trapped in 7 different points in the south of Spain, in the provinces of Granada, Jaen and also in Guadalajara, Albacete, Murcia and Cuenca. The technique employed was that of 'medula osea' (bone marrow) for the study of somatic chromosomes, and Meiosis was studied in testes cells.

With the exception of the population of Granada, with 21 animals examined, which presented a great numeric variety (2n = 38, 39, 40 and 41), the remaining populations from other localities showed a relative stability: 2n = 38 and rarely 2n = 39. The numbers 2n = 39, 40 and 41 are new for the species and were previously reported by us for the first time[12].

In all cases the variation in number was due exclusively to the presence of 1, 2 or 3 supernumerary chromosomes, respectively, which by their morphology and size do not differ from the small metacentric chromosomes of the normal standard, 2n = 38, complement (figures 1–8).

In the population of Granada, the frequency of different kinds of numeric variants was as follows: 2n = 38, ~14.2%; 2n = 39, ~42.8%; 2n = 40, ~38%; 2n = 41, ~5%. In order to know with greater exactitude the nature and origin of these chromosomes, we performed a careful study of Meiosis (figures 9–13).

Early in Prophase these chromosomes appear as strongly heteropicnotic bodies, the arms very strongly contracted and the constrictions well pronounced as in the case of Metaphase chromosomes (figure 9). They collocate themselves generally in the margins of the nucleus. When there are more than 1 of them, they form lateral achiasmatic associations (figure 10) and part precociously, so that in Metaphase they appear separated (figure 12). In the animals 2n = 40, with 2 supernumerary chromosomes, in 80% of all metaphase plates, these chromosomes do not appear in pairs. At this stage it is sometimes possible to observe their negative heteropicnosis (figure 13).

The observations carried out allow us to conclude that the distribution of these chromosomes in the products of Meiosis is irregular. At present we are making crossing experiments in order to elucidate the heredity of these chromosomes. The observations made in Meiosis clearly show the supernumerary character of these chromosomes, being strongly heteropicnotic, because of the lack of pairing at Metaphase and because of their irregular segregation.

Random chromosome breakage by colchicine in *Viscum fischeri* (Loranthaceae)

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Summary. When the shoot-tips of *Viscum fischeri* Engl. were treated with various concentrations of colchicine for different lengths of time, it was found that in this plant chromosome breakage was not localized to centromeric region as reported in other plants. In *V. fischeri* chromosome breakage occurred at random (simulating X-ray-induced fragmentation). The percentage of breakage increased linearly with respect to time at concentrations 0.1, 0.2, 0.3% but parabolically at 0.5%.

Ever since the discovery of colchicine as polyploidising agent in 1937[4], it has been used extensively for duplicating chromosomes of a large number of plant species and plant hybrids; but except for a few reports[5–7] its effect on chromosome damage is not known in detail. The fact that this chemical is used in the treatment of certain human ailments, e.g. gout[8], makes it necessary to investigate its genetic effects. In studies relevent to environmental mutagenesis, various genetic parameters are used; one such parameter is chromosome breakage caused by an agent. Since colchicine is known to cause breaks at the centromeric region of chromosomes[5–7], we undertook further to investigate its effects on the chromosomes, using *Viscum fischeri* which has extraordinarily large chromosomes, ranging from 10 to 44 μm[9].

Material and methods. The shoot-tips of *V. fischeri* Engl. (female) were collected between 09.00 and 10.00 h and immediately placed in aqueous solution of colchicine of 4 different concentrations and for various lengths of time (table 1). The material was washed in distilled water after this pretreatment and fixed in acetic alcohol (1:3) for 1 h. After fixation the material was washed in water for 10 min and then hydrolysed for $7^1/_2$ min in NHCl at 60 °C, then stained in aceto-carmine and squashed.

Results and discussion. A total of 994 cells was scored of which 142 were controls. In the controls as well as in the 0.05% series, breakage was observed in 1 cell only. These cases might be due to the effect of water which is reported to occasionally cause breaks[10]. Higher concentrations in-

Table 1. Observed percentage of chromosome breaks

Time (h)	Percentage of mitoses showing one or more chromosome breaks at 6 concentration levels					
	0.0	0.05	0.1	0.2	0.3	0.5
0.5	0	0	0	0	0	0
1.0	0	0	0	3	12	19
2.0	0	0	0	17	40	77
3.0	3	0	2	26	49	88
4.0	0	2	6	36	66	100

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Table 2. Curve-fitting and test of goodness of fit : X = time and Y = percent of breakage of chromosomes

Concentration level (%)	Regression equation	χ^2	Remarks
0.1	$Y = -1.78 + 1.61\ X$	2.19 (2 df)	not significant
0.2	$Y = -5.83 + 10.59\ X$	0.84 (3 df)	not significant
0.3	$Y = -5.60 + 18.57\ X$	6.15 (4 df)	not significant
0.5 (a)	*$Y = -4.82 + 29.34\ X$	22.71 (4 df)	highly significant
0.5 (b)	*$Y = -36.41 + 71.76\ X - 9.51\ X^2$	3.045 (3 df)	not significant

*The 2 equations are the line and parabola respectively, fitted to data by the 'method of least squares'.

duced breakage at frequencies which became increasingly important as a function of the dose and of the duration of treatment. At 0.1%, breakage was first observed after 3 h of treatment; at 0.5% after 1 h already.

From statistical analysis (table 2) it was found that a more or less linear relationship exists between time and the percentage of breakage induced by colchicine at 0.1, 0.2 and 0.3% concentrations. However, at 0.5% concentration level the relationship is no longer linear but it is parabolic. It should also be mentioned here that no other types of chromosomal aberrations except chromosome breaks were observed in the colchicine-treated material. Earlier workers[5-7] found that the breaks caused by colchicine are not at random but are confined to the centromeric region. For instance, Levan and Rhoades[5] observed that certain chromosomes in maize broke at the centromere and gave rise to isochromosomes. Darlington[6] made similar observation in *Fritillaria camschatcensis* and Karpechenko[7] in barley. The present results show that the chromosome breaks may occur at random and are not confined to the centromeric region alone. One of the reasons why random breaks were detectable in *V. fischeri* may be that this material has much larger chromosomes than those which earlier workers dealt with.

The present results may explain why colchicine-induced 'raw' polyploids are invariably sterile. These results led us to believe that colchicine, besides duplicating the chromosome number, also damages certain chromosomes in the complement, the damage in most cases being undetectable. Further indirect evidence that colchicine may cause damage to chromosomes has been obtained in the colchicine-treated seedlings of the Asiatic cotton, *Gossypium arboreum*, where 1 out of 100 treated seedlings did not show chromosome duplication upon maturity and yet it was completely sterile with no bolls[11]. Pollen mother cells of this unresponsive diploid plant showed regular meiosis of 13 bivalents at metaphase I, and wellstained pollen grains. Such an observation indicates that colchicine may cause damage to chromosomes with or without chromosome duplication; thus undetectable chromosome damage could perhaps be the main cause for seed sterility. It is possibly for this reason that the spontaneously-arizing polyploids are more fertile than the colchicine-induced ones.

In the light of the above results it is suggested that a) when used to induce duplication of plant chromosomes, colchicine should be used only in low concentrations (if a less toxic polyploidising agent is not available for the purpose) and b) it should not be used in the treatment of gout during pregnancy as there is likelihood of congenitally defective births from the prolonged administration of this drug.

11 Unpublished work of the first author in collaboration with his students in Sind University, Pakistan.

Interaction of genotype and learning in the food preference of the flour beetle, *Tribolium castaneum*

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Summary. Selection was combined with training to study learning in the flour beetle, *Tribolium castaneum*. The response was rapid when selecting for both normal (N) and garlic-aversive (G) food preference – which increased for N and decreased for G. N adults can be conditioned to go toward the medium on which they were raised. Preference of G females is explained by habituation and that of N males by conditioning. This suggested that conditioning or habituation depends on the genetic background and sex.

The attraction of animals towards specific foods or odours may depend on either inherited or learned preferences[1]. Genetic variation in preference has been demonstrated by many selection studies, e.g., those for ethanol preference in rodents[2]. But in insects there is a situation where learning can impede the response to selection. This is the phenomenon of 'pre-imaginal conditioning' whereby insects, reared on a normally aversive medium, lose their aversion[3], making it very difficult to select for increased aversion. In *Drosophila melanogaster*, such 'pre-imaginal conditioning' has been explained as habituation to the presence of the aversive substance, rather than as conditioning through an association of the substance with the food medium[4]. The present experiment involved a

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