have only small horizontal cells, we have not yet observed synaptic contacts in the outer plexiform layer (9).

The processes of the horizontal cell which we can identify in the rabbit and cat retinas are neither clearly axonic nor dendritic in appearance. It also seems likely that the same processes of the horizontal cells are postsynaptic to the receptors (1) and presynaptic to the bipolars and other horizontal cells. Thus, the processes of horizontal cells may be quite similar to those of the amacrine cells in the inner plexiform layer, which appear both pre- and postsynaptic along their length (5). Processes of the amacrine cell, like those of the horizontal cell, contain few cytoplasmic organelles, and thus usually appear quite "empty" except near sites of synaptic contact.

With the identification of synaptic contacts, it seems clear that horizontal cells should be classified as neurons. It is true that they may sometimes not be classical neurons possessing clearly differentiated dendritic and axonic terminations. Rather they appear similar to the axon-less amacrine cells of the inner plexiform layer. Recently the axon-less cells in the olfactory bulb, the granule cells, have been examined by electron microscopy, and their processes appear similar to those of the horizontal and amacrine cells of the retina (10). Thus, interneurons of this type, with processes that both receive and transmit stimuli, appear to be a feature of afferent pathways, and probably play an important role in the lateral interactions known to occur in afferent systems (6, 10).

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8 July 1966

## **Polyteny: A Source of Cryptic Speciation among Copepods**

Abstract. A large form of the copepod Pseudocalanus is found in two warm, semi-landlocked fiords in arctic Canada, together with a similar but smaller form attributable to the widespread P. minutus. The large form has the same chromosome number as P. minutus, but has larger chromosomes and a higher nuclear DNA content. There are suggestions in the literature that other similar polytenic and cryptic species occur among copepods.

Students of zooplankton have traditionally made numerous length measurements to follow seasonal changes and, when possible, to distinguish succeeding generations. Seasonal and local variations in size within species are phenotypic responses to temperature, although size-temperature relations differ, presumably genetically, between widely separated populations (1). A number of authors have described and discussed bimodal or polymodal size distributions in species of copepods from the same plankton samples. Sometimes these are attributable to overlap of generations, but in recent years some size forms have been given specific status on the basis of slight, size-independent, morphological criteria.

An example of such a size form (a large one) has been described from Ogac Lake, the warm, semi-landlocked head of a fiord in Baffin Island, at 62°52'N by 67°21'W (1). The eggs and bodies of this large form of the copepod Pseudocalanus are about three times the volume of those of a coexisting small form, which is attributable to the widespread P. minutus (Krøyer). This large form was also shown to develop more slowly in nature. Otherwise the large and the small forms seem morphologically indistinguishable. The large form has since been abundantly found in the semilandlocked head of Winton Bay on the east coast of Baffin Island, at 63°24'N by 64°39'W. No intermediates have been found, and the large form appears to be a reproductively isolated sibling or cryptic species (2).

It was assumed that the large form would prove to be polyploid (1), but collections made from Ogac Lake and elsewhere (1965) indicated that the large form, like P. minutus, has the usual chromosome complement for calanoid copepods (n = 16). The chromosomes, however, are conspicuously larger (Fig. 1). It was therefore of interest to determine whether the larger chromosomes contained more DNA.

Cytophotometric analysis was used to determine DNA content of interphase nuclei in embryos of these two forms from Ogac Lake. The embryos were removed from routine plankton samples, which were fixed and stored in formaldehyde-seawater (5:95). The Feul-



Fig. 1. Aceto-orcein-stained haploid sets of metaphase chromosomes from fresh, undivided eggs of (a) the large form of Pseudocalanus from Ogac Lake, and of P. minutus from (b) Ogac Lake, (c) Frobisher, N.W.T., (d) Millport, Scotland, and (e) Halifax, N.S. All to same scale (in b).

Table 1. Measurements of DNA-Feulgen complex on nuclei of embryos at the 32-cell stage from the large and small forms of Pseudocalanus. Amount of chromatophore is determined from average extinctions and from photometric areas of 532  $\mu^2$  for the small form and 2084  $\mu^2$  for the large form. Average extinctions are from averages of duplicate readings of ten nuclei from each embryo.

Average extraction (mean $\pm$ S.D.)	Total relative amount of chromatophore
Individual	small embryos
$0.122 \pm 0.025$	63.8
$.138 \pm .021$	72.2
$.141 \pm .036$	73.7
$.115 \pm .012$	60.2
$.146 \pm .016$	76.4
Small embr	yos combined
$.132 \pm .022$	69.0
Individual	large embryos
$.241 \pm .030$	502.4
.233 + .028	485.7
$.279 \pm .030$	581.6
$.202 \pm .026$	421.1
$.238 \pm .012$	496.1
Large embr	yos combined
.239 ± .035	498.2

gen reaction (3) was employed as a measure of relative amounts of nuclear DNA. Embryos of both forms were treated concurrently to avoid variability due to the staining procedure. Several nonhydrolyzed embryos served as controls. Measurements were made on five embryos of each form, all at the 32cell stage. Individual embryos were mounted on separate slides in appropriate oil of refractive index. The cytophotometric technique used was the aperture method, and all measurements were made with the two-wavelength method at  $\lambda$  570 m<sub> $\mu$ </sub> and  $\lambda$  500 m<sub> $\mu$ </sub> (4). Ten nuclei were measured from each embryo. Table 1 shows the results.

It is evident that nuclei of the large form have a greater DNA content, which has occurred by an increase of size rather than number of chromosomes. Considering that the ratio of volumes of somatic nuclei among older stages of the large and small forms was shown to be about 3:1, like the ratios of egg and body volumes (1), the ratio of DNA amounts (about 7:1) is somewhat curious. However, the point to be made here is that the two forms are similar except in size and development rates, these being related to their DNA contents. It is known that nuclear size (chromosome number, size, or DNA content) influences cell size and inversely affects both cellular metabolic and division rates, without necessarily affecting qualitative characters of the organism. Several theoretical discussions of these relationships have recently been offered (5). Copepods show determinate growth and probably determinate cell number (1), so that an increase in DNA content may be expected to increase size and to reduce development rate of the entire organism, as found in the large form of Pseudocalanus.

The possible adaptiveness of the large form will not be considered here in detail. It is noteworthy that P. minutus, which is especially large in ordinary arctic waters, is greatly reduced in size by the unusually warm waters of the aforementioned semilandlocked fiords (1). The large form may represent an evolutionary attempt to restore "normal" size and development rates for these high latitudes.

The increase of chromosome size presumably results from polyteny, that is, from multiplication of DNA-containing strands, rather than from differential degrees of tightness of coiling in the chromosomes. Although chromosome size was of sporadic concern to earlier workers, Hughes-Schrader and Schrader (6) were apparently the first to implicate polyteny as a factor in evolution within a closely related group of animals. In the literature there seems to be no explicit demonstration that polyteny is responsible for size forms of the sort demonstrated here, but there are suggestions that others occur among copepods. Three cryptic species of the well-known genus Calanus are now recognized from the North Atlantic (7). Calanus finmarchicus is a mid-latitude form which is smaller than the northern C. glacialis where they occur together in subarctic waters. Similarly, C. finmarchicus is smaller than C. helgolandicus where their ranges overlap in the eastern North Atlantic. Harding (8) reported that both C. finmarchicus and C. helgolandicus have haploid chromosome sets of 17. Noting that the eggs of C. helgolandicus are larger (about 172  $\mu$ ) than those of C. finmarchicus (about 145  $\mu$ ), he suggested that the chromosomes of the former were larger, although his evidence was inconclusive. A form producing large eggs (about 170  $\mu$ ) with distinctive membranes in northern Norway (9) probably belonged to C. glacialis, which was not then described. Similar large (178.6  $\pm$  2.5  $\mu$ ), membraned eggs were produced by C. glacialis in Frobisher Bay (June 1965), and aceto-orcein squashes revealed haploid chromosome sets of 17. It may be noted that the eggs of the two large forms of Calanus are about twice the volume of those of C. finmarchicus. Analogies with the polytenic form of Pseudocalanus are suggested.

Examples of "dualism" among freshwater copepods (10) and seasonal variations in egg size, especially where larger eggs occur in the warm season (11), should be examined more closely, along with bimodal size distributions (7), to see if polytenic and cryptic species are involved.

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15 July 1966

## Circadian Rhythm for Tryptophan **Pyrrolase Activity and Its Circulating Substrate**

Abstract. Hepatic tryptophan pyrrolase and its circulating substrate, whole blood tryptophan, have a circadian rhythmicity in mice. Intact adrenocortical function is required for the normal rhythmicity of both enzyme and substrate although an altered but less apparent rhythm persists in the adrenalectomized state.

Circadian rhythm for the enzyme, kidney transamidinase, has been well described as occurring in mice (1). The lighting regimen was the dominant synchronizer of this enzyme; reversal of day-night relationships could reverse enzyme rhythmicity. A dependent relation to plasma corticosterone rhythmicity was excluded. We now report

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