Table 2. (N = number of chromosomes sampled)

Date	Population	Frequency of Active Allele 1	3	2	4	N
1979	Utah, Natural	.30	.54	.55	.35	100
1980	Control Cage	.28	.61	.54	.17	90
Jun 82	Initial Exp. Cage	.31	.62	.50	.19	1536
Jul 82	Exp. 1st sample	.32	.74	.46	.17	100
Sep 82	Exp. 2nd sample	.41	.81	.64	.16	210
Dec 82	Exp. 3rd sample	.29	.58	.51	.24	252
Mar 83	Exp. 4th sample	.38	.62	.64	.27	254

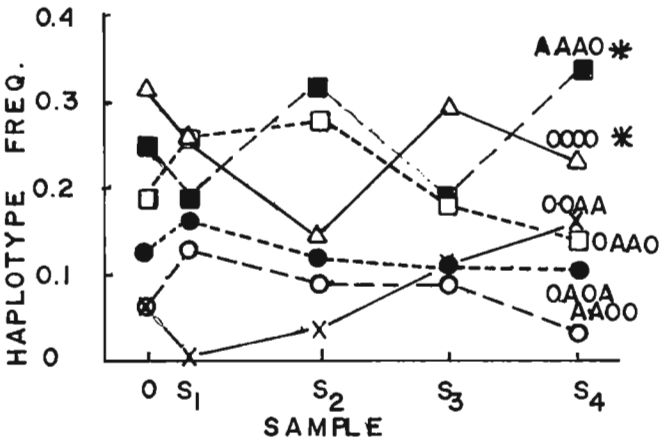

FIGURE 1.

The experimental cage was started with 768 flies comprised of equal numbers of ♂♂ and ♀♀ of the following genotypes: 96 00AA/AA00 + 192 0AA0/0A0A + 96 0AA0/0000 + 384 AAA0/0000. (The four genes are given in their order on the chromosome: 1, 3, 2, 4.) Table 1 gives the numbers of the six haplotypes used to initiate the experimental cage and an asterisk denotes a haplotype in extreme disfavor in both the control cage and the natural population. Thus, at a minimum, over 50% of the chromosomes in the experimental cage were putatively detrimental. The ratio of the six haplotypes initiating the experimental cage was arranged so that the allelic frequencies of active and null alleles at the four loci matched those of the control cage (see Table 2). Note that the cage was started from heterozygotes made from crosses of homozygous lines of the six haplotypes established previously from a population collected in Gothic, Colorado.

The experimental cage was set up in June 1982 as previously described, and in July F_1 larvae were removed, reared, and testcrossed to determine the relative frequencies of the six haplotypes among a sample of 100 chromosomes. Subsequent samples were taken in September 1982, December 1982, and March 1983. The haplotype frequencies observed in these samples are pictured in Figure 1, and the allelic frequencies observed are given in Table 2. There is no indication in these data of any consistent change in either haplotype frequencies or allelic frequencies over the period of experimentation. Certainly the detrimental haploytypes (asterisks) did not decrease in frequency. We estimate that the generation time in the cages was about a month, making a total of around nine generations between the first and the last sample. It was planned to take a last sample after an additional six months but a laboratory accident eliminated the population. These data provide no evidence of selection acting over the limited period of this experiment.

Supported by NSF Grant DEB-79-12336. References: Baker, W.K. & E.A.Kaeding 1981, Amer.Nat. 117:804-809.

Banerjee, I. & A.S.Mukherjee. University of Calcutta, India. Activation of potential initiation sites of DNA replication by Puromycin: evidence from fibre autoradiography.

In eukaryotes replicons occur in clusters (Edenburg & Huberman 1975). Such clusters are organized into replicon families and replicate at a given rate during DNA synthesis (S) phase (Van't Hof & Bjerknes 1978).

In the present study, DNA fibre autoradiography was used to determine the replication properties of polytene DNA at the level of initiation and chain elongation. We have used Puromycin which is a potent inhibitor of protein synthesis for monitoring the property. Our work is based on previous evidence supporting the idea that there are two different protein pools, one responsible for the control of DNA synthesis at the level of initiation and the

Table 1. Replicon properties.

Pre-treatment	Pulse time (^{3}H-TdR) (Sp.act.) (min.)	Mean length of labelled segments (µm±S.E.)	Average of means of length of labelled segments per 100 µm of stretch length (µm±S.E.)	Average No. labelled segments per 100 µm of stretch length (µm±S.E.)	Average of means of gap size per 100 µm of stretch length (µm±S.E.)	Average No. of gaps per 100 µm of stretch length (µm±S.E.)	Rate: fork movement µm/form/min.
Ringer	(Low) 10	4.34±1.08	5.83±0.66	9.90±0.54	5.80±0.97	9.72±0.49	0.434
Puromycin	(Low) 10	2.50±0.60	2.84±0.35	13.10±1.26	6.20±1.50	12.80±1.23	0.250
Ringer	(Low) 30	5.90±1.35	9.64±1.77	9.40±0.60	5.00±0.30	9.03±0.56	0.200
Puromycin	(Low) 30	2.60±0.64	3.42±0.33	16.75±1.56	4.35±1.26	15.90±1.58	0.086
Ringer	(High) 60	10.76±2.63	11.18±1.08	7.24±0.36	5.66±0.46	6.65±0.37	0.180
Puromycin	(High) 60	2.71±0.68	2.91±0.27	11.34±0.88	7.73±0.86	10.80±0.86	0.045
Ringer	(High) 90	12.60±1.94	20.00±2.20	5.33±0.37	8.19±0.76	4.92±0.36	0.140
Puromycin	(High) 90 (fed for 24 hours)	2.61±0.61	2.89±0.15	16.20±0.48	3.59±0.19	16.44±0.50	0.030
Puromycin	(High) 90 (fed for 48 hours)	2.52±0.58	2.69±0.14	20.65±0.53	2.50±0.09	20.80±0.52	0.028
Puromycin	(High) 90 (fed for 72 hours)	2.40±0.59	2.68±0.24	19.64±0.76	2.96±0.16	19.00±0.58	0.026
Ringer	(Low) 60 + (High) 60	13.80±0.68	18.66±2.07	5.14±0.37	5.03±0.32	5.03±0.32	0.115
Puromycin	(Low) 120	2.17±0.58	2.30±0.16	20.96±0.73	2.84±0.16	20.60±0.71	0.018
Ringer	(High) 60 + (Low) 120	20.50±3.60	19.90±1.47	4.20±0.19	3.92±0.19	3.92±0.19	0.114
Puromycin	(Low) 180	3.02±1.10	3.36±0.33	17.66±1.07	2.99±0.16	17.24±1.05	0.010

other, at the level of chain elongation (in preparation, and Mukherjee & Chatterjee 1984).

After Puromycin pre-treatment or incubation in Ringer (control), late third instar larval salivary glands from D.hydei were pulse-labeled in ^{3}H-TdR for different periods of time, starting from 10 mins to 180 mins. The glands were then lysed in lytic buffer (pH 10.8) containing proteinase K, passed through 5% chilled TCA, dehydrated, covered with AR10 stripping film or Ilford emulsion and exposed for a period of 4-8 months in light-tight bakelite boxes. After exposure, the slides were developed in D19b, fixed in X-ray fixer, dried mounted and observed under a Zeiss Photomicroscope III.

The data of all Puromycin treated and control slides are comprehensively pooled in Table 1. From the table, the following facts can be derived: (1) Mean length of labeled segments. In control, whereas a gradual increase in the mean length of labeled segments was observed, in Puromycin treated preparation, it remains more or less constant with increase in the length of pulse time.

(2) Average number of labeled segments/100 µm of stretch length. While in control, this number decreases, in Puromycin treated fibres the number increases (though fluctuating) with increase in the length of pulse time.

(3) Average size of the unlabeled gap and number of gaps/100 µm of stretch length. In control, the gap size remains more or less constant, whereas with Puromycin, the gap size, though fluctuating at the beginning, decreases gradually and then remains constant with increasing duration of pulse time. In control, the number of unlabeled gaps per 100 µm stretch decreases, while with Puromycin, the number tends to increase with increasing time.

(4) The rate of fork movement. In control, the rate is at first high, then drops down sharply and then gradually decreases with increasing pulse time. In Puromycin treated preparations, the rate though 2 to 10 folds less follows the same curvilinear regression as found in the control set.

It appears from the results that Puromycin induces a stage of replication found in early embryogenesis by activating the number of initiation sites, inducing clustering of replicons and reduced replicon size.

Furthermore, the results suggest that there may be two classes of replicon families as suggested by Hori (1979) and others. Puromycin inhibits the rate of fork movement in both types of replicon families.

References: Edenberg, H.J. & J.A.Huberman 1975, Ann.Res.Genet. 9:245-284; Hori, T. 1979, Jap.J.Genet. 55(1):41-54; Hori, T. & K.G. Lark 1974, J.Mol.Biol. 88:221-232; Van't Hof, J., A.Kuniyuki & C.A.Bjerknes 1978, Chromosoma (Berl.) 68:269-285.

Basden, E.B. Leyden Park, Bonnyrigg, Midlothian, Scotland. The Species as a block to mutations.

Mutants (phenotypes) of D.melanogaster and of a few other species of Drosophila have been described in detail. The number discovered since 1907 in melanogaster alone is many, many thousands and of every category.

There are no lists, however, of mutants that might be expected but are not found. One type will be discussed here, and for this purpose the species of Drosophila are grouped into two distinct divisions, viz: (1) The clear-wings. These are species whose wing-blades (including veins) are clear, hyaline, and quite unmarked. Clear wings include affinis, ananassae, funebris, hydei, melanogaster, pseudoobscura, subobscura, etc. (2) The marked-wings. Species whose wings bear a naturally pigmented spot or spots, or cloud, or pattern. Included here are hawaiian picture-wings, immigrans, robusta, quinaria-group, virilis-group, etc.

As far as is known there are no mutants (visible mutations) of any clear-wing species that have pigmented wing marks. Converesely, there are no mutants of marked-wing species that have unmarked wings. Excluded from clear-wing mutants are suffused general yellowing or darkening (as in yellow, black, dusky, ebony, sooty of melanogaster, and the shadowy smudge along the costa of subobscura at certain seasons), melanotic tumors, blood blisters, and developmental disturbances (e.g., black spotted wings (DIS 58:203), dumpy-oblique lethal vortex, and speck).

Wild-type marked-wings have one or more regular precise wing areas that are naturally and discretely pigmented in fully hardened flies. If the marks are multiple, any mutation would have to be assessed on the disappearance of all rather than on some of that particular type.

Thus it appears that at the species level there is a block to the apparently simple shift to or from pigmentation in the wings. In other words, the species is a block to some mutations. However, in a few species the male and female wings differ, one sex being clear-wing, the other marked-wing. Examples are D.tristis of the western palearctic and some species of the melanogaster-group. Evidently many clear-wing species do contain plenty of pigment in their bodies but it does not occur in discrete, localised spots in their wings. Yet two closely related species may belong to the opposite divisions. So where have all these intraspecific mutations gone?

Anyone has my consent to quote this note. I am grateful for information from M.Ashburner, H.Gloor, Oswald Hess, Claude W. Hinton, Costas B.Krimbas, Dan L.Lindsley, K.G. LUning, Dwight D. Miller, Toyohi Okada, D.Sperlich, Lynn H. Throckmorton, L.Craymer, and A-M.Jönsson(née Perje).