here indicates that this type of wing venation variant is relatively common, even in inbred lines, and that F_2 data unsupported by control information of this sort are likely to be misleading.

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Reference

1. R. Milkman, *Science* **131**, 225 (1960).

8 June 1960

In my paper (*1*) I reported examining 1000 flies in the F_2 of each of 21 wild inseminated *Drosophila melanogaster* females. Of these 21 F_2's, 11 contained flies with defective posterior crossveins. There were 119 such flies among the 21,000 examined, and their distribution among the 11 F_2's was far from random. I cited this distribution, in the light of previous information, as evidence for the abundance of genes in natural populations which, in rare combinations, would greatly increase the probability of a fly's having defective posterior crossveins.

Bennett *et al.* (*2*) cite the morphological variation observed in a highly inbred (and ostensibly isogenic) strain to emphasize the point that morphological variation is not necessarily a reflection of underlying genetic variation. This is, of course, true; the question of cause must be put to any such observation.

I should like to confirm my conclusions with more recent information. I should then like to make some comments on the paper by Bennett *et al.*

First, a repeat of the experiment on later generations of the 21 strains gave good agreement, pointing to the persistence of differences among the strains. Second, I have been able so far to obtain a true-breeding, polygenic, crossveinless (*cve*) strain from each of two of the original strains (*3*). In the absence of intrastrain heterozygosity of *cve* genes, this would of course have been impossible. I should mention, in addition, that the crossvein defects of some of the strains were distinguishable from one another, and that this distinction was the same in both experiments.

Now I should like to discuss certain of the statements in the paper by Bennett *et al.* In the abstract the word *phenocopy* is used. Later, the implication is maintained that the only major alternative to genetic variation, as a cause of phenotypic variation, is environmental variation. In many cases, and very probably in this case of venation variation, a third force is extremely important. Wright (*4*) and Reeve and Robertson (*5*) call it "chance variation," and Waddington (*6*) calls it "developmental noise." Chance variation comprises the indeterminate events with developmental consequences. Although these authors discuss chance variation in terms of later development, it must be of equal importance from the start, for even genetically identical eggs are known to vary in size, content, and maternal environment, and adult structures are not independent of such variation.

Such chance variation is what forces one to designate an array of phenotypes for a given genotype under well-controlled environmental conditions. Such morphological variation in spite of apparently uniform genotype and environment is discussed to some extent in several of the references cited in my paper. The problem, then, is to distinguish the causes of morphological variability.

This distinction can often not be made conclusively on the basis of simultaneous controls. In my experiments, I believe the evidence of genetic variability was good. I should have stated that among the vials of any single strain, *cve* flies appeared to be distributed randomly. Thus the nonrandom distribution of *cve* flies among strains meant that the strains were not identical. Conclusive evidence in such experiments comes, as Bennett *et al.* say, by a sorting out and identification of the factors involved. In the two *cve* strains obtained so far, there are apparently 3 and 2 *cve* genes, respectively. The 3 are each on a different autosome but have not been further localized yet. The 2 strains, which come from different grocery stores, seem to share at least one *cve* gene. None of the other 19 strains seems to have it, supporting the possibility (*1*) that many alternative combinations of genes for making *cve* flies exist.

As to the data reported by Bennett *et al.* I find more contrast than comparison with my own. They did not run 21 parallel lines. And, whereas I reported 119 *cve* flies from 21,000, they report only 2 from 28,000. It is difficult to comment on the exact numbers of *cve* flies to have been expected had they raised their animals at 18°C. Nevertheless, the frequency of all venation variants they report is within the range reported for some groups of wild flies in an extensive study by Dubinin (*7*). This supports Bennett *et al.*'s point that morphological variation, even under controlled environmental conditions, is not definitive proof of genetic variation. I have, incidentally, recorded other unusual forms of venation also. Some are strain-specific, which does point to a genetic basis.

I believe it is relevant to cite experiments conducted in parallel on wild and on inbred strains by Waddington (*8*). He produced a variable morphological response to a given type of heat shock in both, but only the wild strain responded to selection for susceptibility. Bateman has done the same thing (*9*).

One of Dubinin's most significant contributions to the defining of the genetic basis of natural variation was his work with 240 lines from wild inseminated females (*7*). Examination of successive generations led to the observation of venation deviants in 163 strains, the response of some of these strains to selection, and the genetic analysis of certain of the selected strains.

I believe we are in a position now to take a census of *cve* genes and thus begin to record the details of the genetic basis of a representative form of natural variation (*10*). I should be delighted to hear from anyone interested in participating.

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References and Notes

1. R. Milkman, *Science* **131**, 225 (1960).
2. J. Bennett, R. L. Capek, T. R. Kallstedt, R. E. Moisand, *Science* this issue.
3. This work was supported by National Science Foundation grant G-9785, and done at The University of Michigan.
4. S. Wright, *Proc. Natl. Acad. Sci. U.S.* **6**, 320 (1920).
5. E. C. R. Reeve and F. W. Robertson, *Z. Induktive Abstammungs-u. Vererbungslehre* **86**, 269 (1954).
6. C. H. Waddington, *The Strategy of The Genes* (Macmillan, New York, 1957); see also A. I. Lansing, *Proc. Natl. Acad. Sci. U.S.* **34**, 304 (1948).
7. N. P. Dubinin, *Zhur. Obshchei. Biol.* **9**, 203 1948); translated by I. M. Lerner. A discussion of this paper may be found in Lerner's *Genetic Homeostasis* (Oliver and Boyd, Edinburgh, 1954).
8. C. H. Waddington, *Evolution* **7**, 118, (1933).
9. K. G. Bateman, *J. Genet.* **56**, 443 (1959).
10. R. D. Milkman, *Genetics* **45**, 35, 377 (1960).
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Demonstration of Canonic Gonial Mitosis and Meiosis in Parascaris equorum

Abstract. It is shown that, contrary to what has been held, separate canonic chromosomes, without fusion into a chromatin mass, occur in the meiotic prophase of *Parascaris equorum*. In mitosis no club-shaped chromosome ends are visible. These results, obtained with a modified fixation procedure, which is described, have been checked by supravital observation.

Mitosis and meiosis in the horse ascaris, *Parascaris equorum* (old name, *Ascaris megalocephala*), have been reported to show several discrepancies in relation to findings in the great majority of animals and plants. Early authors (*1*), described extraordinary phenomena

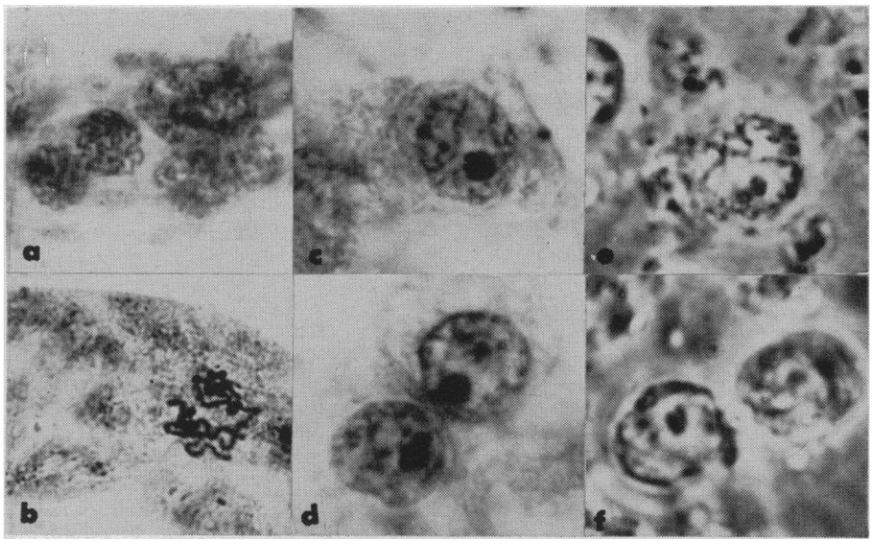

Fig. 1. Photomicrographs of cells of female *Parascaris* (*bivalens*). (*a–d*) Cells fixed in acetic alcohol and stained by Weigert's iron hematoxylin; (*a* and *b*) early and late prophase, respectively, of oögonial mitosis; (*c*) early pachytene stage; (*d*) early diplotene stage. Nucleoli appear as round black bodies. (*e* and *f*) Phase–contrast photomicrographs of living cells in approximately the same stages as *c* and *d*, respectively. (*a* and *b*, × 800; c–f, × 2000)

during meiosis in this species—namely, the aggregation of the two or four chromosomes it possesses (found, respectively, in subspecies *univalens* and *bivalens*) into a common chromatin mass; the occurrence of chromosomes in variable number; and more than one reduction division. Some of these noncanonical aspects have been clarified in more recent times and shown to be due in part to deficiencies in the interpretation of preparations obtained by faulty methods.

Recently it has been reported that *Parascaris* chromosomes are not seen as separate bodies in early meiosis and that they only differentiate themselves from a large chromatin mass, observed in early meiotic prophase, during stages corresponding to late pachytene or early diplotene in other species (*2, 3*). It was reported that before meiosis, in oögonial mitoses, the chromosomes presented large club-shaped ends which consisted of heterochromatin (*3*), and that this same heterochromatin formed the large chromatic mass of early meiosis and was afterwards "diminished" or eliminated in the somatic cells, during embryonic divisions (*3*).

We have found that these noncanonical findings are artifacts produced through deficient fixation. With appropriate fixation, the chromosomes are observed as separate bodies throughout the entire course of meiosis, no chromatin mass being visible in early meiotic prophase. On the other hand, in gonial mitosis the chromosomes do not show club-shaped ends. After several trials we found that in contrast to usual fixation procedure, which must be as rapid as possible, in this material the cells must be fixed gradually, without osmotic shock, to obtain good results. Acetic-alcohol fixative (glacial acetic acid, 1 volume; absolute alcohol, 3 volumes), strong AFA fixative [96-percent alcohol, 2 volumes; 40-percent formalin solution, 1 volume; glacial acetic acid, 0.1 volume (*4*)], buffered osmic acid, several mixtures of formalin, potassium bichromate, and cadmium or mercury chloride solutions all produce defective fixation when employed in the usual way.

We have obtained good results with the following procedure. The animals, secured from freshly killed horses and immediately brought to the laboratory in the intestinal fluid in which they had lived, were immersed in an isosmotic, 0.25*M* saccharose solution. The fixative was gradually dispensed from a dropping funnel into this solution, with stirring, so that 300 ml of fixative was mixed during 1 hour with the 150 ml of saccharose solution. The worms died within about 10 to 15 minutes after the fixative was first added. At the end of this hour of mixing the animals were immersed in pure fixative, and after another hour the cuticle was cut and the genital tract was exposed to the pure fixative overnight. Acetic-alcohol or strong AFA fixative generally gives good fixation. The second of these fluids is better for preserving nuclear morphology, but it may not preserve so perfectly the materials which give the Feulgen reaction (essentially deoxyribonucleic acid).

Figure 1 shows some aspects of oögonial mitosis and early meiosis, in which no chromatin masses or club-shaped chromosome ends are visible. Two photographs of living meiotic cells, obtained by means of phase contrast, are also shown. From rapidly dissected female ascaris, preparations of segments of the genital tract immersed in the body fluid of the worms were readily made and observed with phase-contrast equipment. The chromosomes were then seen to have the same appearance as after the fixation procedures described here. When fixative was added to these preparations, between slide and cover slip, aggregates and masses appeared and the entire nuclear content became distorted, while the materials of the chromosomes were in part dissolved and afterwards precipitated at random. These supravital observations leave no doubt that phenomena hitherto supposed to be the natural state of *Parascaris* chromosomes are indeed artifacts due to fixation. Our procedure opens the way to a detailed study of mitosis and especially of meiosis in these relatively large chromosomes, which are very interesting owing to their multiple, or so-called diffuse, centromere.

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References

1. E. Van Beneden, *Arch. biol.* (*Liége*) **4**, 265 (1883); R. Blanckertz, *Arch. Zellforsch.* **6**, 1 (1911).
2. R. B. Goldschmidt and T. P. Lin, *Science* **105**, 619 (1947).
3. J. Pasteels, *Arch. biol.* (*Liége*) **59**, 405 (1948); T. P. Lin, *Chromosoma* **6**, 175 (1954).
4. J. A. Serra, *Portugaliae Acta Biol. Sér. A*, 1 (1944).

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Callus Tissues from the Mosses Polytrichum and Atrichum

Abstract. Callus-like tissues, isolated from protonemal cultures of two species of mosses, grow vigorously and without marked differentiation on media containing sucrose, casamino acids, and coconut milk. On mineral agar and on media containing sorbitol the tissue from *Polytrichum* (found diploid) reverts to the growth pattern of apparently normal moss plants.

Relatively undifferentiated and continuously proliferating tissues of diverse vascular plants have been induced by one means or another in past years. These have been listed and described by Gautheret (*1*) under the general categories of *callus* and *tumor* tissues. No representative of the nonvascular plants is included. Allsopp (*2*) has described undifferentiated tissue aggregates from two species of liverwort and has noted that such growths, like