Vicine and convicine are present especially in seeds, whereas in senescent leaves they are only 0.26% and 0.11% of d.m. respectively.

Table 2 shows that vicine content, as a percentage, decreases rapidly during seed development (from 3.13% to 1.83% of d.m.); it becomes almost constant in ripe fresh seed (1.5-2.5 g of weight), and then drops during the drying phase. Convicine has a different behavior: values, as percentages, were always uniform until the dry phase starts, then a remarkable decrease was observed.

The table also shows that the production of alkaloids in seeds (mg/seed) steadily increases during development and ripening. Then it decreases during the drying stage, when the color of the cotyledons changes from green to pale yellow (4.5 g of weight). Up to now, it seems impossible to explain the causes of this decrease during the drying phase, as the functions of these compounds in the general metabolism of the plant (perhaps as a nitrogen reserve, or as growth regulators) are unknown. Similar variations have been observed in other cultivars of *V. faba* L., even when the alkaloid content was different.

These results can be considered in relation to the favism problem, a disease related to the use of broad-bean seeds in the human diet. As concerns the oxidization of glutathione (GSH) to GS-SG, vicine, convicine and L-Dopa have not the same activity 'in vitro'; L-Dopa activity is, probably, lower than that of the 2 alkaloids[12]. For this reason the variation of the 2 alkaloids, in relation to the seed age, was studied. In the literature, studies of the variation of the 3 favism-inducing factors, considered together, were not found. At this time it is not clear whether the absence of L-Dopa in ripe fresh seeds is a cultivar characteristic or a general phenomenon of all broad-bean seeds. Longo et al.[10], carrying out a screening in different organs of *V. faba* major, *V. faba* minor, *V. sativa* and *V. narbonensis* plants, found traces of L-Dopa in green seed only and not in dry ones. It must be added, in agreement with what has been observed up to now, that L-Dopa is a precursor in many biosynthetic reactions of the plant; therefore its content falls during seed development. The 2 alkaloids, which are final products of a metabolic process, behave in a different way; vicine and convicine are always present in the seed at various ages. Previous reports[7,8] concerning the vicine and convicine variation in seeds at different physiological stages do not agree with each other, even though they all agree that unripe seeds have a significant toxicity (oxidization 'in vitro' of GSH).

Conclusions. HPLC gives a rapid method for the quantitative determination of the favism-inducing factors in *V. faba* L. Preparation of samples for this procedure is simple, and it is sensitive. The quickness of the method will prevent oxidization and alkaloid precipitation. Furthermore, the high sensitivity makes it possible to obtain more reliable data for the study of antinutritional factors.

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2 A. Bendich and G.C. Clements, Biochim. biophys. Acta *12*, 462 (1953).
3 J. Jamalian, J. Sci. Fd Agric. *29*, 136 (1978).
4 E. Carnovale, M. Cappelloni and G. Zaza, Riv. Agron. *13*, 50 (1979).
5 H.S. Olsen and J.H. Andersen, J. Sci. Fd Agric. *29*, 323 (1978).
6 G. Rivoira, A. Spanu and S. Caredda, Riv. Agron. *13*, 61 (1979).
7 E.G. Brown and F.M. Roberts, Phytochemistry *11*, 3203 (1972).
8 R.S. Andrews and J.B. Pridham, Nature *4977*, 1213 (1965).
9 P.O. Larsen, E. Pedersen, H. Sørensen and P. Sørup, Phytochemistry *12*, 2243 (1973).
10 R. Longo, A. Castellani, P. Sberze and M. Tibolla, Phytochemistry *13*, 167 (1974).
11 J. Jamalian and A. Bassiri, J. agric. Fd Chem. *26*, 1454 (1976).
12 I.E. Liemer, in: Toxic constituents of plant foodstuffs, p. 265. Academic Press, London 1980.

Unstable genetic system in *Drosophila melanogaster:* I. Instability at the *cinnabar* locus

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Summary. A mutant strain containing a *cinnabar* allele *(cn^{rbr}, rojo brillante)* is reported, that produces wild-type revertants at the *cinnabar (cn)* locus. In cn^{rbr}/cn heterozygotes the rate of mutation is highly increased. The presence of a mutator agent acting premeiotically is indicated.

This paper reports studies on an unstable mutation at the *cinnabar* locus of *Drosophila melanogaster,* which arose in a *vestigial* stock with wild-type eye colour, obtained from the Department of Genetics of the Universidad Mayor de San Marcos de Lima (Peru) in January 1970, and since maintained in mass culture in our laboratory. In June 1970, a few flies appeared whose red eyes were more red than those of the wild type; the ocelli were colorless, but the eye color darkened with age. I isolated these flies and demonstrated, using appropriate crosses, that this color was due to the presence of a recessive mutant belonging to the *cinnabar (cn)* series[1] and I called it[2] *rojo brillante*, symbolized by cn^{rbr}.

The behavior of the *rojo brillante* strain was normal. Using a wild strain, I removed the new mutant from the original strain, with the *vestigial* mutant, and I obtained a cn^{rbr} strain on a wild type background. This new strain was maintained in mass cultures but, in some vials, flies with wild-type eye color spontaneously appeared. This wild-type eye color is due to the presence of an allele which is dominant over both cn^{rbr} and *cn.* I thought this allele originated from a spontaneous reversion of the cn^{rbr} allele and named it *normal espontáneo* symbolized by cn^{+e} (the *e* in the symbol indicates its spontaneous origin).

I have studied the spontaneous appearance of wild-type individuals (the reverse mutation from cn^{rbr} alleles to cn^{+e}

Table 1. Appearance of wild-type flies in the offspring of individuals carrying the cn^{rbr} allele. All tested flies were mated with cn/cn individuals

Mutation producer Sex	Genotype	Number of wild-type in progeny, per pair 0	1	2	3	4	5	6	8	9	10	12	16	17	18	22	23	27	Total wild-type	Total flies	Wild-type/ Total
Male	cn^{rbr}/cn^{rbr}	72	7	0	0	1	1	0	4	0	0	0	0	0	0	0	0	0	44	12,136	0.0036 ± 0.0004
Female	cn^{rbr}/cn^{rbr}	29	5	0	2	0	2	2	0	0	0	0	0	0	0	0	0	0	35	7,158	0.0048 ± 0.0008
Female	cn^{rbr}/cn	15	4	2	2	0	0	1	0	0	0	0	0	0	0	0	0	0	20	4,451	0.0043 ± 0.0009
Female	cn^{rbr}/cn	7	1	1	1	2	0	0	1	1	1	3	1	1	1	1	1	1	198	3,188	0.060 ± 0.0041

Table 2. Appearance of mutant flies in the offspring of heterozygous males bearing the marker chromosome (see text for explanation)

Mutational event	Phenotype in progeny b^+, cn^+, vg^+ cn^{rbr} to cn^{+c}	b, cn^+, vg cn to cn^{+c}	Cy^+, cn cn^{+c} to cn	Cy, cn^+ cn^2 to cn^{+c}
0	10	11	16	45
1	3	0	9	3
2	2	0	8	2
3	1	1	6	2
4	3	1	6	2
5	2	0	2	2
6	0	0	0	1
7	0	1	0	0
8	0	1	0	1
9	2	1	0	0
11	0	2	0	0
12	0	3	0	0
17	0	1	0	0
18	0	1	0	0
20	0	1	0	0
Total mutants	54*	144*	77*	45*
Total progeny	3088	3088	2420**	3107**
Mutants frequency	0.016 $\pm$0.0022	0.043 $\pm$0.0035	0.0318 $\pm$0.0036	0.0144 $\pm$0.0021
Parental pairs	24	24	17	28

* Goodness of fit of the data to a Poisson distribution is less than 0.001. ** Data refer number of chromosomes scored.

Table 3. Offspring of cn^{rbr}/cn heterozygotes that contain a substituted chromosome 3

Cross No. 1: $\frac{cn^{rbr}}{cn} : \frac{Sb}{\ln(D)}$ females $\times \frac{cn}{cn} ; \frac{\mathrm{III}(cn)}{\mathrm{III}(cn)}$ males

Replicates 12; total flies 1,155; wild-type 21.

Cross No. 2: $\frac{cn}{cn} : \frac{\mathrm{III}(cn)}{\mathrm{III}(cn)}$ females $\times \frac{cn^{rbr}}{cn} : \frac{Sb}{\ln(D)}$ males

Replicates 8; total flies 620; wild-type 28.

Explanation of some symbols: $\mathrm{III}(cn)$ = 3rd chromosome of the *cinnabar* strain. Sb = the mutant Stubble[1]. $\ln(D)$ = inversion *3 (LR) D*[1].

ones) in the *rojo brillante* strains. For this purpose, I have observed the progeny of 85 cn^{rbr}/cn^{rbr} homozygous males crossed with cn/cn females (the *cn* strain showing stable behavior). The results obtained are presented in line 1 in table 1. I have also mated cn/cn males with 40 cn^{rbr}/cn^{rbr} homozygous females. The results obtained are presented in line 2 in table 1. There it is seen in both classes of crosses that reversion occurs in the offspring of both sexes, and this leads us to suppose that reversion is not due to a recombinational event. Also, it is seen that revertants are concentrated in the progeny of a small number of pairs, but their frequency does not fit a Poisson distribution, from which I deduce that reversion is a premeiotic phenomenon[3].

Thus, I have an unstable strain that shows a strong increase in the rates of mutation and back-mutation at a given locus. This instability could be due to an agent (of unknown structure) that acts before meiosis in both sexes. This makes us think that in *rojo brillante* strains there is a mutator agent that acts against the *cinnabar* locus.

The heterozygote cn^{rbr}/cn shows the same phenotype as *cinnabar* homozygotes, but in its progeny (F_2 and back crosses) wild-type individuals appear. This new phenotype is due to a mutant which is dominant over *rojo brillante* and *cinnabar* alleles. I thought it was a new reversion of the *rojo brillante* allele and I named it *normal de cruce* symbolized by cn^{+c} (the *c* after the plus symbol meaning its origin from cn^{rbr}/cn hybrids). When the progeny of cn^{rbr}/cn heterozygotes was scored, I observed that the frequency of reversions is at 2 levels: a low and a high one. When the reversion occurs at low level, the frequency of appearance of wild type flies is not statistically different from that observed among the progeny of cn^{rbr}/cn^{rbr} homozygotes. But when the reversion occurs at high level, the frequency of their appearance is strongly increased. In order to compare the 2 types of frequencies, I present in lines 3 and 4 in table 1 the offsprings of 2 sets of 24 heterozygous females crossed with cn/cn homozygous males. These results are very different. They show: firstly, that in crosses of high wild-type production (4th line in table 1) there are more pairs of flies (17) producing reversions than in those of low wild-type production (9) (3rd line in table 1); secondly, in addition to this, the pairs of high wild-type production producing reversions, have a larger number of wild-type progeny than the pairs of low production which also produce wild-type flies among their progeny. In other words, in the heterozygotes of high wild-type production, there are more reversion-producing pairs which, in turn, each produce a larger number of wild-type individuals. The increase of the rate of mutations in heterozygotes is probably related to with the presence of a mutator agent. Similar aberrant traits have been defined as hybrid dysgenesis[4,5]. Data both in lines 3 and 4 of table 1 show that revertants have appeared in clusters and this suggests that they have a premeiotic origin.

In order to know which of the alleles *cn* or cn^{rbr} reverts in cn^{rbr}/cn heterozygotes, I have done crosses of *b, cn, vg*/b^+, cn^{rbr}, vg^+ heterozygous males mated with *b, cn, vg*/*b, cn, vg* homozygous females. The expected progeny is $\frac{1}{2}b$, *cn, vg* flies and $\frac{1}{2}b^+$, cn^{rbr}, vg^+ ones. If there are reversions, 2 new types can appear: one showing *b*, cn^+, *vg* phenotype, carrying the revertants from *cn* ton cn^+; and another showing wild-type which would indicate that reversion from cn^{rbr} to cn^{+c} had taken place. Columns 1 and 2 in table 2 indicate the size of clusters where these unexpected phenotypes have appeared. It is clear that both *cn* and cn^{rbr} alleles are affected by the action of the mutator agent. No recombination or reversions were observed involving the outside markers *b* and *cn*, and this strengthens the view that the wild-type revertants of *cn* alleles are not the results of recombination.

Although it is not reflected in table 2, in some of the progeny I have found some individuals which carry the reversion of the cn^{rbr} allele (phenotype b^+, cn^{+c}, vg^+) and some others which show the reversion of the *cn* allele (Phenotype *b*, cn^{+c}, *vg*). This suggests that in the same individual progenitor there may occur more than 1 reversion.

Through appropiate crosses, I substituted the 3rd chromosome in the unstable cn^{rbr}/cn heterozygote, which then remained unstable (table 3). I obtained similar results when I substituted the X chromosome. These results lead us to suppose that the cause of instability is located in the 2nd chromosome which also contains the *cinnabar* locus and the marker loci used.

In order to know whether cn^{+c} revertants alleles are stable or not, I have crossed flies carrying a cn^{+c} allele with a balanced strain (*Cy0, Curly* derivative of Oster, whose genotype is In(2LR), 0, dp^{ive}, *Cy, pr,* cn^2/Bl, *cn, bw).* From the offspring I have selected males with curly wings and wild type eye color (carrying the *Cy*0 chromosome and the cn^{+c} allele in its homologue). These males were mated with *cn/cn* homozygous females. In the offspring of those crosses, 2 types of reversions were noted: 1st, from cn^{+c} to cn^{rbr} and 2nd, from cn^2 to cn^{+c} (this 2nd reversion in interesting because it is a new *cn* allele that reverts by the action of the mutator agent). Without reversions, the offspring expected will be divided into 2 classes: one, with curly wings and cinnabar eyes, another showing wild type wings and eyes. But if reversions take place, 1 or 2 more phenotypes may occur: a) curly wings, wild-type eyes, indicating that a cn^2 allele has reverted to cn^{+c}, and b) wild type wings and cinnabar eyes indicating that a cn^{+c} allele has back-reverted to a *cn* allele. The 3rd and 4th columns in table 2 present the results obtained from such crosses (revertants of both are presented only), and we can see, firts that the mutator agent acts against cn^2 alleles, and secondly that the cn^{+c} alleles are not stable since they can rever again. Appearance in clusters leads us to suppose that both events occur in phases prior to meiosis.

1 D.L. Lindsley and E.H. Grell, Genetic variations of *Drosophila melanogaster.* Carnegie Institution of Washington, Washington, 1968; Publication No.627.
2 E. Valadé del Rio, cn^{rbr}*: rojo brillante.* Drosophila Information Service *51,* 22 (1974).
3 M.M. Green, Genetics *56,* 467 (1967).
4 M.G. Kidwell and J.F. Kidwell, Genetics *84,* 333 (1976).
5 M.G. Kidwell, J.F. Kidwell and J.A. Sved, Genetics *86,* 813 (1977).

Gene controlled condensation in individual chromosomes[1]

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Summary. When cells were irradiated with variable doses of gamma rays, 0.33% showed the appearance of single decondensed chromosomes (SDC) at the moment at which all the other chromosomes of the complement exhibited the normal condensed state corresponding to metaphase stages. Several hypotheses are discussed to explain the origin of SDC. It appears that the most reasonable mechanism to explain our observations is to assume that the process of chromosome condensation is independently controlled in each individual chromosome by a gene/s located in each one of the chromosomes of the complement. A radiation-induced deficiency in one of these genes may produce an impairement in the normal process of condensation of the carrier chromosome which would give rise to SDC.

It is known that changes in the extent of chromosome condensation have a great influence on gene transcription and replication[3]. Genetically active regions are usually decondensed, whereas chromatin condensation is usually found in genetically inactive areas[4]. Likewise, an extended state of the chromatin also seems to be necessary for the chromosome replication to occur[5]. Some time ago, Mazia[6] suggested the existence of orderly changes in the degree of chromosome condensation through the cell cycle. However, due to the difficulties inherent in the analysis of chromosomes in interphase nuclei this suggestion remained to be confirmed. Recently, by using the technique of premature chromosome condensation[7,8] it became possible to visualize the chromosome cycle in interphase HeLa cells[8]. The data obtained demonstrate that there is a progressive chromosome decondensation during the G1 and a progressive chromosome condensation during the G2 phase.

Though the cycle of decondensation-condensation is a general process involving the whole of the chromatin, it is not necessarily simultaneous for all chromosomes or chromosome regions. Since the classical paper of Heitz[10] on heterochromatin it has been well known that certain chromatin areas have an asynchronous cycle of condensation (allocycly) which gives rise to the phenomenon of positive and negative heteropycnosis[11]. Hence, it seems evident that different chromosomes or different intrachromosomal areas may have independent mechanisms controlling the phenomenon of coiling.

In this report evidence is given suggesting that gamma radiation may produce an impairment in the process of chromosome condensation, and the probable causes of this phenomenon are discussed.

Material and methods. Two series of experiments were performed. In the 1st series a total of 8 *Akodon molinae* (Rodentia Cricentidae) were irradiated with 400 R. At 8, 12, 16 and 20 h after irradiation the animals were sacrificed. Colchicine (0.1 µg/g of b.wt) was injected 1.5 h, before

Correlation between the frequency of SDC and gene mutation rates

Gamma-ray doses	Expected mutation rates*	Observed frequency of SDC	Cells analyzed	Relative frequency of SDC
0	-	-	1600	-
100	0.7×10^{-3}	0	400	0
200	1.4×10^{-3}	1	400	2.5×10^{-3}
400	2.8×10^{-3}	3	900	3.3×10^{-3}
800	5.6×10^{-3}	5	700	7.0×10^{-3}

* Estimated with the equation in the text.