 source, compared with non irradiated ones.

Samples		II Chromosomes		III Chromosomes	
analysed		Number tested	Lethals + semi-lethals	Number tested	Lethals + semi-lethals
Last laboratory irradiated population ($6R_1$)		119	77.31 ± 3.84	145	47.59 ± 4.15
Natural	N6R	99	51.51 ± 5.02	162	32.10 ± 3.67
population	3N6R	147	40.81 ± 4.05	150	31.33 ± 3.79
after	4N6R	101	51.48 ± 4.97	41	26.83 ± 6.92
irradiation	5N6R	107	45.79 ± 4.96	139	30.94 ± 3.92
Controls		224	42.41 ± 3.30	389	29.56 ± 2.31

Tab. II. II Chromosome of *D. willistoni's* frequency of allelism, in %, and the number of crosses intra and inter the samples. Righthand column shows the frequencies of lethals + semi-lethals for comparison purposes.

Samples	Last irradiated $6R_1$	Natural populations N6R	3N6R	Controls	Lethals + semi-lethals
$6R_1$	6.14% 1009				77.31 ± 3.84
N6R	4.01% 1520	3.57% 588			51.51 ± 5.02
3N6R	0.46% 1499	0.64% 1255	0.46% 648		40.81 ± 4.05
Controls	0.12% 1622	0.15% 1319	0.55% 1456	0.79% 758	42.41 ± 3.30

Tab. III. III Chromosome *D. willistoni's* allelism frequency, in %, and the number of crosses intra and inter the samples. Righthand column shows the frequencies of lethals and semi-lethals for comparison purposes.

Samples	Last irradiated $6R_1$	Natural populations N6R	3N6R	Controls	Lethals + semi-lethals
$6R_1$	3.88% 309				47.59 ± 4.15
N6R	2.94% 781	3.07% 522			32.10 ± 3.67
3N6R	0.31% 642	0.12% 856	0.00% 373		31.33 ± 3.79
Controls	0.00% 1034	0.07% 1435	0.16% 1240	0.22% 910	29.56 ± 2.31

Summing up, the return to lethal equilibrium frequency in irradiated natural populations is attained quickly, probably by selection against heterozygous bearing individuals. However there is a partial incorporation of new lethals and discharge of 'wild' ones demonstrated here,

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[12] A. B. DA CUNHA, J. S. TOLEDO, C. PAVAN, H. L. DE SOUZA, and S. A. TOLEDO, Comunication to the I. Simpósio Sul-Americano de Genética (São Paulo 1960).

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for the first time, throughout the allelism tests. A further step is reached when allelism frequency drops down to control values, also. Nevertheless, there are indications that a small amount of newly induced lethals remains incorporated even when all these frequencies appear to be equal to non-irradiated populations.

Zusammenfassung. Eine isolierte natürliche Population von *Drosophila willistoni* erhielt während eines Jahres eine beträchtliche Beimischung von F_I-Co 60-bestrahlten Fliegen. Die genetische Analyse ergab, dass die Häufigkeit der letalen und semiletalen Allele nach 5 Generationen rasch wieder auf das Normale sank. Die Frequenz der Letalallele war allerdings noch hoch, gleich hoch wie in der in Massenkultur gehaltenen bestrahlten Zucht. Erst nach 15 Generationen sank sie zum Niveau der natürlichen Kontrollpopulation. Einzelne durch Bestrahlung erhaltene Letalallele blieben in der Population erhalten.

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Paradoxical Findings in Ouchterlony Tests

Ouchterlony's 'reaction of identity' in agar gel diffusion precipitation tests is sometimes misleading[1]; whenever such a reaction is obtained, it should be carefully evaluated. Diffusion of precipitins present in extracts of *Ricinus communis* or *Abrus precatorius* seeds, and in horse antiserum specific for type XIV pneumococcus, against a 1/1000 (w/v) aqueous solution of purified type XIV pneumococcus polysaccharide results[2] in the formation of a single continuous precipitation line (Figure 1); therefore it may be concluded that the precipitins in the horse serum and the seed extracts are identical. However, in view of the heterogenous origin of the precipitating reagents, the 'reaction of identity' should not be accepted entirely at its face value.

Extracts of *Ricinus communis* or *Abrus precatorius* seeds agglutinate the erythrocytes of many animal species and form precipitates with their sera[3]; it is believed that the structure which reacts with the seed principle is present both on the erythrocyte surface, and, in soluble form, in the sera of these animals; one such animal is the horse. Thus it would appear that horse anti-XIV serum contains both a precipitin identical with the seed precipitins, as well as the structure specifically precipitated by the seed reagents. This paradox requires explanation.

The 'reaction of identity' (Figure 1) is obtained by using horse anti-XIV serum in a dilution at which it does not form precipitates with the seed extracts, but can still strongly precipitate the type XIV polysaccharide. When the undiluted serum is used instead, a 'reaction of partial identity' is obtained (Figure 2); this probably occurs because the precipitate formed by the pneumococcus polysaccharide and the anti-XIV serum is slightly deflected in an effort to join up with the precipitate formed by the seed extract and the antiserum. In view of the fact that the two precipitating reagents react with one another, it would be better not to attempt to interprete the precipitation pattern obtained under these circumstances (Figure 2), but to accept the 'reaction of identity' which was obtained when the two precipitating reagents did not precipitate each other (Figure 1). Moreover, it can only be claimed that the 'reaction of identity' indicates merely that the seed and serum precipitins precipitate the same molecules, and not that the precipitins are chemically identical.

The 'precipitinogen' in horse serum is in fact related to type XIV pneumococcus polysaccharide. When a sample of normal horse serum, which does not contain anti-XIV antibodies, is allowed to diffuse against the type XIV polysaccharide, and either *Ricinus communis* or *Abrus precatorius* seed extracts, a 'reaction of partial identity' is obtained (Figure 3). Nevertheless, anti-XIV antibodies appear in horse serum after immunisation with pneumococcus type XIV; they are thus highly specific and probably combine with some part of the type XIV pneumococcus polysaccharide molecule which is not common to the partially-related substance in horse serum. The seed precipitins probably combine with another part of the type XIV polysaccharide molecule. Whatever the explanation may be, the precipitins of *Ricinus communis* and *Abrus precatorius* seeds, when considered in relation to a system of pneumococcal polysaccharides, appear to be specific for type XIV pneumococcus polysaccharide[2]. Extracts of *Ricinus communis* and *Abrus precatorius* seeds could thus be used in selected serological studies as substitutes for horse antipneumococcus XIV sera.

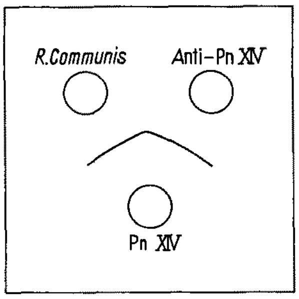

Fig. 1

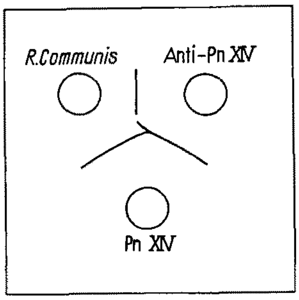

Fig .2

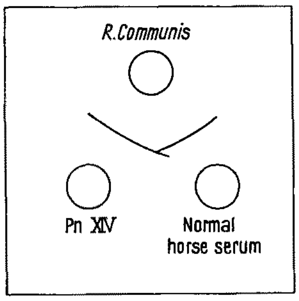

Fig. 3

Zusammenfassung. Mit Präzipitationsversuchen im Agargel wird gezeigt, dass Präzipitine aus *Ricinus communis*- oder *Abrus precatorius*-Samen und die spezifischen Anti-Pn XIV-Präzipitine des Pferdeserums mit verschiedenen Seitenästen der Strukturformel von Pn XIV-Kapselpolysaccharid reagieren können.

G. W. G. BIRD

Armed Forces Medical College, Poona (India), December 21, 1960.

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