LSD. The absence of the metabolic activity of the liver and the greater sensitivity of cells in vitro may be causes for the discrepancy in results between in vivo and in vitro systems. Psilocybin has not been investigated as extensively as LSD. Addition of psilocybin to human lymphocytes in vitro led to an increase of chromosome gaps[11]. This type of chromosome abnormality, however, is usually not seen as representative for chromosome breakage. In contrast to the publication of Eberle and Leuner[12], where an increased number of chromosome breaks was found in 4 patients treated with psilocybin, results of our experiments with humans on psychotherapy and Chinese hamsters[20] gave no indication for an in vivo chromosome-damaging activity of psilocybin and are in good agreement with the negative findings in the micronucleus test. The results with THC in the micronucleus test correspond well with literature findings. Neither Δ^8- nor Δ^9-THC induced chromosome aberrations when added to human lymphocytes in vitro[13, 14]. Δ^9-THC taken orally or smoked in different dosages in cigarettes by humans, did not cause an increase in chromosome breaks[15, 16], nor did s.c. application in Syrian hamsters[10]. In vitro addition of *Cannabis* resin to embryonic rat fibroblasts or human lymphocytes, in vivo application to pregnant rats or smoking by humans did not lead to an increase in chromosome abnormalities[17]. In a large group of marihuana users, on the other hand, a statistically significant increase in chromosome aberrations was found[18, 19]. The conflicting data from marihuana smokers may be explained by assuming a component, other than THC, being the cause of the chromosome damage. In mutagenecity testing it is not possible at present to prove the mutagenic potential of a compound in a single test system. Results of other tests will be needed to confirm our negative results with the micronucleus test.

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Chromosomal polymorphism caused by supernumerary chromosomes in *Rattus rattus* ssp. *frugivurus* (Rafinesque, 1814) (Rodentia, Muridae)

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Summary. A chromosomal numeric polymorphism 2n = 38, 39, 40 and 41 in the species *Rattus rattus* ssp. *frugivurus* (Rafinesque, 1814) is reported for the first time for this subspecies. The numbers 2n = 39, 40 and 41 are new for the species. The polymorphism is due to the presence of 1, 2 or 3 B-chromosomes, which are all small metacentrics of the size and shaped very close to the other autosomes of the normal complement, and whose character of being supernumeraries is shown in Meiosis.

While chromosomal polymorphism due to supernumerary or B-chromosomes is widely extended in plants and among some groups of insects[1], its incidence among the mammals is rather rare. And yet, some cases have been described, among which stands out one reported for the marsupial *Schoinobates volans*[2], which contains in its cells a variable number of small additional metacentric chromosomes (microchromosomes), ranging from 1 to 3. In *Echymipera kalabu* was pointed out by Hayman[3], in parallel with a sex chromosome mosaicism. Certain extra or supernumerary chromosomes were also reported in other mammalian species, such as *Vulpes vulpes*[4-6]. In rodents, *Reithrodontomys megalotys*[7], *Rattus rattus* ssp. *diardii*[8], *Rattus rattus*[9] and *Apodemus giliacus*[10].

The numeric polymorphism of *R. rattus* ssp. *diardii*, described by Yong[8] in the individuals with standard karyotype 2n = 42 of Malaysia, is due to the presence of some small additional chromosomes, which appeared all to be small metacentrics, indistinguishable from small metacentrics of the normal complement. Gropp et al.[11] found in

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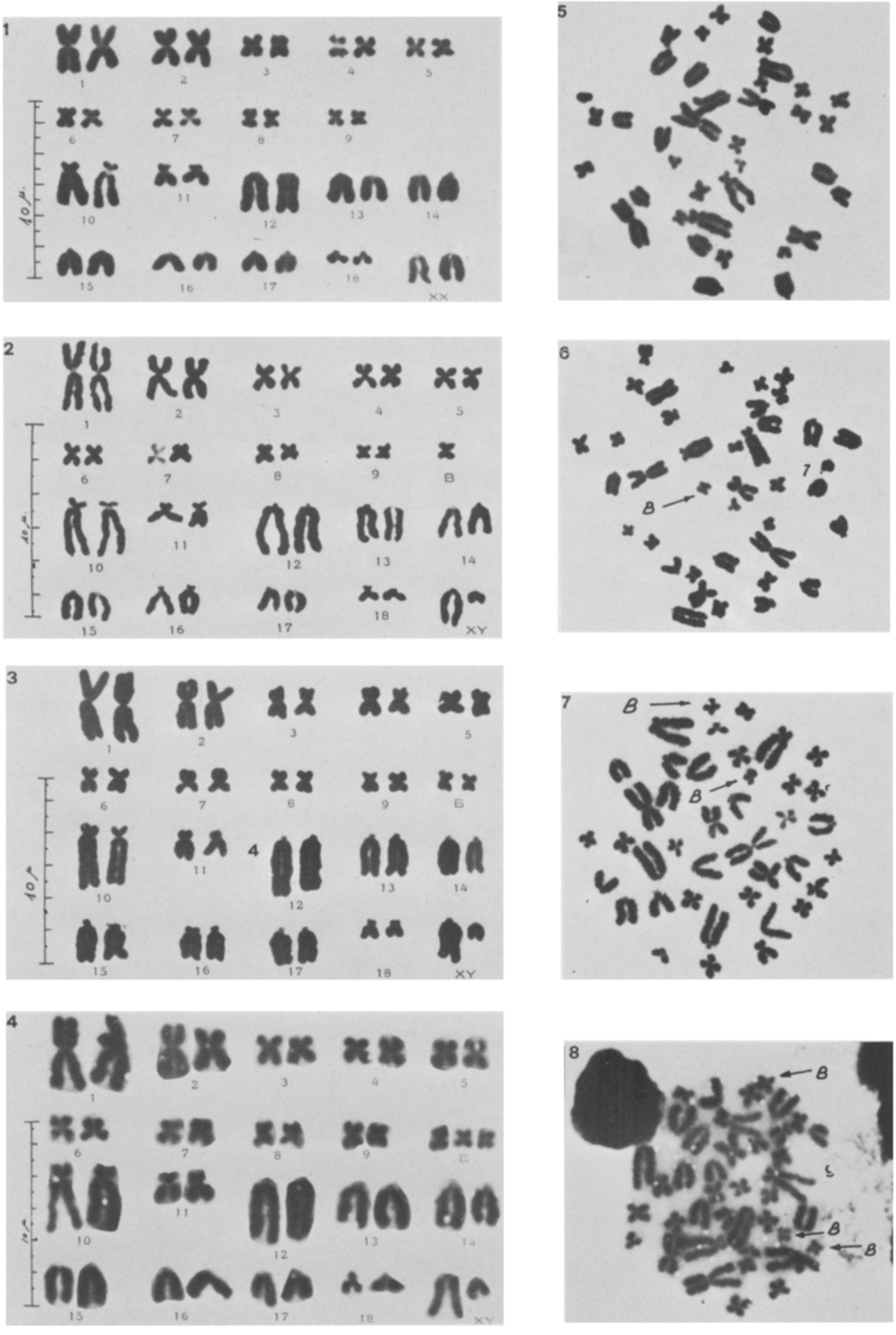

Figures 1–8. Karyotypes of *Rattus rattus* ssp. *frugivurus* (Raf. 1814).

Fig. 1. Standard female karyotype, 2n = 38. Fig. 2. Male, 2n = 39, with 1 B-chromosome. Fig. 3. Female, 2n = 40, with 2 B-chromosomes.

Fig. 4. Male, 2n = 41, with 3 B-chromosomes. Fig. 5. Mitotic metaphase plate, normal, 2n = 38. Fig. 6–8. Mitotic metaphase plates with 1,2 and 3 B-chromosomes respectively.

Asiatic population of *Rattus*, identified as *R. rattus* ssp. *thai* (Kloss, 1971), 2n = 42, a new type of numerical variation, due to the presence of 1, 2, 3 or 4, respectively, pairs of additional small metacentrics. The 2 latter authors do not go very deeply into the studies of the nature of these additional chromosomes, limiting themselves to the mere statement of their presence.

Hayata[10] describes wide individual variation ranging from 48 to 59 of the chromosome number in *Apodemus giliacus*. Such extreme variability in chromosome number was ascribed to the differences in number of several biarmed chromosomes and microchromosomes. The heteropicnotic character of these additional elements, as well as their irregular meiotic behaviour in this species, revealed that the unstable elements represented certain complex supernumeraries of hitherto undescribed nature.

The presence of B-chromosomes in the species *Rattus rattus* ssp. *frugivurus* (Rafinesque, 1814) has been previously reported by us[12]. In this paper, a case of numeric polymorphism in this proceeding from different localities, mostly in the south of Iberic Peninsula, is described. A total number of 44 animals had been analysed, all of them live-

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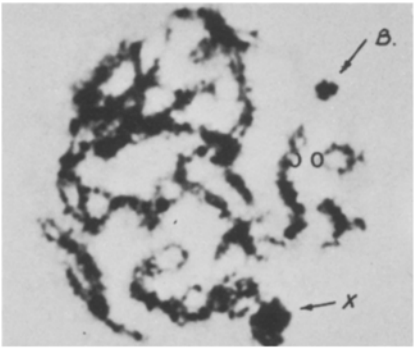

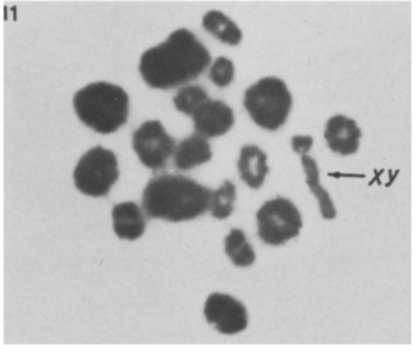

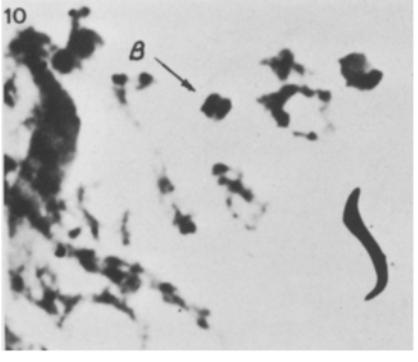

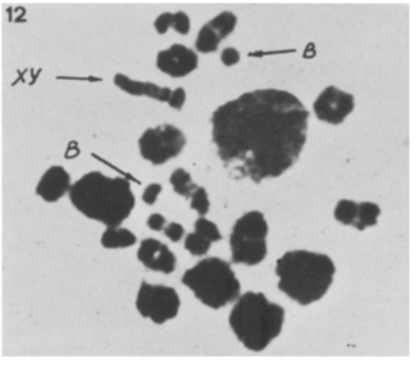

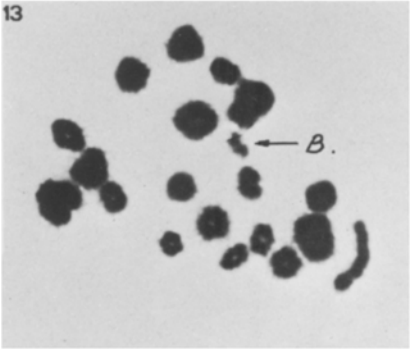

Figures 9–13. Meiosis in *Rattus rattus* ssp. *frugivurus*.

Fig. 9. Pachytene with 1 B-chromosome. Fig. 10. Diplotene. Lateral aquiamatic pairing of B-chromosomes, 2n = 40. Fig. 11. M-I, with 19 II. Normal, 2n = 38 individual. Fig. 12. M-I, with 2 unpaired B-chromosomes, 2n = 40. Fig. 13. B-chromosome showing negative heteropicnosis, 2n = 39.

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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