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.

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[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

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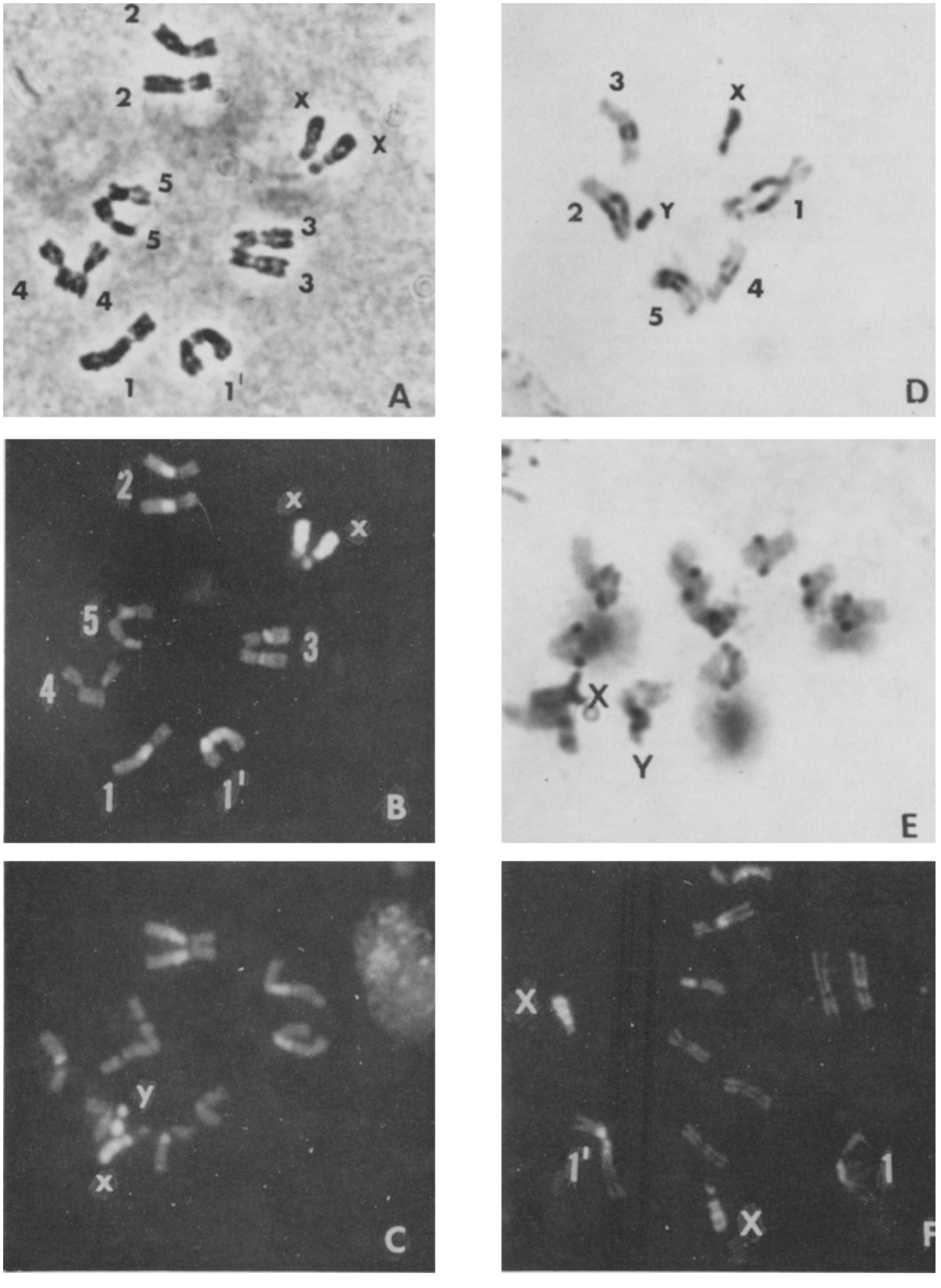


Metaphase karyotypes of *Voria ruralis*, $2n = 12$, prepared from larval neuroblast tissue. A) Unstained female karyotype. B) Same preparation stained with QM. Sex chromosomes are predominantly brightstained and heteromorphic. A band polymorphism occurs in the long autosomal pair. C) QM stained chromosomes of male exhibiting characteristic fluorescence bands on homologues and bright-staining *X* and *Y*. D) C-banded chromosomes of male with *X* and *Y* entirely dark-stained. E) C-banded late metaphase in male with sister centromeres initiating poleward movement. *X* and *Y* are separating at their ends first. Terminal one-third of *X* is more darkly stained than remaining nonseparated portion. F) QM stained chromosomes of female. *X* chromosomes are obviously heteromorphic with respect to the lengths of their secondary constrictions. Note particularly the band polymorphism of autosomal pair 1 resulting from the very bright spot on 1′.

longer sex chromosome. Females of the species are therefore the homogametic sex while males are heterogametic. The shorter sex chromosome ($Y$) is characterized by a constriction at its mid-region, while the $X$ has a constriction near one end (Figure C).

Centromeric heterochromatin staining (C-banding) was performed to verify the location of the centromere in each pair (see Figure D). The $X$ and $Y$ chromosomes appear darkly stained, again indicating their predominantly heterochromatic composition. In Figure E is shown a late metaphase cell stained for centromeric heterochromatin. Chromatids are beginning to move toward opposite poles, led by their centromeres. The ends of the $X$ chromosome are separating first, indicating that the $X$ is telocentric. Similarly, the $Y$ chromosome is separating at one end first, and therefore is probably also telocentric. Both $X$ and $Y$ chromosomes have been seen to be associated with the nucleolus at their visible constrictions. These constrictions therefore are nucleolar organizer regions.

Although completely dark-staining when treated for centromeric heterochromatin, the $X$ chromosome does not stain uniformly over its entire length (Figure E). The telocentric one-third of the chromosome stains darker than the remainder. This difference in affinity for stain may reflect differences in heterochromatic composition; hence, the $X$ chromosome may be composed of more than one type of heterochromatin. The two $X$ chromosomes are not morphologically identical but differ especially with respect to the lengths of their secondary constrictions (Figures A and F). In QM-treated preparations from 8 of 9 females, the secondary constriction in one $X$ was always approximately 3 times longer than the constriction in its homologue.

A band polymorphism in autosomal pair number 1 was observed in 9 of 13 male and female QM-treated preparations (Figures A and F). When present in one cell of a fly, this polymorphism was seen in all other favorably spread cells from that individual.One chromosome displays that typical banding pattern described previously with bright fluorescence adjacent to the centromere on the q arm. Its homologue, however, exhibits a brightly staining spot on the p arm next to the centromere in addition to the fluorescent bands on the q arm. In addition, one number 1 chromosome often appears more metacentric than its homologue. Preliminary measurements from a limited number of negatives show the q/p arm ratio is significantly different for chromosomes exhibiting this polymorphism (see Table).

Arm ratios (q/p) for chromosome pair No. 1 in two larvae exhibiting polymorphism

| Specimen | | Homologue 1 | Homologue 1′ |
|---|---|---|---|
| Larvae A, ♂ | | 2.719 | 1.777 |
| | | 2.162 | 1.589 |
| | | 2.451 | 1.579 |
| Larvae B, ♀ | | 1.946 | 1.765 |
| | | 3.079 | 2.246 |
| | | 2.119 | 1.647 |
| | | 1.696 | 1.272 |
| | | 2.209 | 1.335 |
| | | 2.200 | 1.455 |
| | Mean[a] | 2.288 | 1.631 |

[a] Mean difference between homologues significant at 0.05 level.

*Discussion.* An explanation for the band polymorphism observed in chromosome pair number 1 is problematical. The apparent change in arm ratio suggests a pericentric inversion. Although rare in *Drosophila*, pericentric inversions are not uncommon in other insects. WHITE[5] has reported that heterozygous pericentric inversions exist in at least 4 species of grasshoppers. Because pericentric inversions generally lead to reduced fertility in chiasmate meiosis, an examination of zygote lethality in *Voria* would be one way to test for such an inversion. *Voria*, however, is ovoviviparous rendering this procedure difficult. Analysis of polytene chromosomes would provide a more feasible means of verifying the presence of an inversion. The salivary gland chromosomes of this fly spread poorly, however, making them difficult to analyze. The primary difficulty with the inversion hypothesis is that the bright fluorescing region in the p arm of one chromosome exhibits greater fluorescence than that of any part of its homologue (Figure F). Another explanation for the polymorphism is the addition of heterochromatic material to the p arm of one homologue. In a recent paper JOHN[6] proposed such a mechanism to explain supernumerary segments in grasshopper and locust chromosomes. The additions suggested by JOHN occur terminally on the chromosome arms. Such a mechanism in *Voria* would require an intra-chromosomal insertion of a heterochromatic segment to account for the observed banding differences. A deletion of a heterochromatic region on the p arm of the number 1 chromosome is an unlikely explanation for the polymorphism. Members of the population lacking the proposed deletion (i.e., chromosomally 'normal' individuals with a fluorescent band on the p arm of both homologues) have not been found, whereas members having the suggested deletion on both homologues, or no bands on either p arm, have been found. These findings seem contrary to the expected mode of genic action since the probability of a viable fly occurring with a double deletion should be much less than that of a viable fly occurring with no deletion at all. An interchromosomal translocation is eliminated as a possible mechanism because, in flies carrying the polymorphism, banding in all other chromosome pairs is homologous. We believe that the more probable explanation for the polymorphism is the addition of heterochromatic material.

Heteromorphic $X$ chromosomes similar to those observed in this study have been reported in other Dipteran flies. WOLF, as cited by JOHN and LEWIS[7], and ULLERICH et al.[8] both reported $X$'s differing in total length in craneflies. In *Voria* the differences in the length of the secondary constriction might be the result of a differential uncoiling phenomenon. This would be analogous to the uncoiler factor in humans. On the other hand, the difference in lengths could be due to the addition or deletion of chromatin at the site of the secondary constriction. Assuming crossing over to occur between the sex chromosomes, an exchange of material between the $X$ and $Y$ during spermatogenesis would manifest itself in the offspring as heteromorphic $X$'s. Not all combinations of $X$'s have been observed to date, which suggests that perhaps not all are viable.

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*Zusammenfassung*. Aufgrund des Fluoreszenzbandenmusters können die einzelnen Chromosomen der parasitischen Fliege *Voria ruralis* identifiziert und unterschieden werden. Die diploide Chromosomenzahl beträgt 12, und die Geschlechtschromosomen sind vorwiegend heterochromatisch. Nach der Fluoreszenzfärbung mit Quinacimmustard wurden ein Bandenpolymorphismus eines Chromosomenpaares und morphologische Abweichungen des *X*-Chromosoms in 8 von 9 Weibchen beobachtet.

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## Identification of Chromosomes Involved in the 9 Robertsonian Fusions of the Apennine Mouse with a 22-Chromosome Karyotype

CAPANNA, CIVITELLI and CRISTALDI[1] have recently discovered in the Central Apennines a population of house mice that may be classified as *Mus musculus* and are characterized by a 22-chromosome karyotype (Figure 1a). This karyotype consists of 18 metacentric chromosomes, obtained by the centric fusion of 18 pairs of acrocentric autosomes of the standard 40-chromosome mouse karyotype, plus a pair of small acrocentric autosomes, as well as the pair of heterochromosomes.

The population of 22-chromosome mice has a rather large area of distribution which includes part of the northeast basin of the Tiber and stretches as far as the Adriatic coast. The southwest boundaries of this area have been ascertained by the finding of both 40-chromosome border populations and by a number of natural hybrid populations intermediate between these two karyological situations[2].

A survey of the meiotic diakineses in laboratory hybrids obtained by cross-breeding 40- and 22-chromosome mice has revealed the existence of 9 trivalents. The latter clearly display a correspondence between the autosomal arms of the metacentrics of the 22-chromosome karyotype and the single chromosomes of the 40-chromosome karyotype (Figure 1b).

It thus seemed worth while making an accurate identification of the elements involved in the Robertsonian fusion processes and comparing the arrangement of the mutation events in our Apennine material with the arrangement of those in other natural populations of *Mus musculus*[3] and in *Mus poschiavinus* Fatio[4]. In our view, this approach has two interesting features. The first, and most obvious one, is the possibility of characterizing cytologically material that may be very useful in in vitro cytogenetic research both because of the low diploid number, which is in complete contrast with the high number of chromosome markers and because the species in question is *Mus musculus*, i.e. a species whose standard karyotype characters have been identified perfectly and whose linkage groups related to each chromosome are also well known[5]. The second feature is the possibility of adding further cytological data to the problem of the chromosomal 'polymorphism' of *Mus musculus* and its evolution.

For this purpose, primary cultures of fibroblasts were prepared in the cell culture laboratory of the Study Centre on Evolutionary Genetics of the National Research Council, starting from the kidneys of 2 hybrid female individuals obtained by crossing a 40-chromosome female with a 22-chromosome male in the laboratory. The in vitro cell lines had a 31-chromosome karyotype consisting of 20 chromosomes from the maternal set and 11 from

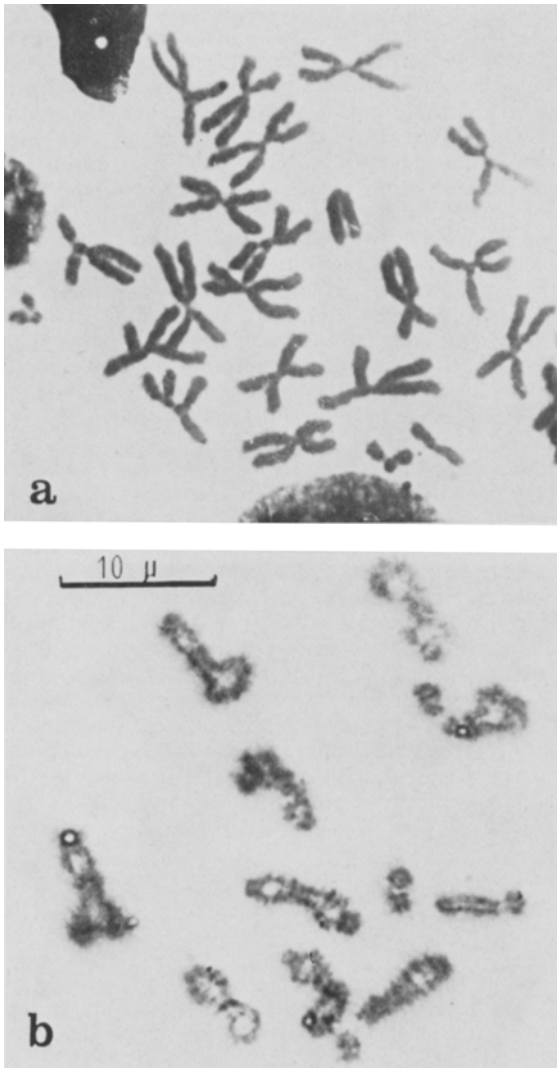


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Fig. 1. a) Somatic metaphase from bone marrow of a 22-chromosome Apennine *Mus musculus*; b) first meiotic diakinesis of a hybrid mouse obtained crossbreeding a 22-chromosome male with a 40-chromosome female.