(*V. rufa*)[2,5], we found that *V. germanica* was the closest to *V. rufa*. Yamane[3] concluded on the basis of larval characters that *Dolichovespula* species share some derived characters and are less closely related to *Vespa* than to *Vespula* species. In our study we found, in accordance with Green[20], that the ancestral genus of *Vespa* is more related to the genus of *Dolichovespula* than to the genus of *Vespula*. This is in agreement with the thesis that the *Vespula* group represents the most recently derived group.

The present study gives further evidence for the phylogenetic relationship of six European social wasps, based on genetic data. The results are largely in agreement with morphological studies[2,20], with the exception of dividing *Paravespula* and *Vespula* into distinct groups. The next step in the study of wasp mtDNA should be the analysis of restriction site maps, which will give us further details of Vespinae phylogeny.

Acknowledgments. This work was supported by the Deutsche Forschungsgemeinschaft (Grant No. Mo 373/4-1; RFAM).

1 Linnaeus, C., Systema Naturae, 10th edn. Holmiae Laur Salvii (1785).
2 Beaumont, J. De, Mitt. schweiz. ent. Ges. *31* (1958) 168.
3 Yamane, S., Ins. Matsumurana New Ser. *8* (1976) 1.
4 Varvio-Aho, S. L., Pamilo, P., and Pekkarinen, A., Insectes soc. *31* (1984) 375.
5 Carpenter, J. M., Syst. Ent. *12* (1987) 413.
6 Richards, O. W., Biol. Rev. *46* (1971) 483.
7 Carpenter, J. M., Syst. Ent. *7* (1981) 11.
8 Densmore, L. D., Wright, J. W., and Brown, W. M., Genetics *110* (1985) 689.
9 Bermingham, E., Lamb, T., and Avise, J. C., J. Hered. *77* (1986) 249.
10 Merril, C. R., and Harrington, M. G., Trend genet. *5* (1985) 150.
11 Solignac, M., Monnerot, M., and Mounolou, J. C., J. molec. Evol. *23* (1986) 31.
12 Moritz, R. F. A., Hawkins, C. F., Crozier, R. H., and Mackinlay, A. G., Experientia *42* (1986) 322.
13 Maniatis, T., Fritsch, E. F., and Sambrook, J., Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 1982.
14 Nei, M., and Li, W. H., Proc. natl Acad. Sci. USA *76* (1979) 5269.
15 Upholt, W. B., Nucl. Acid Res. *4* (1977) 1257.
16 Tukey, J. W., Exploratory Data Analysis. Addison-Wesley, Reading, Massachusetts 1977.
17 Sokal, R. R., and Michener, C. D., Univ. Kansas Sci. Bull. *28* (1958) 1409.
18 Nei, M., Molecular Evolutionary Genetics. Columbia University Press, New York 1987.
19 Carpenter, J. M., Insectes soc. *34* (1987) 58.
20 Green, A., Ann. ent. Soc. Am. *72* (1979) 614.

0014-4754/90/101068-05$1.50 + 0.20/0

Induction of chromosome aberrations and chlorophyll mutations in plants by methylisocyanate (MIC) gas

G. Kumar, A. N. Sahi and S. K. Roy

Laboratory of Cytogenetics, Department of Botany, Banaras Hindu University, Varanasi-221005 (India)
Received 11 September 1989; accepted 13 March 1990

Summary. Seeds of *Solanum surattense* Burm. f. collected from areas of Bhopal (India) affected by methylisocyanate gas showed chromosome aberrations in root cells, and growth retardation and chlorophyll mutation of seedlings, the frequencies of which varied from one locality to another.
Key words. Solanum surattense; methylisocyanate gas; chromosome aberrations; chlorophyll mutants; interlocality variations.

Though hazardous in nature, the actual lethal potency of methylisocyanate (MIC) gas was not known before the fateful event on December[2-3], 1984, at the Union Carbide Factory at Bhopal. The venomous vapour killed more than 3000 people and numerous animals and plants[1,2]. Besides its lethal effects, it produced morphological lesions and cytogenetic abnormalities in living organisms[3-6]. In vivo study revealed that MIC and its reaction products cause mutagenicity in cultured mammalian cells[7]. It was shown to inhibit gametophyte morphogenesis and caused chlorophyll deficiency in fern gametophytes[4,8], and induced chromosome aberrations in pollen mother cells[5]. In order to understand the extent to which cytogenetic damage is carried into the progeny plants, MIC-exposed seeds were sown and the seedlings studied cytomorphologically.

Materials and methods

Wild *Solanum surattense* seeds were gathered from five areas known to be gas-affected and also from unaffected ones (control area)[5] in August, 1985. Five different sites in each area were randomly selected and from each site 10–15 ripe fruits of five different plants were collected and their seeds stored in a desiccator for a couple of months at room temperature. These were grown in the following November on sand beds. Seedling growth was measured on 15-day-old seedlings on a dry weight basis, and seedling survival was recorded three times a week until no more deaths occurred. For cytological observations germling root-tips were fixed in Carnoy's solution (acetic acid: alcohol, 1:3) overnight, and squashed in 1% acetoorcein. About 250 cells per sampling area were analysed for each kind of chromosome aberration.

Table 1. Effects on *S. surattense* seedlings from MIC exposed seeds.

Sampling areas	Germination (%)	Seedling survival (%)	Growth performance (Dry wt mg/ plant)	No. of chimeric plants**	Frequency of chlorophyll mutants			Frequency of mosaic plants
					Viridis (%)	Chlorina (%)	Total (%)	
Firdaus Nagar	95.48 ± 1.52	100.00	38.40 ± 2.15	–	–	–	–	–
J. P. Nagar	92.40 ± 1.38	92.60 ± 2.65	28.68* ± 2.38	47	4.20	3.00	7.20	2.20
Cholakenchi	92.80 ± 1.62	97.50 ± 2.38	32.50 ± 2.71	32	2.60	2.40	5.00	1.40
Nishatpura	93.40 ± 1.42	96.67 ± 2.40	34.60 ± 2.68	15	1.40	1.60	3.00	-
Railway colony	92.80 ± 1.28	93.66 ± 2.68	30.33* ± 2.33	40	3.60	2.60	6.20	1.80
Central School	95.60 ± 1.39	95.33 ± 2.75	32.78 ± 2.57	18	2.00	1.60	3.60	-

*Significant at 0.05 level; **500 plants observed in each case.

Chlorophyll mutants were scored at the 3–5 leaved seedling stage, and classified after Gustafsson[9].

Results

Seeds from the affected areas showed no significant inhibition either in germination or in the survival of seedlings, but their growth performance was severely affected (significant at 0.05 level, table 1). Two kinds of chlorophyll-deficient plants, namely *Viridis* and *Chlorina* were frequently observed among them. Mosaic plants from highly affected areas showed the worst performance (table 1; fig. 1).

Chromosome aberrations of root-tip cells are assembled according to area in table 2. Of these aberrations stickiness, breakage, bridge formation and laggards (figs 2–7) were prominent and more numerous in places near the factory and along the wind current than in those away from it (fig. 1). Up wind from it the effects were less and less marked. While an affected area, e.g. J. P. Nagar, showed 2.77% stickiness, 1.03% breakage and 2.30%

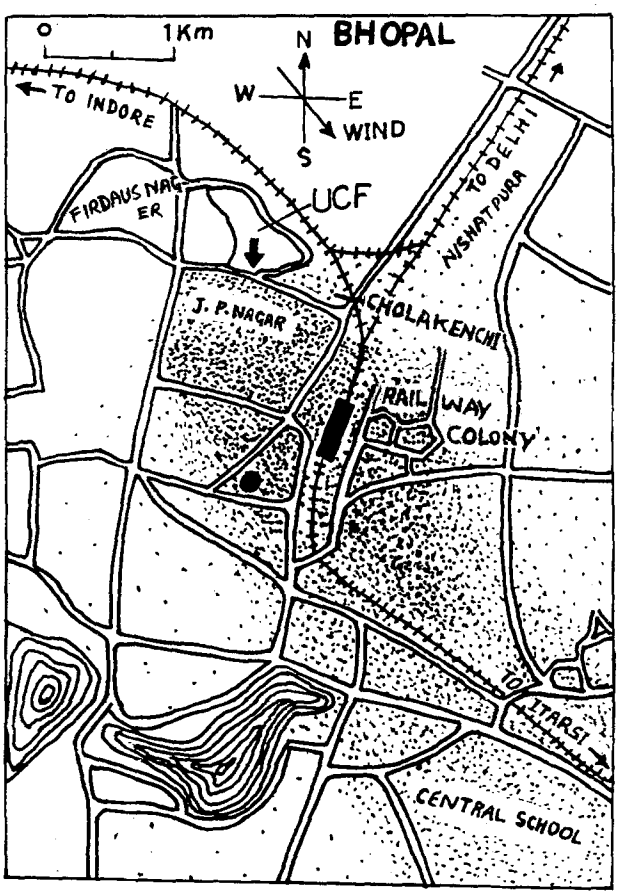


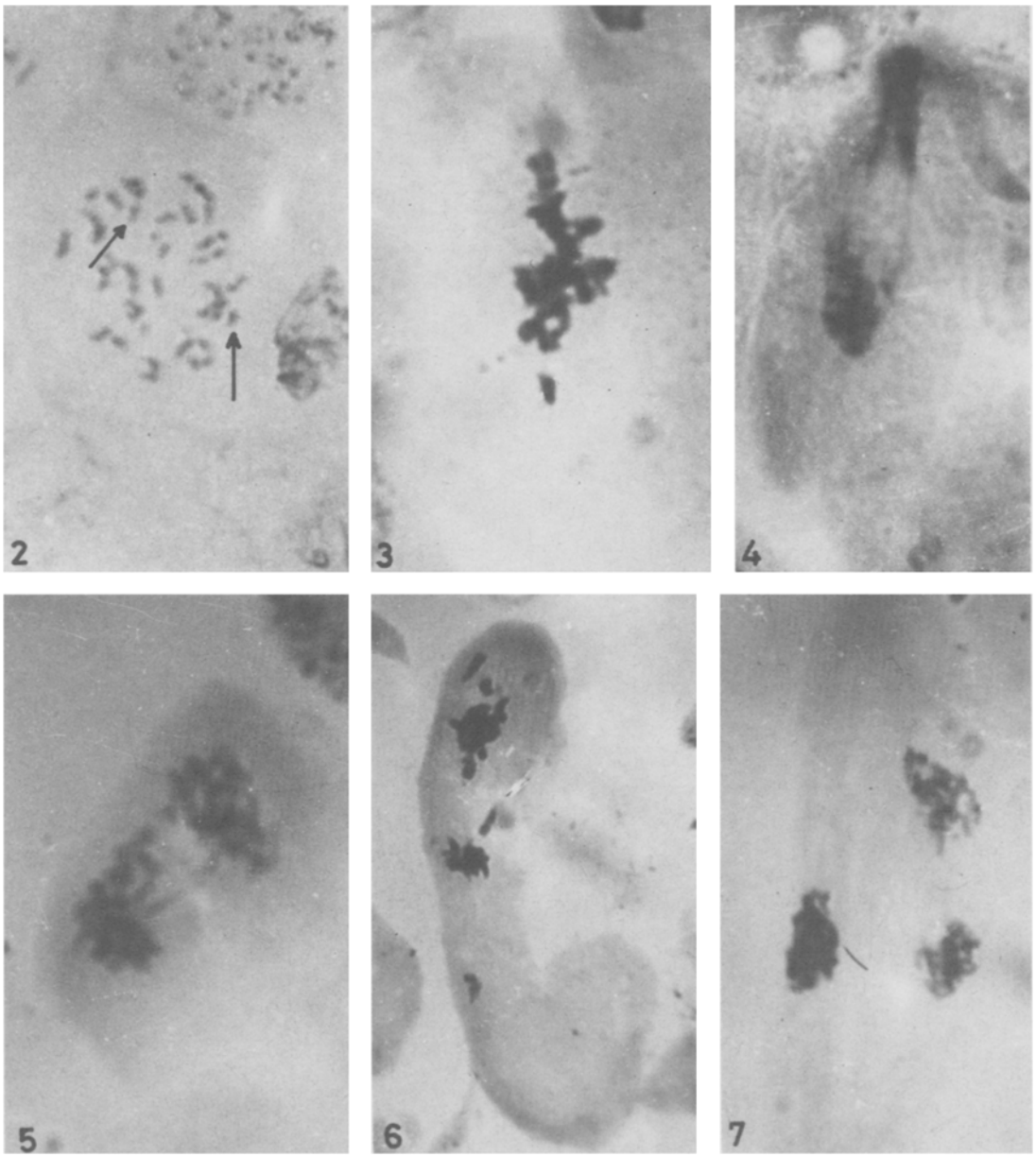
Figure 1. MIC gas affected areas of Bhopal. Arrow indicates Union Carbide Factory from where gas leaked.

Highly affected area; ● Bus stand; Lake; Lightly affected area; Rly. Station

Table 2. Effects of MIC on root cells of *S. surattense* (mean ± SD).

Sampling areas	No. of roots analysed	Chromosomal aberrations			
		Stickiness	Breakage	Bridge	Laggard
Firdaus Nagar	10	0.10 ± 0.05	–	0.16 ± 0.03	0.16 ± 0.03
J. P. Nagar	10	2.77*** ± 0.09	1.03*** ± 0.08	2.41*** ± 0.03	2.30*** ± 0.04
Cholakenchi	10	1.20*** ± 0.06	0.37*** ± 0.15	0.94*** ± 0.03	0.69*** ± 0.03
Nishatpura	10	0.64** ± 0.06	0.12** ± 0.03	0.53*** ± 0.04	0.32*** ± 0.03
Railway colony	10	2.30*** ± 0.67	0.87*** ± 0.06	2.09*** ± 0.03	1.99*** ± 0.05
Central School	10	1.69*** ± 0.07	0.52*** ± 0.03	1.69*** ± 0.04	1.25*** ± 0.04

Significant level **$p < 0.01$; ***$p < 0.001$. Sticky – includes clumped chromosomes; bridges – include sticky bridges; laggards – include eliminated chromosomes.



Figures 2–7. Microphotographs of MIC-induced aberrant root-tip cells of *S. surattense*. All ×2000 except fig. 2 which is ×1600. *2* Metaphase showing broken chromosomes (arrow). *3* Sticky metaphase. *4* Anaphase showing breakage of sticky bridge. *5* Anaphase bridge. *6* Lagging chromosomes at anaphase. *7* Anaphase showing trinucleate formation.

laggards, the figures from an unaffected area were 0.1, 0.16 and 0.16%, respectively. Even where dilution of the gas with air had lessened the effects, these still occurred at significant levels ($p < 0.01$, table 2).

Discussion

The present observations revealed that MIC retards growth and induces chlorophyll deficiency and mosaicism in plants. Root-tip cells of these plants exhibited various kinds of chromosomal aberrations at significant levels ($p < 0.01$) which, however, varied with the locality, the highest level being near the factory.

MIC vapour is more toxic than phosgene gas[1] and is heavier and denser than air, with the consequence that the gas could easily come into contact with the ground vegetation to cause devastation[5]. Although the mechanism of mutagenicity by MIC is not clearly understood, the induction at significant frequencies of chromosome aberrations and chlorophyll deficiency in MIC-affected plants suggest that it does indeed have mutagenic properties on plants. In vivo studies on mammalian cells have proved that MIC and its derivatives can cause mutagenesis[7] and genetic sterility[3]. The damaging property of MIC gas is reported to be due to its reactivity with water and the exothermic characteristics of this reac-

tion[1,10-12]. The exothermic reaction of MIC gas with cellular water is thought to cause molecular[11-15], cellular[1,5], cytogenetic[5,16,17] and morphological damage[4,8]. Most of the abnormalities could not be discarded by self-eliminating processes in successive generations of plants; this indicates that MIC has long-term effects on biological systems.

1 Picot, A., The World Scientist *10* (1986) 46.
1 Jayaraman, K. S., Nature *337* (1989) 679.
3 Agarwal, A., New Scientist *38* (1985) 1484.
4 Singh, I. P., and Roy, S. K., Curr. Sci. *56* (1987) 679.
5 Kumar, G., Tripathi, D. S., and Roy, S. K., Envir. exp. Bot. *29* (1989) 261.
6 Gupta, B. N., Rastogi, S. K., Chandra, H., Mathur, A. K., Mathur, N., Mahendra, P. N., Pangetey, B. S., Kumar, S., Kumar, P., Seth, R. K., Dwivedi, R. S., and Ray, P. K., Ind. J. exp. Biol. *26* (1988) 149.
7 Caspary, W. J., and Myhr, B., Mutat. Res. *174* (1986) 285.
8 Singh, I. P., and Roy, S. K., Curr. Sci. *58* (1989) 381.
9 Gustafsson, A., K. Fysiogr S allsk. Lund. Forh. *36* (1940) 1.
10 Gruzdyev, G. S., Zinchenko, V. A., Kalinn, V. A., and Slovtsov, R. I., in: Chemical protection of plants, p. 227. Ed. G. S. Gruzdyev. MIR Publisher, Moscow 1983.
11 Mericle, L. W., and Mericle, R. P., Envir. Mutat. Soc. News-sheet *5* (1971) 28.
12 Lindgreen, D., Hereditas *70* (1972) 165.
13 Stark, G. R., Biochemistry *4* (1965) 1030.
14 Coutino, R. R., Envir. Health Perspect. *31* (1979) 131.
15 Kihlman, B. A., in: Action of chemical on dividing cells, p. 96. Prentice Hall Press, New York 1966.
16 Vogel, E., and Natarajan, A. T., Mutat. Res. *62* (1979) 101.
17 Wade, D. R., Airy, S. C., and Singheimer, J. E., Mutat. Res. *58* (1978) 217.

0014-4754/90/101072-04$1.50 + 0.20/0

Cytogenetic and biochemical comparison of *Mus musculus* and *Mus hortulanus*[1]

L. V. Yakimenko, K. V. Korobitsyna, L. V. Frisman and A. I. Muntianu*

*Institute of Biology and Pedology, Far East Branch, Academy of Sciences of the USSR, Vladivostok 690022 (USSR), and * Institute of Zoology and Physiology, Academy of Sciences of the Moldavian SSR, Kishinev 277028 (USSR)*
Received 29 June 1989; accepted 15 June 1990

Summary. Two chromosome markers of *Mus hortulanus* are described: a dotted Y chromosome exceeding half of the length of autosome 19, and the 'domesticus' type of C-banding in the X chromosome. In *Mus musculus* from distant regions of the USSR (more than 200 specimens of various subspecies), the Y chromosome is equal to autosome 19, and the X chromosome is of the 'molossinus' type. Specific biochemical characteristics of house mice of the USSR are shown.
Key words. Mus musculus; *Mus hortulanus*; karyotype; X chromosome; Y chromosome; protein electrophoresis.

The relative uniformity of all chromosomes of the house mouse complement hinders cytogenetic studies of these animals. Beginning from 1970 the newest techniques have been used for analysis of chromosome structure in *Mus musculus*. Nevertheless, the problem of chromosome markers for differentiation of laboratory strains, and as subspecific and specific diagnostic criteria for various forms of house mice, has not been solved.

Comparative electrophoretic studies[2-4] provided the framework for the recognition of five genetic units in Europe: *Mus domesticus, M. musculus, M. spretus, M. hortulanus* and *M. abbotti. M. domesticus* and *M. musculus* hybridize in Central and South Europe[5], and from there *M. musculus* extends all over the USSR. *M. hortulanus* inhabits southern Europe, but the eastern limit of its range has not been determined. *M. abbotti's* range extends at least close to the Caucasus, but remains to be clarified. Also, the relationships between these species and Asian house mice are not known[6,7].

M. hortulanus differs from *M. musculus* in several ecological and ethological traits, even though the two species are very similar in morphology[7,8]. The hitherto-described karyotype of *M. hortulanus*, when compared to that of the house mouse, reveals no peculiarities by conventional staining, or in C-banding[7,9].

The paper presents the results of comparative studies of *M. musculus* s. str. and *M. hortulanus*, thus completing the data of Mezhzherin[10]. The species assignment of animals was determined according to Bonhomme et al.[11].

Materials and methods

More than 200 mice from 35 localities in the USSR were studied (table). Specimens from Kalmykia, Tuva and Transbaikalia were obtained both indoors and outdoors in summer. Animals from Moldavia were obtained in winter.

The chromosomes were prepared from cells of the bone marrow using standard procedures. C-banding for revealing the heterochromatin regions of chromosomes was performed according to Sumner[12]. The horizontal electrophoresis of proteins was carried out in starch gel with the buffer systems described by Selander et al.[13].