Mean number of flies and standard error for the total number of males of *D. nasuta*, *D. neonasuta* and the total population size including the males and females of the 2 competing species

No.	Frequencies of founder population *D. nasuta* : *D. neonasuta*	Mean number of males of		Mean of the total population size including the males and females of *D. nasuta* and *D. neonasuta*
		D. nasuta	*D. neonasuta*	
	Experiment 1			
1	1:1	50.57 ± 6.93	14.09 ± 3.67	135.71 ± 18.17
2		54.75 ± 5.68	11.20 ± 2.13	140.29 ± 14.06
3		61.73 ± 7.18	23.57 ± 3.67	168.73 ± 22.49
4		44.42 ± 4.95	19.00 ± 3.14	137.07 ± 18.14
Average		52.86	16.96	145.45
	Experiment 2			
1	1:4	22.92 ± 5.80	36.48 ± 9.60	118.62 ± 29.89
2		17.00 ± 3.89	41.10 ± 7.42	119.13 ± 22.38
3		26.13 ± 3.88	39.36 ± 7.76	140.10 ± 22.02
4		33.29 ± 7.65	44.12 ± 9.98	152.41 ± 33.19
Average		24.83	40.26	132.56

In the light of this, the universality of frequency dependent selection and the natural selection favouring a sparse species ramains to be further investigated.

[3] The authors are deeply indebted to Dr. M. R. RAJASEKARASETTY, Professor and Head of the Department of Zoology, for his help and encouragement. This work is financially supported by Mysore University Research grants and C.S.I.R. New Delhi.

Zusammenfassung. In Mischpopulationen von Drosophila nasuta und D. neonasuta dominiert die erste Art, wenn das Anfangsverhältnis der Arten 1:1 beträgt. Beim Verähltnis 1:4 stirbt jedoch D. nasuta aus.

H. A. RANGANATH and N. B. KRISHNAMURTHY[3]

Department of Post-graduate Studies and Research in Zoology, Manasa Gangotri, Mysore 570006 (India), 1 August 1974.

Induction of Androgenetic Embryoids in the in vitro Cultured Anthers of Several Species

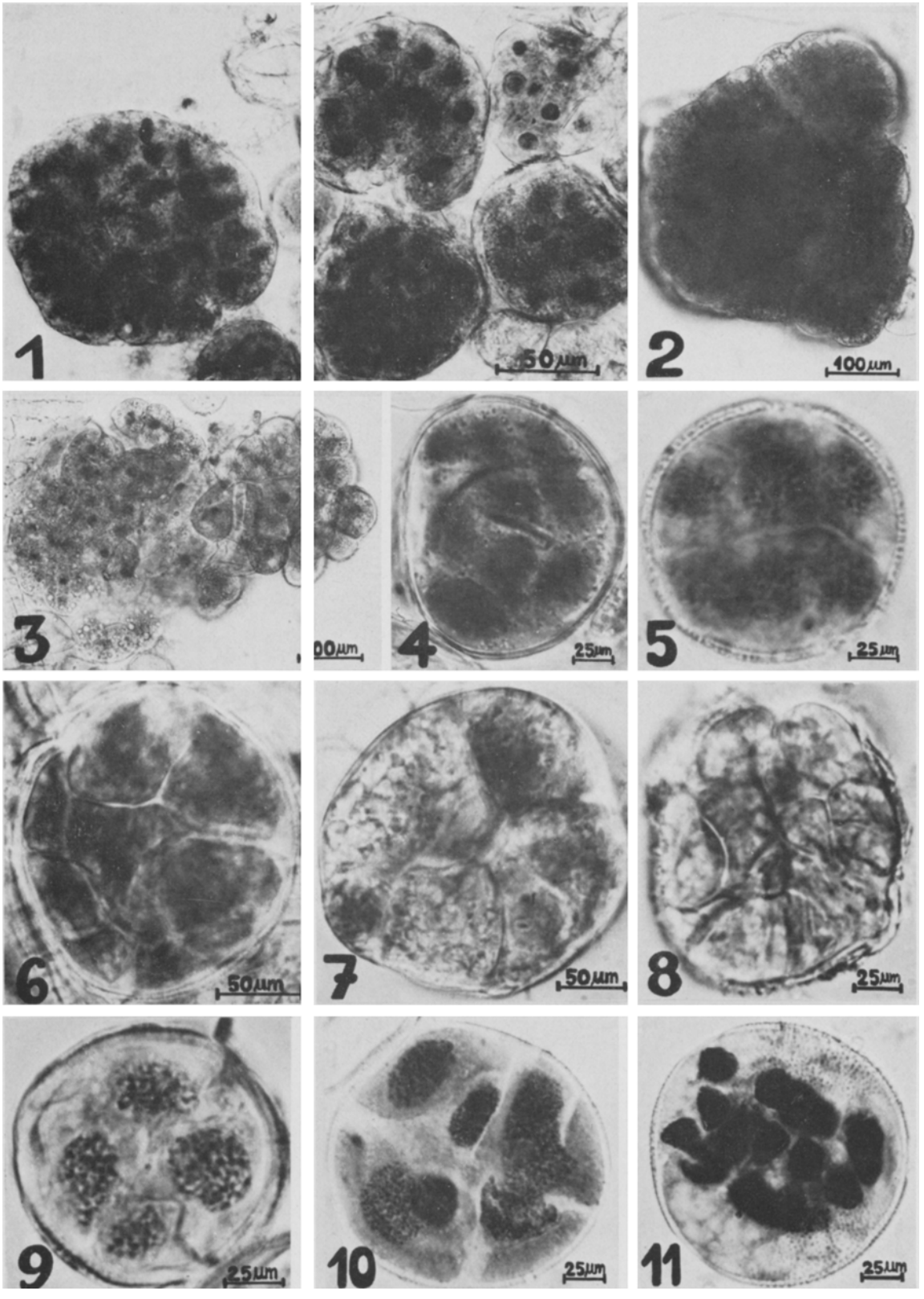
The importance of the in vitro culture of anthers in plant breeding and genetic research was stressed by several authors[1–4]. Up to date, pollen grains of a limited number of species when cultured in vitro are capable of growing directly into embryoids and eventually into plants, or of producing undifferentiated calluses which may in turn give rise to shoots and roots. This report is concerned with the successful development of androgenetic embryoids through culture of anthers of 7 species.

Anthers of the following species at the stage of uninucleate pollen grains in the given number were inoculated and cultured in vitro: *Helleborus foetidus*, 2900; *Paeonia lutea* v. *superba* and *P. suffruticosa*, 400; *Prunus avium*, 360; *Bromus inermis*, 2200; *Agropyron repens*, 350; *Festuca pratensis*, 1840; *Hordeum vulgare* (4 varieties: *Alsa, Damazy, Skrzeszowicki* and *Wiza*), 11600.

Flower buds of *Helleborus*, *Paeonia* and *Prunus* were sterilized in 70% ethanol for 30 sec, and later with chlorine water for 6–12 min. Flower spikes of species belonging to Gramineae were sterilized by chlorine water only, for 2–5 min. Material of all the species after sterilization was thoroughly washed with sterilized water. The basal media of MURASHIGE and SKOOG[5] and LINSMAIER and SKOOG[6] were used with an increase of sucrose concentration up to 12%. Growth supplements such as IAA, 2,4-D, BAP (benzylaminopurine) and casein hydrolysate were added to the media in various concentrations and combinations. The cultures were kept exposed to constant cool-white fluorescent illumination at temperature 22–25 °C and relative humidity of 70–80%. Squash preparations were made in order to determine the developmental stages of pollen grains. The whole inoculated material was cultured up to the 10th week, with the exception of anthers of *Hordeum*, *Bromus* and *Festuca* which are still kept in freshly prepared media.

After about 10 days of culture, anthers of *Paeonia* and *Helleborus* contained about 80% of 2-nuclei pollen grains. Some pollen grains were in a stage of nuclear division and after 14–21 days multinuclear and multicellular pollen grains were seen (Figures 5, 9, 10, 11). In *Paeonia*, after 6 weeks multicellular embryoids developed from 2–3% of pollen grains; however, they were still enclosed by exine. Microscopical observations revealed the presence of up to 30 cells in some embryoids. In *Helleborus* only about 1% of pollen developed into embryoids. It is worth noticing that, beside the presence of embryoids in anthers of *Paeonia* and *Helleborus*, many pollen grains were still fully developed possessing 1 or 2 nuclei situated in a densely stained cytoplasm. In *Prunus*, after 2 weeks of culture nearly all pollen degenerated, only few (about 0.1%) looked normal and possessed 1 or 2 nuclei. Pollen embryoids containing

[1] J. P. NITSCH, Z. Pflanzenzücht. *67*, 3 (1972).
[2] K. K. PANDEY, New Phytol. *72*, 5 (1973).
[3] H. H. SMITH, BioScience *24*, 5 (1974).
[4] N. SUNDERLAND, Sci. Prog., Oxford *59*, 527 (1971).
[5] T. MURASHIGE and F. SKOOG, Physiologia Pl. *15*, 473 (1963).
[6] E. M. LINSMAIER and F. SKOOG, Physiologia Pl. *18*, 100 (1965).



Figs. 1–3. Numerous pollen embryoids (Figure 1), an advanced embryoid (Figure 2) and pollen callus (Figure 3) in anthers of *Hordeum vulgare* after 2–3 weeks of culture on LINSMAIER and SKOOG[6] (LS) medium with 0.2 mg/l IAA, 200 mg/l CH and 12% sucrose. 4. Multicellular pollen grain of *Bromus inermis*, 2 weeks on LS with 2 mg/l K, 1 mg/l IAA and 6% sucrose. 5. Multicellular pollen grain of *Helleborus foetidus* after 18 days of culture on MURASHIGE and SKOOG medium[5] (MS) with 0.02 mg/l K, 1 mg/l IAA, 0.2 mg/l 2.4-D, 400 mg/l myoinositol and 3% sucrose. 6. Multicellular pollen grain of *Festuca pratensis* after 17 days of culture on MS with 1 mg/l K, 0.5 mg/l IAA, 2 mg/l 2.4-D and 6% sucrose. 7. Multicellular pollen grain of *Agropyron repens* cultured for 18 days on MS with 2 mg/l K, 1 mg/l IAA, 1000 mg/l CH and 8% sucrose. 8. Multicellular pollen grain of *Prunus avium* after 6 weeks of culture on MS with 1 mg/l IAA, 1 mg/l BAP and 12% sucrose. 9. 4 nuclear pollen grain of *Paeonia suffruticosa* after 2 weeks of culture on MS with 1 mg/l K, 1 mg/l IAA, 500 mg/l CH and 3% sucrose. Figs.10–11. Multicellular (Figure 10) and multinuclear (Figure 11) pollen grain from anther of *Paeonia lutea* v. *superba* after 3 weeks of culture on MS with 1 mg/l K, 1 mg/l IAA, 500 mg/l CH and 3% sucrose. K, kinetin; IAA, indol-3yl-acetic acid; 2,4-D, 2,4-dichlorophenoxyacetic acid; CH, casein hydrolysate; BAP, benzylaminopurine.

several or more than 30 cells were found after 6 weeks of culture. In some cases, as is shown in Figure 8, the exine burst and embryoids assumed more irregular shape.

Embryoid formation occurred in all species of grasses but most frequently in barley (Figures 1–4, 6 and 7). The percentage of anthers which formed pollen embryoids ranged from 1 to 5, eg. in *Bromus* 1–2%, in *Agropyron* 1% and in *Hordeum* up to 5%. In anthers of *Hordeum* inoculated in medium composed of 1 mg/l IAA, 200 mg/l CH and 12% sucrose, an abundant number of pollen grains underwent mitosis and developed embryoids. In some anthers, more than 50% of pollen grains formed embryoids.

In all species of Gramineae, the division of 1-nucleated pollen started after 3–4 days of culture. Multicellular or multinuclear pollen were seen after 4 days of culture, and after 2–3 weeks the highest number of cells still enclosed by exine was found. Within the multinuclear pollen, haploid, triploid, diploid, tetraploid and higher polyploid mitoses were observed. In the case of *Agropyron*, *Bromus* and *Festuca*, embryoids were usually enclosed by the exine. However, there were exceptions where callus was formed. More advanced developmental stages of androgenetic embryoids were found in anthers of *Hordeum* in which pollen calluses composed of abundant number of cells were seen in all 4 varieties (Figure 3). Those pollen calluses have been transferred in fresh media with a higher concentration of sucrose in order to induce the process of organogenesis. More caryological details of the early stages of the division of microspores will be published later.

Zusammenfassung. Es wurden Antheren im einkernigen Pollenstadium von 7 Gattungen in vitro kultiviert, und in den Pollen von *Helleborus*, *Paeonia* und *Prunus* wurden nach 6 Wochen vielkernige oder vielzellige Pollen beobachtet. In sämtlichen Gattungen von Gramineae wurden verschiedene Embryonalstadien gefunden.

M. Zenkteler[7], E. Misiura and A. Ponitka

Institute of Biology, University, ul. Stalingradzka 14, 61-713 Poznań (Poland), 13 November 1974.

[7] The authors express their thanks to Prof. A. Szweykowska, Head of the Laboratory of General Botany, for critical reading of this paper. Thanks are also due to Polish Academy of Sciences for the financial support.

The Fluorescent Karyotype of the Tachinid Fly *Voria ruralis* Fallén (Diptera)

Voria ruralis Fallén (Tachinidae: Diptera) is parasitic on several larvae of the family Noctuidae, especially those of the cabbage looper, *Trichoplusia ni* Hübner (Lepidoptera)[1]. A study of the mitotic chromosomes of the parasite was undertaken as part of an investigation into the host-parasite relationship. Because identification of homologous chromosomes based on morphology alone is difficult when working with more than 1 chromosome pair of almost equal arm lengths, the fluorochrome quinacrine mustard (QM) and the technique of C-banding were employed to facilitate homologue identification.

Materials and methods. Parasitized *T. ni* were obtained on the 3rd day after infection from the Entomological Research Division, Agricultural Research Service, Mesa, Arizona. Neuroblasts from *Voria* maggots were used to study mitotic metaphase cells. Larval brain ganglia were dissected in insect saline, placed in 45% acetic acid on slides subbed by Hsu's technique[2], heated gently, and squashed. The slides were frozen briefly on dry ice, dehydrated in 95% and 100% ethanol baths (1–5 min in each), and air dried. For fluorescence air-dried slides were stained 20 min in a 50 µg/ml aqueous solution of QM (pH 4.5) in the dark, rinsed 3 min in running tap water, and mounted in pH 4.5 *tris*-maleate buffer saturated with glycerin. A Leitz fluorescence microscope with a BG 12 exciter filter and a 510 nm barrier filter was used to enhance fluorescence. Metaphase cells with favorable fluorescence were photographed on Kodak Tri-X Pan film. Giemsa staining of centromeric heterochromatin was performed according to the procedure of Evans et al.[3]. Unstained preparations were observed and photographed using phase microscopy.

Results. The diploid chromosome number of *Voria ruralis* is 12. The karyotype consists of 5 autosomal pairs and 1 pair of sex chromosomes (Figure A). The autosomes include 1 pair of long sub-metacentric chromosomes[1], 3 pairs of submetacentric chromosomes of intermediate length[2–4], and 1 pair of short metacentric chromosomes[5]. The somatic pairing seen in Figure A is typical of Dipterans. The distribution of heterochromatin in the autosomes as detected by QM bands (Figures B and C) verifies the homologue assignments suggested by the somatic pairing. The longest autosomal pair, number 1, has 2, sometimes 3, closely-placed fluorescing bands on the long (q) arm proximal to the centromere. Autosomal pair number 2 has two closely-placed intense bands on the q arm adjacent to the centromere, and pair number 3 has one, less brightly fluorescing band in a similar location. Autosomal pairs 4 and 5 are less easily distinguished, each having a dimly fluorescing spot on the q arm proximal to the centromere. These chromosomes are distinguishable, however, in that the band on number 5 is less distinct than that on number 4 and chromosome number 5 is shorter and more metacentric than number 4. Identification of the sex chromosomes was based in part on QM banding patterns. One chromosome pair is almost entirely bright-staining (Figure B) and therefore is predominantly heterochromamatic[4]. As sex chromosomes in Dipteran flies are frequently heterochromatic, it was expected that this pair would be involved in sex determination. In other QM-treated preparations the largely heterochromatic (i.e., entirely fluorescing) chromosomes constitute a nonhomologous pair in some individuals (Figure C). Gonadal preparations of pupal and adult tissue show that tissue undergoing spermatogenesis contains the nonhomologous pair of sex chromosomes. In oogenic tissue all cells contain a matching pair of chromosomes corresponding to the

[1] D. G. Jackson, G. D. Butler jr. and D. E. Bryan, J. econ. Entom. *62*, 69 (1969).
[2] T. C. Hsu, J. Heredity, *62*, 285 (1971).
[3] H. J. Evans, R. A. Buckland and A. T. Sumner, Chromosoma *42*, 383 (1973).
[4] K. P. Adkisson, W. J. Perreault and H. Gay, Chromosoma *34*, 190 (1971).
[5] M. J. D. White and K. H. L. Key, Austral. J. Zool. *5*, 56 (1957).