pendent synthesis. A mixture of the three synthetic components, IV, V,

$$CH_3(CH_2)_5CO_2CH_3$$
(VII)

$$CH_3O_2C(CH_2)_{10}OCOCH_3$$
(VIII)

and IX, gave an infrared spectrum indistinguishable from that of the unfractionated hairpencil secretion.

$$CH_3(CH_2)_5-\underset{H}{\overset{}{C}}=\underset{H}{\overset{}{C}}-(CH_2)_{10}OCOCH_3$$
(IX)

Pyrrolizidinones have not previously been isolated from insects or other animals, although this ring system, in a lower oxidation state, is a characteristic moiety of the senecio alkaloids (*7*). In contrast, the two acetate esters bear striking, and possibly meaningful, similarity to the two known pheromones from moths (I, II).

Whether the three components (together with the elusive odorous factor) serve in "unison" to convey a single message, or whether they constitute a medley of distinct signals, some perhaps not even related to courtship (*8*), remains to be determined. Work on the behavior of *Lycorea* is hampered by the fact that this species apparently courts in dense tropical forest. Studies on the biological "meaning" of danaid hairpencil secretions might therefore best be carried out on a species such as the queen (*Danaus gilippus berenice*), which courts in a more open habitat.

J. MEINWALD, Y. C. MEINWALD
J. W. WHEELER, T. EISNER*
*Department of Chemistry* and *Division of Biology, Cornell University, Ithaca, New York*

L. P. BROWER
*Biology Laboratory, Amherst College, Amherst, Massachusetts*

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8. *Lycorea*, in common with some but not all other danaid butterflies, everts its hairpencils in response to handling (Fig. 1*B*). This suggests that the secretion, or perhaps only one of its components (possibly the pyrrolizidinone), might have a defensive role.
9. Supported by grant AI-02908 from NIH (T.E. and J.M.), NIH training grant 5TI-GM-A34-02 (Chemistry, Cornell), unrestricted funds from the Upjohn Co. (T.E.), and grants 20152 and GB 2291 from NSF (L.P.B.). Jocelyn Crane, Department of Tropical Research, New York Zoological Society, H. Croze and T. Pliske, undertook the laborious task of collecting and shipping most of the butterflies. We thank Drs. A. F. Thomas and B. Willhalm, Firmenich & Cie., Geneva, for the mass spectra, and Dr. R. Pitcher, Varian Associates, for the nuclear magnetic resonance spectrum.
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## Nuclear Number in the Rotifer Asplanchna: Intraclonal Variation and Environmental Control

Abstract. *The gastric glands and vitellarium of* Asplanchna *are exceptions to the rule of constancy of the number of nuclei in rotifers. The two glands of a female may have different numbers of nuclei. Nuclear number in both organs varies widely among different individuals in a single culture. The mean nuclear number characteristic of a clone may be modified by dietary changes.*

Rotifers, the rest of the Aschelminthes, and certain other invertebrate phyla show the phenomenon of eutely (*1*): each adult organ contains a constant, species-specific number of cells (or nuclei, in syncytial tissues). The gastric glands and vitellarium of rotifers of the genus *Asplanchna* are exceptions to this principle. We have studied the variability in number of nuclei in gastric glands of individual animals, and the number in the glands and vitellarium among individuals of a clone in identical environments. We have also demonstrated an environmental influence on nuclear number in both organs.

All studies were done on females, as the vitellarium is lacking and the gastric glands are degenerate in the male. Most or all of the females examined were amictic. The rotifers were grown in a baked-lettuce infusion (*p*H 7.4–7.6) and fed *Paramecium aurelia* (Medium 29) (*2*). In some experiments, cultures grown on Medium 29 were compared with cultures fed green *Paramecium bursaria* or *Eudorina elegans* suspended in the baked-lettuce infusion. In one experiment, animals were fed *Paramecium bursaria* in Gilbert's solution (*3*). All cultures were kept at 23°C in constant light, either in plastic tissue culture dishes or in glass culture tubes.

We have examined three species of *Asplanchna*. *A. Girodi* from Tennessee (one clone) and *A. brightwelli* (*sensu latissimo*) from Indiana and Tennessee (four inbred clones) have been described (*2*). Data from the Indiana and Tennessee stocks were identical and are considered together. The most extensive experiments were done with clone 7B1 from Pennsylvania and with its offspring by selfing (clone 7B1-1). This clone has been identified as *A. sieboldi* (*4*); it is morphologically, physiologically, and serologically similar to, but not identical with, the Indiana and Tennessee *A. brightwelli*.

For nuclear counts, a few live females were placed in a small drop of culture fluid on a slide and covered with a coverslip, so that the animals were slightly squashed. The nuclei were counted under a phase objective at a magnification of 400 or 500×. We counted nuclei only in those organs in which it was reasonably certain that all nuclei were visible. Any errors arising from failure to count all nuclei or (in gastric glands) from counting nuclei in other, overlying tissues were insignificant compared to the variability observed.

A large proportion (70 to 80 percent) of the females of all stocks had different numbers of nuclei in their two

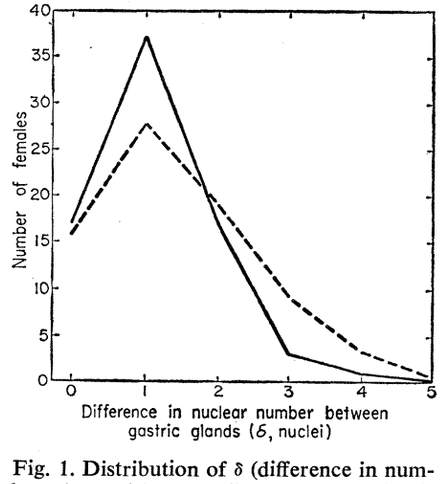


Fig. 1. Distribution of $\delta$ (difference in number of nuclei between the two gastric glands of one female) in pooled data from two samples of clone 7B1-1 fed *Paramecium aurelia*. Solid line: observed distribution. Broken line: distribution expected if the population of glands were paired randomly in females.

gastric glands. We attempted to determine if there was a significant tendency for the two glands of one female to have identical nuclear numbers. Our method of recording data precluded the calculation of correlation coefficients, so the following method was used. The parameter $\delta$ is defined as the difference in nuclear number between the two glands of a pair; in the three samples analyzed in detail, $\delta$ ranges from zero to four nuclei. In each sample, the number of individuals having a given value of $\delta$ was compared with the distribution expected if the glands were paired randomly. For clone 7B1-1, two samples fed *Paramecium aurelia* and one sample fed *Eudorina* were analyzed. Each showed an excess of low values of $\delta$ (zero and one), but the deviations from the random distributions were not significant. However, the pooled chi-squares, as well as the chi-square for the pooled data from the two paramecium-fed samples, were highly significant ($P < .01$); the distributions for the pooled data are shown in Fig. 1. We thus conclude that the nuclear numbers in the two gastric glands of a female show partial correlation.

When characterizing intraclonal variation, we have ignored the pairing of gastric glands in individuals and considered each culture as a population of individual glands and vitellaria. Alternative procedures, such as using the sum or the mean number of gastric-gland nuclei for each female, limit the useful data to cases where both glands of a female can be counted accurately. Such methods yield distributions similar in form to ours, but modify the variance somewhat. Figure 2 gives frequency distributions for nuclear numbers found in glands and vitellaria, with pooled data from six or seven samples of cultures fed *Paramecium aurelia* being used. Distributions for other stocks and for cultures on other diets were similar in form, but differed in the modes and mean. For the gastric glands, most distributions have an excess of items near the mean. This observation was verified by a statistical test on one large sample, which showed a significant positive kurtosis. The standard deviations of gastric-gland populations, estimated by sample standard deviations, typically fall in the range of one to four nuclei per gland in large samples, while the ranges in such samples typically vary from five to nine nuclei per gland. Nuclear numbers in vitellaria are even more variable within a single sample, with large-sample standard errors usually in the range four to six nuclei per vitellarium and ranges of from 9 to 24 nuclei per vitellarium. For these two organs, it is thus impossible to specify a single characteristic nuclear number, and comparisons between different populations must use sample means with appropriate confidence tests. This mean nuclear number fluctuates somewhat from day to day, even for samples from the same culture reared under "constant" environmental conditions. Such fluctuations are due to reproduction and to the appearance in the culture of new females with a slightly different mean nuclear number, because the nuclear number in an adult organ cannot change. In only one instance, however, have we found these fluctuations statistically significant (Fig. 3: the mean number of vitellarium nuclei is significantly higher on day 7 than on days zero and 13 in the paramecium-fed culture). It has thus proved relatively easy to detect effects of an environmental factor, namely diet, on nuclear number. Figure 3 shows the results of two experiments on clones 7B1 and

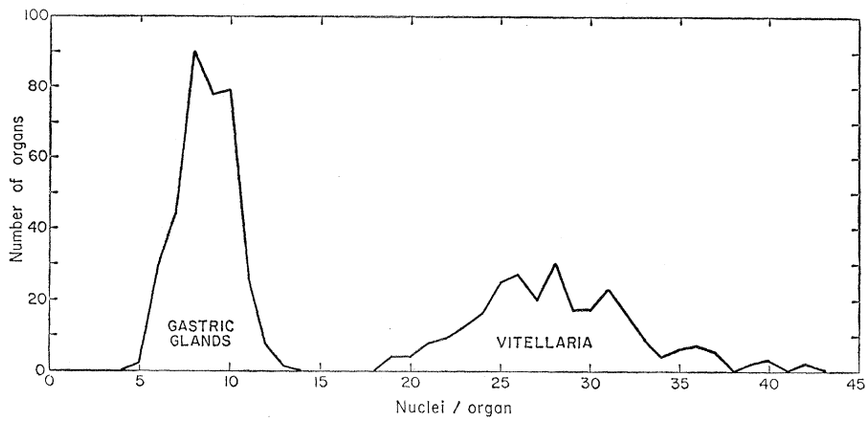


Fig. 2. Frequency distribution of nuclear numbers in gastric glands and vitellaria; clone 7B1-1 fed *Paramecium aurelia*. Pooled data from six samples (gastric glands) or seven samples (vitellaria) from one culture, taken over a 17-day period.

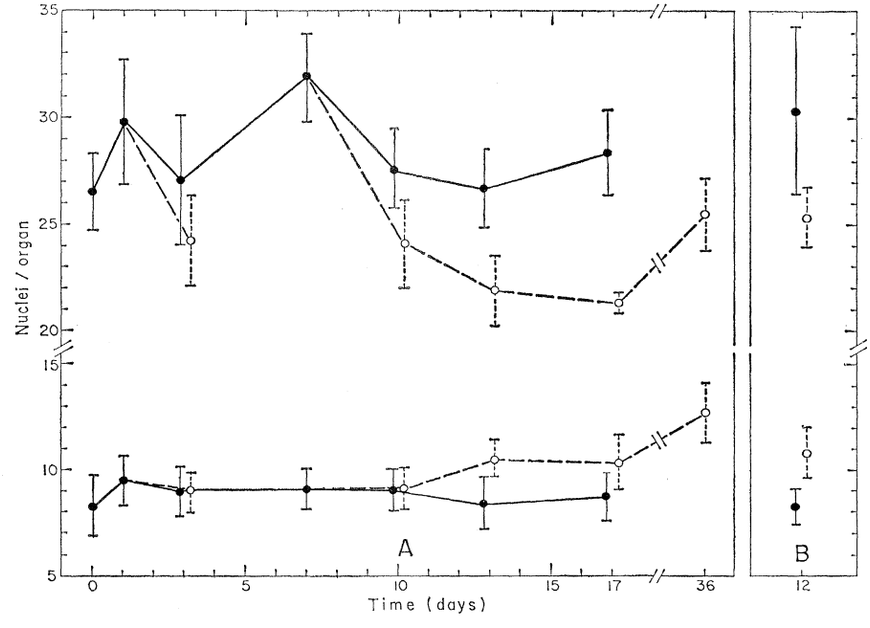


Fig. 3. Effect of diet on nuclear number in vitellaria (upper curves) and gastric glands (lower curves). Each point gives the mean and 95-percent confidence limits for one sample; successive samples from the same culture are connected by horizontal lines. (*A*) At 1 and 7 days, a portion of a culture of clone 7B1-1 fed *Paramecium aurelia* (solid lines) was removed and fed *Eudorina* (broken lines) for 2 and 29 days, respectively. The paramecium-fed culture was too small to give an accurate count on day 29. (*B*) Cultures of clone 7B1-1 were fed *Paramecium aurelia* (solid lines) or *P. bursaria* (broken lines) for 12 days, then counted.

7B1-1. Cultures fed *P. aurelia* have in general fewer nuclei per vitellarium and more nuclei per gastric gland than parallel cultures fed *P. bursaria* or *Eudorina elegans*. Differences between parallel cultures fed different diets are not always significant but are always consistent in direction. The reduction in number of vitellarium nuclei can be detected after as little as 2 days, or about two generations, after feeding *Eudorina;* an increase in gastric-gland nuclei was not detectable after 3 days but was significant at 6 days (about six generations). Similar results have been obtained with *Asplanchna brightwelli* from Indiana, but the samples were too small to allow definite conclusions.

These variations in nuclear number occur between two organs in an individual, and among organs in different individuals all descended by diploid parthenogenesis from a single female. Such individuals are believed, on the basis of cytological evidence, to constitute a clone. The variations seem far too great to be explained by mutation or by any recombination which might occur during the mitosis-like maturation divisions of the parthenogenetic eggs. The parthenogenetic female embryos of *Asplanchna* undergo their entire embryonic development within the uterus of their mother; mitosis ceases early in development, and the embryo is born with a fixed number of nuclei. Environmental effects, such as diet, which have at least partial control over nuclear number, must thus act indirectly through the mother. Possibly dietary changes may first affect the vitellarium and subsequently the egg whose cytoplasm is derived largely from the vitellarium during oogenesis. Differences between organs in the same individual or between different individuals in the same culture container are not readily explained by environmental effects, and may reflect imperfections in the mechanism or mechanisms which control mitosis during embryogenesis. They could also indicate variations in the proportion of embryonic cells which are set aside to form the primordia of these organs. Possibly information about the mechanism of eutely will be more easily obtained from studies on organs in which the mechanism shows some variability, especially when that variability can be partially controlled by the experimenter, than from studies on other organs where the mechanism operates almost perfectly.

We have also looked for interclonal differences that might be utilized in studying the genic control of nuclear number. No significant differences have been found between the Indiana and Tennessee stocks of *A. brightwelli*, which can be crossed. These stocks usually have slightly larger numbers of nuclei in both the vitellarium and gastric glands than do the Pennsylvania stocks. Unfortunately, we have been unable to attempt to mate these stocks, because of difficulties in obtaining miotic females in large numbers from clones 7B1 or 7B1-1. *Asplanchna girodi*, which cannot be crossed with *A. brightwelli*, has a mean of about 34 nuclei per vitellarium and may not differ significantly from the other species. It has, however, about 32 nuclei in each gastric gland (too many to count accurately with our method); in this respect it clearly differs from all our *A. brightwelli* stocks.

We do not intend to imply that our findings on the gastric glands and vitellaria of *Asplanchna* cast doubt on the existence on eutely as a general phenomenon in the rotifers. Nachtwey (*5*) found a somewhat lesser degree of variability in these same organs in *Asplanchna priodonta*. However, in *Epiphanes senta* Van Cleave (*6*) and Shull (*7*) found that 100 and 92.5 percent respectively of all gastric glands had six nuclei and 99.7 and 92.5 percent of all vitellaria had eight nuclei. We have also looked at certain other organs in *Asplanchna* which typically have one or a few nuclei and have found the nuclear numbers in these organs to be quite constant.

C. W. BIRKY, JR.
BONNIE FIELD*

*Department of Zoology,*
*University of California,*
*Berkeley*

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* Present address: 728 Luring Drive, Glendale 6, California.

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## Auxin Effects on the Mobility of Kinetin in the Plant

Abstract. *Kinetin (6-furfurylaminopurine) is generally considered to be relatively immobile within plants. Kinetin labeled with $^{14}C$ was applied to the stumps of decapitated bean plants, with or without simultaneous application of indoleacetic acid. Significantly greater amounts of kinetin moved downwards in the stem in the presence of added indoleacetic acid than in its absence.*

Kinetin (6-furfurylaminopurine) is generally considered to be relatively immobile within plants (*1*). In experiments on hormone-directed transport, it was found that when indoleacetic acid (IAA) was applied to the internodes of decapitated pea plants it induced the movement of $^{32}P$ from the base of the stem toward the point of application (*2*). Kinetin alone had no effect upon the movement of $^{32}P$ in this system, but when it was applied in combination with IAA kinetin enhanced the IAA-induced movement (*3*). Since kinetin affects the level of $^{14}C$-IAA in the stems of decapitated bean plants (*4*), we have investigated whether IAA, in turn, affects the movement of $^{14}C$-labeled kinetin.

Young seedlings of French bean (*Phaseolus vulgaris*), grown in pots in a heated greenhouse for 3 to 4 weeks, were decapitated just below the third node so that the primary leaves and one fully expanded trifoliate leaf and the internode above it were left on each plant. Immediately after decapitation, 0.5 $\mu$c of kinetin-$^{14}C$ (6-furfurylaminopurine-8-$^{14}C$) in 0.01 ml of 80 percent aqueous ethanol was applied to the side of the decapitated stump after scraping away a small area of epidermis. Equal amounts of lanolin

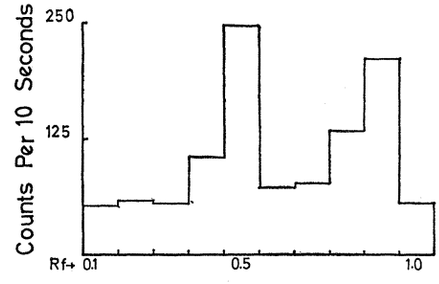


Fig. 1. Chromatogram of extract of internodes of decapitated bean plants to which kinetin-8-$^{14}C$ and IAA were applied. There has been some metabolism of the kinetin-$^{14}C$ but the fraction occurring at $R_F$ 0.7 to 1.0 appears to be unmodified kinetin.