centric) autosomes. The $2n = 38$ karyotype had a pair of distinctively large metacentric autosomes. This pair of extremely large metacentric autosomes was not present in the $2n = 40$ karyotype. The $2n = 40$ karyotype, however, possessed 2 extra pairs of large acrocentric autosomes. An intermediate condition was found in the $2n = 39$ karyotype with only 1 extremely large metacentric autosome but with an extra pair of large acrocentric autosomes when compared with $2n = 38$ karyotype (1 pair less than $2n = 40$ karyotype). These differences in the 3 karyotypes could be ascribed to Robertsonian translocation.

In addition to numerical chromosomal polymorphism, the present results obtained from the Malayan species indicate that the karyotype of the Malayan house shrew differs from that of the Indian, Japanese and South Vietnamese taxa (Table II). Direct comparisons, however, would be fallacious due to the undefined nomenclature that were employed.

Chromosomal polymorphism in insectivores has been extensively reviewed (BORGAONKAR[1]; GROPP[2]; FORD[10]). The family Soricidae, to which belong *Suncus murinus*, is one of the most extensively and widely studied. Robertsonian polymorphism had been found in *Sorex araneus* (SHARMAN[11]; FORD, HAMERTON, and SHARMAN[12]; MEYLAN[13,14]) and *Blarina brevicauda* (MEYLAN[15]; LEE and ZIMMERMAN[16]). The present finding is another concrete example of Robertsonian polymorphism, and it resembles that of *Blarina brevicauda*. Circumstantial evidence seems to favour centric fusion rather than fission as the mechanism giving rise to the observed numerical polymorphism. Whether two or more pairs of autosomes are involved, however, cannot be resolved by the present data. Meiotic and population studies are now being conducted to unravel this and other related problems[17].

Zusammenfassung. 15 Exemplare von *Suncus murinus* aus Kuala Lumpur und Petaling Jaya, Selangor (Malaysia) zeigten 3 Exemplare mit einer diploiden Zahl von 40, 9 weitere mit $2n = 39$ und 3 Exemplare mit $2n = 38$. Der Unterschied der Chromosomenzahl innerhalb einer Intrapopulation wird mit der Robertson'schen Translokation in Zusammenhang gebracht.

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[10] C. E. FORD, *Human Population Cytogenetics* (Eds. P. A. JACOBS, W. H. PRICE and P. LAW; University of Edinburgh Press 1970).
[11] G. B. SHARMAN, Nature, Lond. *177*, 941 (1956).
[12] C. E. FORD, J. L. HAMERTON and G. B. SHARMAN, Nature, Lond. *180*, 392 (1957).
[13] A. MEYLAN, Rev. Suisse Zool. *71*, 903 (1964).
[14] A. MEYLAN, Rev. Suisse Zool. *72*, 636 (1965).
[15] A. MEYLAN, Canad. J. Zool. *45*, 1119 (1967).
[16] M. R. LEE and E. G. ZIMMERMAN, J. Mamm. *50*, 333 (1969).
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In vitro Induction of Vegetative Buds by Tobacco Smoke Condensate[1]

We reported earlier[2] that benz(a)anthracene (BaA), a tobacco smoke carcinogen, replaced the morphogenetic effect of 3-indoleacetic acid (IAA) and kinetin on the callus derived from the stem tissue of haploid tobacco plants. Recently, we have been able to induce vegetative buds on a similar callus grown in a nutrient medium supplemented with water-soluble extract of tobacco smoke condensate. Neither IAA nor kinetin was present in the medium.

Haploid plants were obtained by culturing immature surface sterilized anthers of *Nicotiana tabacum* (cv. 'Burley 21') on the nutrient medium used by NITSCH and NITSCH[3]. The callus was obtained by inoculating small pieces (4–5 mm long) of stems of these plants on modified MURASHIGE and SKOOG's medium[4] supplemented with 0.2 mg/l of α-naphthaleneacetic acid (NAA) and 0.2 mg/l of IAA. This callus served as experimental material. Calli weighing about 300–350 mg were planted (Figure 1a) on modified MURASHIGE and SKOOG's medium supplemented with various concentrations of water-soluble extract of tobacco smoke condensate (TSC).

The TSC was prepared under the direction of Dr. J. F. BENNER, Department of Agronomy, University of Kentucky. The University of Kentucky Reference Cigarettes 1R1[5,6], equilibrated at 20 °C and 60% relative humidity, were smoked on a Borgwaldt smoking machine employing a standard smoking cycle (a 35 ml puff volume of 2 sec duration at 1 min intervals). The smoke was collected in a 3 l flask containing 100 ml of water cooled at 0 °C. A specially designed pump, Chemap vibromixer, was used to obtain maximum contact of the smoke with water. After 840 cigarettes were smoked, the water solution was transferred to a cooled graduated cylinder. The non-volatile residue weight was determined by evaporation of a 5 ml portion of the solution on a rotary evaporator at a pressure of 30 mm at 35 °C with a 50 ml/min stream of nitrogen. The smoking flask was then rinsed with sufficient water in several portions to give a final concentration of 50 mg/ml of non-volatile residue. The TSC was subsequently diluted to have final concentrations of 5, 10, 15, 20, and 25 mg/l in the nutrient media. The pH of the medium was adjusted to 5.8. Sterilization was achieved by autoclaving at 18 lb ψ for 20 min. For each treatment 16 replicates were main-

[1] This study was carried out under contract No. 12-14-100-341(73) with the Agricultural Research Service, U.S. Department of Agriculture, administered by the Eastern Utilization Research Development Division, Philadelphia.
[2] T. S. KOCHHAR, P. R. BHALLA and P. S. SABHARWAL, Planta *94*, 246 (1970).
[3] J. P. NITSCH and C. NITSCH, Science *163*, 85 (1969).
[4] M. J. KASPERBAUER and R. A. REINERT, Physiologia Pl. *20*, 977 (1967).
[5] W. O. ATKINSON, Proceedings of the Tobacco and Health Conference, University of Kentucky, Lexington, Kentucky (1970), p. 28.
[6] J. F. BENNER, Proceedings of the Tobacco and Health Conference, University of Kentucky, Lexington, Kentucky (1970), p. 30.

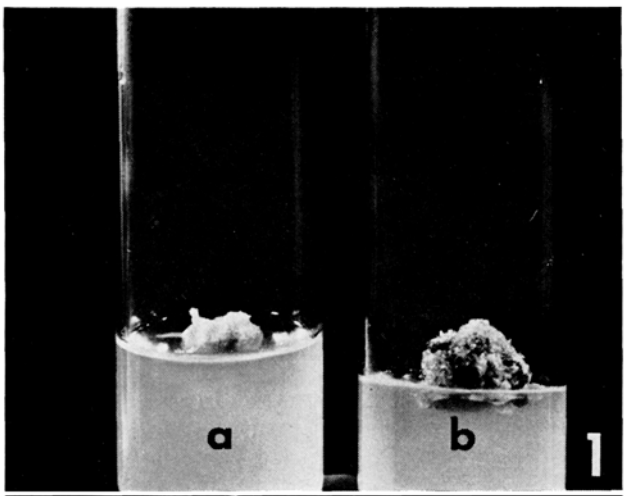

Fig. 1. a) Callus derived from haploid plants of *Nicotiana tabacum* at the stage of inoculation on the nutrient medium supplemented with 5 mg/l of water-soluble fraction of tobacco smoke condensate (TSC). ×0.88. b) Note the growth of the callus on the nutrient medium containing 5 mg/l of TSC 2 weeks after inoculation. ×0.88.
Fig. 2. Induction of a shoot on a medium fortified with 5 mg/l of TSC after 8 weeks of inoculation. ×0.84.

Observations on the induction of vegatative buds by TSC[a] on the tobacco callus

Weeks after inoculation	Cultures[b] differentiated (%) in various concentrations of TSC (mg/l)					
	0 (control)	5	10	15	20	25
2	0	0	0	0	0	0
3	0	25	31	13	6	0
4	0	56	44	38	31	13
5	0	56	44	44	31	13
6	0	56	44	44	31	13
7	0	75	50	44	31	13
8	0	75	50	44	38	13

[a] Water-soluble extract of tobacco smoke condensate. [b] Each treatment consisted of 16 replicates.

tained at 225–250 foot candle intensity. The experiment was repeated twice. The cultures were observed for growth and morphogenesis for 8 weeks.

Slight growth of the callus was observed in the control. As the cultures became old, parts of the callus showed brown color. In TSC media the callus showed growth in all the concentrations tested (Figure 1b). The callus retained green color in most of the cultures. After 15 days, a few roots started emerging from the callus on the media containing 5 and 10 mg/l of TSC. However, at higher concentrations (15, 20 and 25 mg/l), the rooting was seen about 3 weeks after inoculation.

Vegetative shoots appeared at TSC concentrations of 5, 10, 15 and 20 mg/l after 3 weeks of inoculation. The shoots originated from the surface of the callus which was in contact with the medium. They grew in the medium for some time but finally assumed normal polarity to give rise to plants (Figure 2).

After 4 weeks of inoculation, vegetative shoots were seen in all concentrations of TSC (Table). The number of cultures with vegetative buds increased with the time. In the lowest concentration (5 mg/l), 75% of the cultures produced vegetative buds 7 weeks after inoculation. However, the number of cultures with vegetative buds declined with the increasing concentrations of TSC. Only 13% of the cultures showed differentiation in 25 mg/l of TSC. No morphogenesis was observed in the control.

Investigations on the effect of cigarette smoke on cells in vitro have shown changes in chromosomes[7], alteration in mitotic processes[8], increased mitotic abnormalities[9,10] and significant cell damage[11,12]. However, in no case until now has tobacco smoke condensate been shown to induce vegetative buds in higher plant tissues. The results described in this paper show that water-soluble extract of tobacco smoke condensate induces morphogenesis in the absence of IAA and kinetin. Information on the effect of tobacco smoke on plant tissues may lead to a better understanding of its mode of action in eliciting biological responses.

Zusammenfassung. Es wird gezeigt, dass wasserlösliche Extrakte aus Tabakrauchkondensaten vegetative Keimlinge auf Kallusstücken aus Stämmen von haploiden Tabakpflanzen (*Nicotiana tabacum*) induzieren und dass der gleiche Effekt mit Kinetin und 3-Indolessigsäure erzielt werden kann.

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[7] Y. H. NAKANISHI, M. MIZUTANI and C. M. POMERAT, Texas Rep. Biol. Med. *17*, 542 (1959).
[8] G. VENEMA, Chromosoma *10*, 679 (1959).
[9] C. IZARD, D. VALADAUD and P. MOREE-TESTA, C. r. Acad. Sci., Paris *270*, 2457 (1970).
[10] J. BOUCHARD and R. M. MAY, Archs Anat. microsc. Morph. exp. *49*, 307 (1960).
[11] M. MIZUTANI, J. Fac. Sci. Hokkaido Univ. (Ser. VI) *15*, 18 (1962).
[12] A. AWA, Y. E. OHNUKI and C. M. POMERAT, Texas Rep. Biol. Med. *19*, 518 (1961).
[13] The authors are thankful to Dr. R. B. GRIFFITH, Director, Tobacco and Health Research Institute, University of Kentucky, for critical review of this paper and valuable suggestions.