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Stablization of unstable X chromosomes.

Lim (1979, 1981) has described the behavior of an unstable X chromosome, called Uc, which was derived from an EMS mutagenesis experiment. The instability of this chromosome was evident from its high rate of lethal mutation and from its propensity to accumulate rearrangements, including deficiencies, duplications, inversions and combinations of these. Detailed genetic and cytological analysis of mutant and rearranged chromosomes established that there was a hot spot for mutation between 6F and 7C on the polytene chromosome map and that there was a corresponding hot spot for breakage in the 6F1-2 doublet. A later study by Lim et al. (1983) showed that a derivative of Uc could impart all these properties to a stable chromosome by co-existing with it in females for one generation. However, this phenomenon of homologue destablization did not involve recombination between Uc and its partner; rather, the stable partner of Uc acquired its Uc-like instability, including the mutational and breakage specificity, without any detectable recombination. All of these properties suggested the activity of a transposable element, designated the L factor, which resided in Uc.

This note reports experiments which show that derivatives of Uc which had previously been unstable have now stabilized. The method in each experiment was to measure the rate of occurrence of recessive X-linked lethal mutations. Other chromosomes unrelated to Uc were also tested to serve as controls. In every case, the lethal mutation rate was low, implying that the agent which previously had caused many lethals had been inactivated or lost. Further tests established that a high lethal mutation rate could not be restored by outcrossing Uc-bearing males to an unrelated stock, and that Uc could no longer destabilize another X chromosome in heterozygous females.

The genotype of the Uc chromosome is y^{59b} z w^{i} ct^{6} f; we studied three derivatives with this genotype (referred to as H3, H4, and H7) and one derivative in which the ct mutation had reverted. The latter chromosome is called Uc-ct^{r82}. The chromosomes denoted as H3, H4, and H7 were used in the experiments reported by Lim et al. (1983) and were collectively referred to in that paper simply as Uc-l^{r}. All three of these X chromosomes were descended from a single X, which, in turn, was derived from the original Uc. The name Uc-l^{r} was used by Lim et al. (1983) because the progenitor of H3, H4, and H7 carried a reversion of a lethal mutation which had occurred on Uc (see Laverty & Lim 1982).

The experiments reported by Lim et al. (1983) were performed in 1981 and 1982. Since the beginning of those experiments, two independent sets of stocks with the chromosomes H3, H4 and H7 have been maintained, one set in our laboratory and the other in the laboratory of J.K. Lim. We shall refer to the set maintained by Dr. Lim with the cognate labels H30, H40 and H70.

A stock carrying Uc-ct^{r82} was established by Dr. Lim in 1982 from ct^{+} males which appeared in a culture of one of the many Uc derivatives he maintains. This ct reversion did not appear in any of the stocks of H30, H40 or H70. Our ct^{r82} stock was derived from Lim's stock in 1984. Throughout their history, all the Uc derivatives discussed in this report have been maintained in the male line by using C(1)DX, y f/Y females.

The procedure for detecting newly arisen recessive X-linked lethal mutations made use of the FM7, y^{31d} sc^{8} sn^{x2} B balancer chromosome. The males whose mutation rate was to be measured were mated individually to FM7/sc^{7} females at 21°; the sc^{7} chromosome in these females has a recessive lethal mutation that was induced with EMS (see Simmons et al. 1980). Then single FM7/"X" daughters which had been able to mate with their FM7 brothers were placed individually in culture tubes to produce the next generation. The culture methods of Simmons et al. (1980) were used. The flies emerging from these cultures were scored for "X"/Y males, where the "X" chromosome is the one under test. The absence of these males indicated that a recessive X-linked lethal mutation had occurred. All cases of suspected lethals were retested to confirm the initial result. A chromosome was classified as lethal if the frequency of the indicator males was less than 0.025 in the retest. For convenience, we staggered the tests at weekly intervals. Therefore, in each experiment measurements were made on males of two age classes: young males, mated within two days of eclosion, and old males, mated exactly one week later. This permitted a check for any possible effects of age on the X-linked lethal mutation rate. Since no such effects were found, the data from the two age classes have been pooled in the results that follow.

The first set of experiments involved males collected directly from the various Uc stocks and from unrelated stocks that served as controls. The latter included a stock homozygous for the X chromosome In(1)Δ49, pn v B^{M1}, abbreviated simply as Δ49, and two stocks homozygous for the m mutation. One of these had the background of the Canton S wild-type strain and was therefore called m-CS, while the other was obtained from Dr. Lim and was therefore called m-Lim. This latter stock was the one used by Lim et al. (1983) in their homologue destablization experiments; their results showed that in the absence of any association with Uc, the m-Lim chromosome had a very low mutation rate and therefore could be regarded as intrinsically stable.

Table 1. X-linked lethal mutation rates by strain.

Experiment	No.males tested	Chromosomes tested, No.	No.indep. events	No. lethals	Mutation rate (%)
Uc derived:					
H3	309	3,105	2	2	0.06
H4	331	3,357	1	1	0.03
H7	426	3,628	0	0	--
H30	312	2,493	0	0	--
H40	306	2,605	1	1	0.04
H70	349	3,502	0	0	--
ct^{r82}	126	970	0	0	--
Controls:					
Δ 49	304	2,568	0	0	--
m-CS	263	2,148	2	2	0.09
m-Lim	287	2,482	1	1	0.04

Table 2. X-linked lethal mutation rate produced by outcrossing.

Experiment	No.males tested	Chromosomes tested, No.	No.indep. events	No. lethals	Mutation rate (%)
H3	433	4,268	3	3	0.07
H4	454	4,570	2	2	0.04
H7	458	4,652	4	4	0.09
m-CS	297	3,263	0	0	--
m-Lim	304	3,269	0	0	--

Table 3. X-linked lethal mutation rates from induction experiments.

Experiment	No.males tested	Chromosomes tested, No.	No.indep. events	No. lethals	Mutation rate (%)
Series A:					
m-CS	324	3,566	0	0	--
H7(CS)	360	2,539	1	2	0.08
m-Lim	209	2,019	5	10	0.49
H7(Lim)	364	2,619	4	4	0.15
Series B:					
m-CS	414	3,968	5	6	0.15
m-Lim	417	4,141	0	0	--
Series C:					
m-CS	399	4,033	3	3	0.07
m-Lim	210	2,186	2	2	0.09

Table 4. X-linked lethal mutation rates by line from experiments with derivatives of Uc-l^r (Lim et al. 1983). Mutation rates were calculated using the unweighted procedure of Engels (1979).

Uc-line	No.males tested	Chromosomes tested, No.	No.indep. events	No. lethals	Mutation rate ± s.e. (%)
H3-9	68	2,051	15	48	2.29 ± 0.64
H4-21	70	2,064	7	13	0.57 ± 0.24
H4-24	70	2,186	2	2	0.08 ± 0.06
H4-27	67	2,013	14	71	3.83 ± 1.20
H4-39	68	1,962	25	118	5.96 ± 1.70
H4-40	70	2,204	23	116	4.84 ± 1.25
H7-65	70	2,086	14	43	2.33 ± 0.72
H7-66	70	2,054	9	29	1.57 ± 0.61
H7-72	70	1,974	17	90	4.79 ± 1.60
H7-72	70	2,125	16	55	2.60 ± 1.03

The results of X-linked lethal tests with males taken from these various Uc and control stocks are given in Table 1. The mutation rates are uniformly low so there is no evidence for any mutational instability.

The second set of experiments involved males collected from various outcrosses. Males from a particular stock were crossed at 25° to C(1)DX, y f/Y females from an unrelated stock and their sons were used in the X-linked lethal tests. The purpose was to see if outcrossing induced X chromosome instability. The results of these experiments are given in Table 2. Again, the mutation rates are low so there is no evidence that this outcrossing procedure induces chromosome instability.

The last set of experiments involved males collected from various crosses between the H7 stock and the two m stocks. Two of these crosses were between m males (either m-CS or m-Lim) and C(1)DX, y f/Y females from the H7 stock; these produced m sons whose X-linked lethal mutation rates were estimated. We refer to these males as belonging to the "B" series of experiments. The purpose of this series was to see if any autosomally or maternally transmitted factor from the H7 stock could cause instability of the m chromosome. As is evident from the data given in Table 3, no instability was detected. Therefore, we conclude that no such autosomal or maternal factor is present in the H7 stock.

There were two other series of experiments in this final set. All were derived from crosses between H7 males and m/m females, which in turn came from either the m-CS or m-Lim stocks. One series of experiments made use of the m sons derived from these matings; in Table 3 these are referred to as the "C" series. Series "C" was carried out to see of some paternal contribution from the H7 stock could induce instability in either of the m chromosomes. Another series involved sons derived from the daughters of these matings. Since the daughters are genotypically Uc/m, they would be expected to produce many types of recombinants. We mated these females to their m brothers and collected only two types of sons (Uc or m), which were then tested for X-linked lethal production. The tests of these males comprise the "A" series of experiments. The purpose of this series was to see if the H7 chromosome became unstable in H7/m females and, further, to see if H7 could destabilize either of the m chromosomes without recombining with them.

The results of series "A" and "C" are given in Table 3, along with those from series "B". In no case was there any indication that the X-linked lethal mutation rate was high. Therefore we conclude that the H7 chromosome is not destabilized in females carrying one of the m chromosomes, and furthermore, that in such females, H7 does not destabilize either of these m chromosomes.

All of these results demonstrate that the previously unstable chromosomes H3, H4 and H7 have stabilized. In addition, Uc-ct^{r82}, although derived from an unstable chromosome, is now stable. This extensive evidence for the loss of instability was foreshadowed by some of the data collected by Lim et al. (1983), who measured the X-linked lethal mutation rates of seven genotypic classes of males derived from Uc/m females, as in series "A" above. See their paper for the details of the genotypes. Here we simply note that the Uc chromosome which produced these males came from either the H3, H4 or H7 stocks. An analysis of the mutation rates of the ten sublines which Lim et al. (1983) used is given in Table 4. While eight of the ten lines had X-linked lethal mutation rates greater than 1.5%, two of the lines had conspicuously lower rates. These were both derived from stock H4, suggesting that the agent responsible for the high mutability of that stock had disappeared or been inactivated in these two lines.

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Drosophilidae of Uttarakhand, U.P., India.

The Uttarakhand region includes eight border districts of Uttar Pradesh, viz. Dehra Dun, Tehri-Garhwal, Uttarkashi, Pauri-Garhwal, Chamoli, Nainital, Almora and Pithoragarh. Uttarakhand region is peculiar in its animal as well as plant fauna.

Despite the fact that the family Drosophilidae and that the genus Drosophila occupy a very important position among the organisms which are used as a material for genetic studies, very little has been known so far about the Drosophilid fauna of India. About 170 species belonging to different genera have been discovered so far by different workers in this country (Parshad & Paika 1964; Gupta 1969, 1970, 1971, 1972, 1973, 1974a,b; Singh & Gupta 1974, 1977a, b,c, 1979). During the present studies a thorough survey of Uttarakhand region, which is a completely virgin field from the above viewpoint, was undertaken. A preliminary survey of this region has yielded some interesting results regarding the distribution of different Drosophilid species.

A total of 2689 species were collected. The name of the species, their number and collection locality is shown in Table 1 and Figure 1. From our collection data we desire to point out that some of the species, viz. **D.immigrans, D.kikkawai, D.lacertosa, D.melanogaster, D.jambulina, D.nepalensis** and **D.malerkotliana**, were collected in large number while some species, viz. **D.ananassae, D.bipectinata** and **D.nasuta** which are very common in other parts of the country, were completely absent.

Table 1. Species name, number and collection locality.

Species	No. flies	Collection locality
D.immigrans	402	Pithoragarh, Nainital, Maikoti (Chamoli), Rampur
D.buski	110	Pithoragarh, Nainital, Maikoti (Chamoli)
D.kikkawai	220	Nainital, Pithoragarh, Thalasu (Chamoli)
D.repleta	52	Nainital, Pithoragarh
D.lacertosa	215	Nainital, Pithoragarh, Thalasu (Chamoli)
D.melanogaster	405	Nainital, Pithoragarh, Satarakhal (Chamoli)
D.jambulina	327	Nainital, Almora, Maikoti (Chamoli)
D.nepalensis	510	Nainital, Tanakpur, Chamoli
D.malerkotliana	206	Nainital, Pithoragarh, Rampur
D.takahashi	120	Nainital, Pithoragarh, Almora
Leucophenga interrupta	10	Pauna (Chamoli)
* **D.** sp.	35	Chamoli
* **D.** sp.	11	Tanakpur
* **D.** sp.	21	Tanakpur
* **D.** sp.	16	Tanakpur
* **D.** sp.	2	Chamoli
* **D.** sp.	25	Chamoli
* **Mycodrosophila** sp.	2	Chamoli
Total	2689	

* species not identified; supposed to be a new species.