POLYTENY AND THE FUNCTIONAL SIGNIFICANCE OF THE POLYTENE CELL CYCLE

M. J. PEARSON

Department of Zoology, Downing Street, Cambridge, England

SUMMARY

Polyteny is a special nuclear differentiation reported in larval and adult Diptera, Collombola, Protozoa, and angiosperm ovular nuclei. In not all cases, however, do discrete giant chromosomes represent homologous pairs as in the salivary gland nuclei of Drosophila or Chironomus larvae, and in Calyptratae (e.g., Calliphora) a degree of homologue separation and dissociation of the constituents results in a reticular chromosomal organization within which banded polytene regions are prominent. The correlation previously shown between the polytene cycle of replication without mitosis and the growth habit of larval Calliphora suggests that the significance of the polytene phenomenon may lie, not in a special physiology of discrete giant chromosomes, but in the cell cycle responsible for the polytene condition; chromosome morphology may not be functionally significant. The significance of polyteny versus endopolyploidy is considered in terms of the cell cycle. Whereas the endopolyploid cycle is endonuclear and thus non-disruptive of cytoplasmic syntheses, the polytene cycle further departs from a normal mitotic cycle in that there is no M-phase: the nucleus is in permanent 'interphase', and clearly the cycle should not be referred to as 'endomitotic'. The physiology of polytene chromosomes is discussed: there is no evidence that the intense activities of hypertrophied salivary cells in Diptera depend on continuous monitoring at transcription level. The acetic-orcein squash technique has been used to describe the chromosomal organization in two cell types known to hypertrophy without sign of an M-phase: oenocytes of the locust Schistocerca gregaria, and the giant trophoblast cells of mouse concepti. In both cases, there is cytological evidence of polytene lateral pairing over short regions. In Schistocerca oenocytes, this organization is similar to the reticular fibrillation of polytene chromosomes seen in calyptrate epidermal and salivary nuclei. The functional significance of the polytene cell cycle is discussed in the light of its known incidence among eukaryotes, and the use of terminology examined.

INTRODUCTION

Cellular hypertrophy as a basis of tissue growth is frequently encountered among insects, and in particular in the larvae of Diptera, where it is associated with the nuclear phenomenon of polyteny. 'Polytene chromosomes of Diptera arise during development of definite cell types and in most typical cases are maintained as a permanent differentiation of their nuclei' (Matuszewski, 1965). That is, giant chromosomes are formed within hypertrophying nuclei by successive cycles of replication without segregation of daughter chromosomes, and remain morphologically distinct.

These giant chromosomes were first described in larval chironomids by Balbiani (1881) but only accorded proper significance 50 years later by Heitz & Bauer (1933).
and by Painter (1933) who showed independently, in the larval Malpighian tubules of *Bibio hortulensis* and in the salivary gland of *Drosophila melanogaster* respectively, that the nuclear structures described by Balbiani were in fact derivatives of mitotic chromosomes. Koltzoff (1934) put forward the polytene hypothesis: that these giant chromosomes attain their enormous size by multiplication of the axial threads (the 'genoneme', 'axoneme' or 'chromoneme') which remain paired, and that the visible banding of the polytene chromosome is the effect of alignment of corresponding chromomeres along the sister chromomata.

This polytene condition is a special nuclear differentiation only occasionally reported outside the order Diptera, and a departure from the common diploid DNA constancy of somatic cells which needs a functional explanation. Despite the many studies of polytene chromosomes that have contributed to the literature of developmental cytology (see Beerman, ed., 1972), it is still not clear why Diptera have so exploited this method of DNA replicative organization as a basis of growth; and further, as noted by Ashburner (1970) in his review of polytene puffing in insect development, 'the functional significance of polyteny v. endopolyploidy is obscure'.

It has been shown recently (Pearson, 1974) in the epidermis of the acephalous larva of *Calliphora* that the rapid increase in nuclear DNA content (resulting from cycles of polytene replication) during early larval life is closely correlated with the growth habit of the larva. It was suggested that polyteny here is a cellular adaptation to the function of the larval period; that is, the extreme growth required by continuous, rapid food ingestion and the subsequent accumulation in the fat body of those reserves which will be later deployed in the complex morphogenetic expense of metamorphosis. However, in Calyptratae (e.g. *Calliphora*) it is characteristic that the classical polytene chromosome morphology seen in lower Diptera is considerably modified by separation of the homologues and dissociation of constituent chromomata, which results in a nuclear reticulum within which typically banded polytene regions alternate with fragmented oligotene – or finer – chromonomal strands. Nevertheless, it was shown that the replicative organization of DNA in such 'atypical' polytene nuclei is the same: that each chromonomal element participates in each round of replication, so that Feulgen densitometry of nuclei at different stages of larval life shows a geometric series of DNA values.

According to Swift (1950), 'polycny is a morphological concept' and 'since both polyploidy and polyteny apparently result in a duplication of genetic components, in physiological terms the two processes may be regarded as essentially identical'. This view has remained apparently without criticism until, by implication, Ashburner's remark quoted above; and while polytene chromosomes have been exploited as gratuitous chromosomal structures whose banding patterns and puffing phenomena allow an experimental approach to the problem of differential gene activity, interest has been concomitantly restricted to the classical case of polytene morphology exemplified by the salivary gland chromosomes of *Drosophila* and *Chironomus*. According to this predominant attitude, the calyptrate condition is a special case. However, if, as suggested, the significance of polyteny lies not in the banded polytene chromosomal morphology, which depends on lateral pairing, but in the cell cycle responsible...
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for this condition, then the reticular oligotene pattern of chromatin seen in calyptrate larvae may not be a very significant modification; indeed, it may be that the highly precise pairing of chromonemata and homologues seen in most dipteran larval tissues itself represents the special case.

If such functional significance is to be found in the cell cycle, polyteny is clearly not physiologically identical to polyploidy, and in this case it may be asked why polyteny should arise in some hypertrophying nuclei, but not others; that is, what is the functional correlation between those cells which are characteristically polytene? And what is the pattern of the polytene cell cycle?

The purpose therefore of this article is to review the incidence of polyteny as reported in the literature of the subject; to examine the notion, by reference to cell cycles, that in physiological terms the polytene and polyploid processes are identical; to describe by the acetic–orcein squash technique the chromosomal organization in two further cell types whose nuclei are known to hypertrophy without evidence of mitotic chromosomes; and in the light of current understanding of such cell types to attempt a reassessment of the concept of polyteny and its functional significance.

THE INCIDENCE AND MORPHOLOGY OF POLYTENY

Polytene chromosomes have been reported, not only in dipteran larval and adult tissues, but also in angiosperms, protozoa, in another insect order, Collembola, and in different carcinomas.

Polytene chromosomes in Protozoa

Ciliates. Several workers have reported polyteny in the macronuclear development of various ciliates (Grell, 1949; Golikowa, 1964; Alanzo & Perez-Silva, 1965; Radzikowski, 1967; Ammerman, 1964), protozoa which contain a diploid micronucleus and a so-called 'polyploid' macronucleus. ('Polyploid' here should be taken merely to indicate a DNA content > 2C, since the chromosomal organization in macronuclei is not well understood, differs certainly during the life-cycle, and may differ through the class.) The macronuclear events after conjugation which lead up to this condition are not the same in Tetrahymena and Styloynchia (Ammerman, 1971). In Styloynchia, from thin despiralized threads at the periphery of the nucleus, there develop large polytene chromosomes of the same banded appearance as in dipteran salivary glands, but often joined end to end. By 40 h after syngamy, however, these structures have begun to disintegrate, and more than 90% of the macronuclear DNA is eliminated before the final reorganization, involving further DNA synthesis, which leads to a mass of small unconnected chromatin granules, apparently distributed at random to daughter nuclei. In the macronuclear development of Nyctotherus cordiformis (Golikowa, 1964) and of Chilodonella cuculus (Radzikowski, 1967) the same bizarre events—of polyteny followed by elimination—occur. It is impossible however, to draw functional significance from these reports until more information is available on the metabolic activities of the macronucleus during its development. Ammerman (1968, 1969) has shown that the breakdown of poly-
tene chromosomes at the end of the first phase of macronuclear development in *Stylonychia* exconjugants leads to a loss of labelled DNA degradation products, via the cytoplasm, to the external culture medium. When this labelled medium was used for the culture of new exconjugants, the label was not taken up in polytene replication, suggesting that these breakdown products are not DNA precursor material, but probably much smaller molecules than the originally labelled thymidine. It is clear also that the significance of the polytene phase does not lie in the provision of a large template surface for transcription, since no incorporation of tritiated uridine was observed until the later 'polyploid' stage (Ammerman, 1968).

*Dinoflagellates.* Polyteny has been described in dinoflagellates (Grasse & Dragesco, 1957) but these 'chromosomes' show prokaryote characteristics — circularity, lack of histones (Haapala & Soyer, 1973) — and are not banded in the typical manner of eukaryote giant chromosomes. It is hardly possible to draw close comparison between the cell cycle of these and eukaryotic organisms.

**Polyteny in the ovular cells of angiosperms**

Flowering plants show no trace of the gametophyte generation, already reduced in gymnosperms, except for the postmeiotic nuclear divisions of the megaspore that lead to the formation of the egg apparatus: egg nucleus, polar nuclei, synergids and antipodal cells. At fertilization, the polar nuclei engage in a triple fusion with the vegetative male nucleus leading to endosperm development, but the function of the synergids and antipodal cells is obscure. Coe (1954) fed *Zephyranthes drummondi* with $^{14}$CO$_2$ during production of flowering scapes, and after 36 h collected ovaries. Autoradiography of frozen material showed highest $^{14}$C concentrations in nucellus, synergids and antipodal cells, indicating that these cells are metabolically intensely active.

*The antipodal cells.* The antipodals are ephemeral and show signs of degeneration shortly before or after fertilization (Maheshwari & Sachar, 1963). After completion of mitotic divisions and before this degeneration begins, in a number of angiosperms studied by Geitler's coworkers, the antipodal nuclei enlarge and either undergo endopolyploidy, as in *Clivia* (Tschermak-Woess, 1957) or, as in *Papaver* (Hasitschka, 1957) develop giant polytene chromosomes. This latter report is interesting in several features. (i) At lower levels of polytene replication, typically banded polytene chromosomes are apparent, whereas (ii) later, sister chromatids separate, retaining close contact only through densely staining chromocentres — a situation which recalls the fragmentation of polytene chromosomes during growth of ovarian nurse cells in several higher Diptera (Bauer, 1938). (iii) Although Hasitschka does not describe endomitotic activity in these later polytene nuclei, as is the case with nurse cells (Bier, 1957), she did find one antipodal nucleus in endomitosis, 128-ploid, and with chromosomes of normal size. It is not clear, however, whether this metaphase derived from a polytene nucleus by fragmentation or whether it had reached this ploidy through earlier endomitoses. In *Aconitum* (Tschermak-Woess, 1956) the giant antipodal nuclei increase through endomitosis, giving rise to a high degree of polyploidy but in a few instances the nuclei were found with the haploid number of giant polytene chromosomes.
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The synergids. In Orchidaceae, at least, which undergo the common Polygonum type of monosporic gametogenesis, the synergids are either destroyed by the male generative nucleus or degenerate before zygote division (Rao, 1967), but in many angiosperms one of the synergids persists after fertilization and often hypertrophies. In Allium one of the synergids regularly enlarges, and has been shown to contain large unbanded polytene chromosomes with loosely organized chromonemal strands (Håkansson, 1957) which, during final nuclear degeneration, separate to give the nucleus a finely fibrillar appearance.

The embryo. Banded polytene chromosomes are found in the early embryogenesis (i.e. development of the sporophyte) of Phaseolus vulgaris (Nagl, 1969). In the suspensor cells, homologues are unpaired, and the structure is diffusely granulated rather than banded – as in the primary polytene chromosomes of Calliphora ovarian nurse cells – but when bred at lower temperatures typically banded chromosomes develop. The suspensor is a temporary organ, the rapid growth and enlargement of which serves to force the embryo into intimate contact with the nutritive tissues of the gametophyte.

All these ovular cells serve an ephemeral function and then degenerate. In all cases, nuclear growth is rapid and DNA synthesis intense. Bennet, Rao, Smith & Bayliss (1973) have measured the DNA content of Triticum antipodals. In the 5 days previous to dehiscence, after which there is no further increase, a 1C DNA content reaches 200 C, and Bennett suggests that this antipodal DNA is merely synthesized as a metabolic store for the subsequently developing endosperm. It is interesting that for the first 6 or 7 mitotic cycles of the endosperm nuclei the cycle time at 20 °C is 4.5 h (which compares with the first root tip meristem cycle of 12.5 h, with 12.0 h for haploid embryo sac nuclei, and 9.0 h for the diploid nuclei of the embryo) of which 3.5 h is S and 1 h is M (Bennett et al. 1973). It is possible that the concomitant breakdown of the antipodal nuclei provides DNA precursor material which obviates a long G-phase in the endosperm.

While hypertrophied synergid nuclei may serve a similar purpose, the polytene growth of suspensor cells is correlated with the rapid cell hypertrophy which is their function.

The great variety of morphology among these polytene chromosomes indicates that if we are dealing primarily with a morphological concept, it is a very loose one.

The polytene nuclei of Diptera

Since their first discovery giant polytene chromosomes have been described in the nuclei of many dipteran larval tissues (Makino, 1938) where growth by cell hypertrophy is associated with histolysis at metamorphosis and replacement by a stock of imaginal cells. Other polytene larval cells, however, persist through metamorphosis into the adult – in Malpighian tubules, brain, heart, and pericardium (Makino, 1938). Histolysis, then, does not inevitably result at metamorphosis from the polytene condition, but neither is there, in the persistent cell types cited, redifferentiation – except, arguably, in Malpighian tubules where although there is no gross reorganization, there are changes in the pattern of enzyme synthesis at metamorphosis (M. Berridge, personal communication). In either case, it is likely that the polytene cycle
in the larval tissues is correlated with the extreme growth habit of larval forms whose
special feeding habit is independent of, and a preparation for, the complex processes
of holometabolous metamorphosis (Ribbert, 1967).

It has recently been shown also that polyteny may arise in adult epidermal cells at
metamorphosis. In both footpad cells (Whitten, 1964) and the cells of the macro-
chaete apparatus (Ribbert, 1967) of Calyptratae, polyteny is associated with an early
rapid cell hypertrophy, which is a prerequisite of their function in secreting large
cuticular structures at an early stage of adult life. Unlike the polytene nuclei of larval
Calyptotrate, these chromosomes are discrete structures with reproducible patterns of
bands which have been mapped by Ribbert (1967).

Collembola

Polytene chromosomes have been described in the salivary glands of different
species of Collembola (Cassagnau, 1966, 1968). In *Bilobella massoudi*, in the large
nuclei of the salivary gland, the 2n (14) number of banded polytene chromosomes
appear, either end to end, or with partial pairing of homologues. Structural differences
are produced by different conditions.

Carcinomas

Reports of polyteny in various vertebrate carcinomas (Biesele, Poyner & Painter,
1942; Biesele, 1943; Biesele & Poyner, 1943) refer to enlarged nuclei where the
individual 2n chromosomes are double or quadruple the usual volume, and presumed
to be multistranded. These estimations are based on such chromosomes
which were seen in metaphase plates; that is, in mitotic nuclei. Since larger polytene
chromosomes were not regularly evident, it is probable that there is no controlled poly-
tene cell cycle in these carcinomas, but rather irregular aberrations of the mitotic
cycle such as might be expected in malignant lines.

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Polyteny and endopolyploidy: the polytene cell cycle

Bauer’s (1938) description of the disintegration of primary polytene chromosomes
in the nurse cells of *Lucilia, Polenia* and *Musca*, into their constituent chromonemata,
provided the first direct evidence for the polytene hypothesis of dipteran giant chromo-
somes. These chromosomes are the result of a replicative process which has often
been referred to as endomitotic, but which, unlike the cycle of a mitotic cell, is not
followed by the reorganization and separation of daughter chromatids. The term
‘endomitosis’, was introduced by Geitler (1939) to describe rather different events.

Growth through cell hypertrophy occurs in Hemiptera (Geitler, 1939), in Nema-
tocera (Risler, 1950) and in Coleoptera (Romer, 1964) but unlike those instances
discussed already, the concomitant increase in nuclear size and DNA content follows
a process involving the replication and separation of each pair of daughter chromo-
somes within an intact nuclear envelope, with a consequent doubling of the number of
chromosomes at each such replication, giving rise to *endopolyploidy*. Thus, after
x cycles of this endomitotic replication, an originally diploid cell (2n) will have $2^{x+1}n$ chromosomes whose individuality is maintained throughout the endopolyploid process. In a polytene cell, after x cycles of replication, there are n polytene chromosomes (since, in Diptera, the homologues are paired) but the DNA content has increased from 2C to $2^{x+1}C$. It is clear that this mechanism for the increase of nuclear DNA content should not be referred to as mitotic, or endomitotic, since the essence of any mitosis is the organized separation of daughter chromosomes. The replicative aspect of chromosomal DNA organization belongs to the S-phase of the cell cycle, and is completed well before mitosis (M) (Pelc & Howard, 1952).

Ashburner (1970) lists the manner in which the chromosomal duplication of polytene development differs from that of a normal mitotic cycle: '(i) the homologues remain unpaired; (ii) the chromosomes fail to participate in the normal mitotic cycle of coiling and uncoiling; (iii) daughter chromosomes do not segregate but remain intimately paired with each other at the end of each replication cycle; and (iv) the nuclear membrane and nucleoli remain intact throughout the replication cycles.'

In features (i), (ii) and (iii) polyteny also differs from the endomitotic cycles studied in Hemiptera by Geitler (1939, 1953) and Nur (1968). Nur looked at the Malpighian tubules of the mealybug, Planococcus citri (Homoptera) where, in recently moulted adult females, many of the binucleate cells are in stages of endomitosis. In this species the diploid number 2n = 10. In some of the endopolyploid nuclei he found over 300 condensed chromosomes, suggesting that 32n chromosomes were in division, i.e. a fifth endomitotic cycle. In this endomitosis the nuclear membrane does not break down, spindle fibres are absent, and the chromosomes show little or no kinetic activity. However, Nur was able to identify endoprophase, endometaphase, endoanaphase and endotelophase on the basis of the state of chromosomal contraction and the distance between sister chromatids/chromosomes, indicating that in the Malpighian tubules of Planococcus citri the chromosomes do undergo the same cycle of coiling, chromatid...
separation and uncoiling as in the normal mitotic cycle of diploid cells in Hemiptera.

Polytene duplication of chromosomes departs further from the normal mitotic cycle in that there are no such changes of chromosomal organization, so that the cell cycle, no longer showing any trace of the M-phase, can be represented by Fig. 1 b. Whereas the endopolyploid process avoids the disruption of cytoplasmic syntheses by spindle formation, with breakdown of nuclear membrane and nucleolus, the duplication of polytene chromosomes eliminates M altogether, and thereby any disruption of chromosomal syntheses of the G-phase due to contraction and coiling of the chromosomes. The polytene nucleus, unlike the endopolyploid nucleus, is thus in permanent 'interphase' (Fig. 1).

The synthetic activity of salivary gland polytene chromosomes

Polytene chromosomes and the puffing phenomenon have contributed greatly to our understanding of the mechanisms of gene action. In wider aspect it may be asked whether a peculiar physiology of polytene chromosomes provides the correlation between those cell types which systematically exploit a polytene cell cycle as a basis of growth.

There is now a wealth of autoradiographic evidence to show that for the greater part of the cell cycle polytene chromosomes in Diptera are highly active in the synthesis of RNA (Pelc & Howard, 1956; Pelling, 1959, 1964; Sirlin, 1960; Rudkin, 1962; Ritossa, 1964), and that the sites of this activity are those bands which form puffs. Lara, Lopes & Forestri (1965) isolated RNA from the salivary gland nuclei of Rhynchosciara angela after tritiated uridine labelling, and found that most of the newly formed RNA was of high molecular weight, with sedimentation constant 45s, which suggests that it might be r-RNA precursor. Microelectrophoretic determinations by Edström (1964) showed that r-RNA precursor is synthesized at the nucleolar organizers only, and accumulates in the enormous nucleoli, but comparable rates of RNA synthesis are found in the other large chromosomal formations, the Balbiani rings (BR) (Sirlin, 1960). In Chironomus salivary nuclei Edström could show that the base composition of RNAs from different nucleolar organizers was identical, but that RNA from different chromosomes, and even from different segments of the same chromosome, each bearing a single BR (which were found to be responsible for 75–85% of the RNA on each of these segments), showed markedly different base compositions (Edström & Beerman, 1962). BRs are merely exaggerated puffs (Beerman, 1952) and it is in fact the puffs which are the sites of chromosomal RNA synthesis. Unpuffed bands never synthesize RNA, whereas puffed bands invariably do so (Pelling, 1966). Further, the structural condition of the puff is dependent on RNA synthesis since actinomycin D blocks puff formation (Ritossa & Pulitzer, 1963).

Salivary gland nuclei labelled at any point of the cell cycle show RNA synthesis. If polytene nuclei are engaged in DNA replication for more than 50% of the cell cycle – as in the epidermal cells of Calliphora (Pearson, 1974), or in the salivary glands of Chironomus (Plaut & Nash, 1964) – the question arises: what is the relation between DNA and RNA synthesis during this S-period?

Ritossa (1964) incubated Drosophila salivary glands in a medium containing tritiated
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cytidine and/or thymidine. After incorporation of both RNA and DNA precursors together for 3–4 min, some chromosomes were labelled only at puffs, while others labelled continuously along the whole length. This labelling pattern was altered by RNase digestion, leaving some cells totally unlabelled, some with continuous labelling of the chromosomes, and others with discontinuous labelling of chromosomal bands. The ratio of continuously: discontinuously labelled nuclei was now the same as that produced by tritiated thymidine incorporation alone, and was therefore due to DNA replication. After longer periods of thymidine incorporation the proportion of continuously labelled chromosomes rose, suggesting that the discontinuity merely represents a phase in DNA replication along the whole chromosome. In those chromosomes which displayed the discontinuous pattern of thymidine incorporation no DNA synthesis was found in any puffs, whereas, of course, puffs were labelled in continuously labelled chromosomes. The evidence of Ritossa, and of Pelling (1964) and Plaut, Nash & Farring (1966) fits the hypothesis that DNA replication begins simultaneously in all bands but continues longer in some bands than in others, giving rise to the discontinuous labelling pattern. Ritossa’s experiments also show that syntheses of DNA and RNA are not mutually exclusive within whole nuclei; and that in Drosophila salivary glands at least, the period of DNA replication in the puffs is brief, whereas RNA synthesis is evident for the rest of the cell cycle.

It might be supposed, then, that the function of cells with polytene nuclei is dependent on a constant high rate of chromosomal RNA synthesis, and therefore under immediate control of the active genes. Clever and his associates have examined this possibility in the polytene salivary glands of last-instar Chironomus larvae (Clever, Storbeck & Romball, 1969; Clever, 1969; Darrow & Clever, 1970). In the case both of total protein (Clever et al. 1969) and a special secretory product in Chironomus pallidivittatus (Clever, 1969), 95% inhibition of RNA synthesis by actinomycin does not significantly lower protein synthesis. Apart from some special proteins synthesized in the first few hours of the last instar (Darrow & Clever, 1970), the synthesis of proteins during the last instar is not immediately controlled by the rate of synthesis of corresponding RNAs. Rather, m-RNA here appears to be stable and long-lived, just as in many other differentiated cell types investigated (see, for example, Revel & Hiatt, 1964; Tyler, 1967). Although the significance of polyteny in these last instar salivary glands cannot therefore lie in any continuous chromosomal synthesis of RNA, such data on the physiology of polytene cells during the earlier more critical period of shortest cell cycle are not available.

Oenocytes of Schistocerca gregaria (Orthoptera)

If polytene chromosomes occur in such a primitive insect order as Collembola, it is surprising perhaps that reports should be common only among Diptera. However, the pairing of diploid homologues is a distinctive feature of the metaphase karyotype in mitotic cells in this order, and it is possibly the same phenomenon of chromosomal attraction which is responsible for the highly distinctive banded giant chromosomes in dipteran polytene nuclei. It is possible that less distinctive cases of polyteny have been overlooked.
Insect oenocytes are large ovoid cells with hyaline acidophilic cytoplasm (Wigglesworth, 1970) which is seen by electron microscopy to be packed with smooth endoplasmic reticulum (Locke, 1969). Oenocytes derive from the epidermis either in embryogenesis or at the moult cycles of larval life; and although their function has not been fully described, it is clear that they are important in the synthesis of paraffins which later appear in the cuticular lipids (Diehl, 1973). In Calpodes (Lepidoptera) the permanent oenocytes have been described as large polyploid cells whose nuclei hypertrophy through larval life by repeated endomitoses (Locke, 1969). On the basis of nuclear volume, Locke estimates that already in the first instar oenocyte nuclei have undergone 4 or 5 cycles of replication, and that later increase could be accounted for by one cycle of replication per stadium.

In Schistocerca also oenocyte nuclei hypertrophy without sign of mitotic chromosomes (Diehl, personal communication). In acetic-orcein squashes of oenocytes taken from the peripheral fat body of mid fifth-instar females, which on the basis of volume changes may be expected to have undergone 5 or 6 replication cycles, they are of diameter about 40 \( \mu \text{m} \), compared with 10 \( \mu \text{m} \) diameter of epidermal cell nuclei. Figs. 17–19 are typical squash preparations in which the chromatin shows banded chromosome regions which fragment into oligotene fibrils and reassociate in a reticular manner just as in the polytene larval cells of calyptrate Diptera (Figs. 2–8; Bier, 1957; Pearson, 1974).

**Giant trophoblast cells from mammalian embryos**

Trophoblast cells of rodent and other mammalian embryos are known to hypertrophy during early implantation stages without any sign of mitosis (Zybina, 1961; Barlow & Sherman, 1972). In mouse blastocysts it becomes cytologically evident that all the cells of the mural trophoblast are committed to hypertrophic specialization by 4-5 days (Dickson, 1963), by which stage they also display a marked increase in phagocytic activity when cultured in vitro with melanin granules (Gardner, unpublished observations). This rise of phagocytosis is not shown by the embryonic polar trophoblast whose cells remain diploid, and Gardner (unpublished) has suggested that it is an important function of the mural trophoblast in early development. The induction of uterine decidua by transplanted trophoblast vesicles, or by oil droplets, results in a large chamber of degenerate cells or debris within which are seen a few non-proliferating giant cells. After transplant of a normal or reconstituted blastocyst, however, which promotes the development of extensive trophoblast, this space is occupied by the embryonic cylinder and no debris is seen (Gardner, 1972); it is apparently phagocytosed by the giant cells, which are also known to be responsible for inflation of the blastocyst (Gamow & Daniel, 1970).

Squash preparations of trophoblast tissue from 8-5-day mouse concepti given to me by Dr Gardner show a highly diffuse chromatin reticulum with a number of heterochromatic foci. In some preparations a banded polytene articulation appears over short regions; specifically in preparations which have not been fully flattened, and have presumably suffered less distortion in the squash (Figs. 11–13). The banded appearance is usually adjacent to a chromocentre, into which chromatin fibrils may
be seen converging. Barlow & Sherman (1974) have also described the reticulate nuclear morphology, and observed instances where chromatin threads run in parallel to give thicker strands. They ‘do not consider the apposition of the strands close enough or consistent enough to merit the use of the term “polytene”’. However, the same convergence of threads into banded territory associated with the chromocentres is evident in their micrographs. Barlow & Sherman (1974) have compared the number of chromocentres in diploid and giant trophoblast nuclei, and finding that the numbers in both fall within a similar range, they suggest that this result is ‘consistent with a model in which homologous chromosomes remain aligned throughout the numerous cycles of endoreduplication, but mainly by the centromeric chromatin’.

Despite the failure to corroborate Zybina’s (1970) claim that polytene chromosomes can be distinctly seen, the evidence of total genome reduplication in giant trophoblast nuclei (Sherman, McLaren & Walker, 1972), the absence of mitotic figures, and the similar number of chromocentres throughout nuclear growth (Barlow & Sherman, 1974) support the view that these cells replicate through a ‘polytene’ cell cycle (endoreduplication). The observed convergence of fibrillar chromatin into banded regions provides cytological evidence of a polytene phenomenon.

Oligotene chromatin fibrils and the banding of polytene chromosomes

It may be significant that oligotene fibrils – in Calliphora epidermal nuclei, or in Schistocerca oenocytes – do not show clear banding (Figs. 6–9). Possibly the characteristic banding of polytene chromosomes is a product or concomitant of some further structural ordering of the chromomeric DNA regions consequent upon a high degree of lateral alignment. In those nuclear types where regions of polytene banding are evident among a more prevalent oligotene, or reticular organization, such alignment would be critically achieved only over more or less short lengths.

Cases of interrelated polyteny and endopolyploidy

In both ovarian nurse cells of calliphorid flies, which degenerate after transfer of metabolic reserves to the egg, and in the polytene ovular nuclei of angiosperms which may function in a comparable manner, the discrete banded chromosome morphology is lost when the chromonemata separate to give a fibrillar nuclear reticulum. Bier (1957, 1960) describes this fibrillation at the 16–32C stage in ovarian nurse cells of Calliphora, and in particular shows how at this and subsequent duplications, the separated chromosomes pass through an endometaphase involving longitudinal contraction of the chromosomes. In these cells, which in earlier stages of replication formed normal polytene chromosomes and showed no trace of $M$, an endopolyploid cycle has appeared. Later, some of these endopolyploid chromosomes may reconstitute polytene chromosomes (Bier, 1960): it seems that the state of attraction between sister chromonemata may vary through the life of the same cell, and that, although responsible for the morphology of polytene chromosomes, may not be functionally important.

The disintegration of salivary gland chromosomes in Cecidomyiidae (White, 1946) has been described as a polyploid condition derived from polyteny (Henderson, 1967),
although it is not clear that the number of elements seen corresponds strictly with ploidy; and in *Dasyneura urtica* the polytene chromosomes undergo disintegration not to individual chromonemata, but to oligotene fibrils, a process which is 'not accompanied by any appreciable contraction of the resulting fibrils, comparable to the mitotic contraction of chromosomes' (Matuszewski, 1965).

An even more extraordinary situation occurs in the metamorphosis of the ileum in *Culex pipiens* (Berger, 1938). Large larval epithelial nuclei do not show polytene chromosomes, but are filled with fine reticular threads; the 'polyneme' condition. At metamorphosis these larval cells divide, with the production of multiple complexes – at first cells containing 96 or 48 chromosomes, later 24, 12, and finally 6, the diploid number. At the end neither large cells nor multiple complexes are seen: that is, a stock of diploid cells has been retrieved from highly polyneme nuclei by somatic reduction divisions. This unique report is most interesting in that the high ploidy of the original larval cells, according to Berger, is not achieved through endomitosis, but by repeated replications in typical resting nuclei. Here, a polytene/polyneme cell cycle changes to a unique cycle of mitotic reduction to produce diploid adult cells.

**DISCUSSION**

A morphological criterion of polyteny (Swift, 1950) is perhaps only a useful one in the strictly descriptive context. The polytene chromosomes of dipteran salivary glands provide a 'natural system in which differential gene activity, and its control, can be analysed directly at the level of the genes themselves'; and while the experimental significance of this particular polytene system is the obvious and reproducible banding pattern of the homologue doubles, nevertheless in terms of function this morphological type may not be important. It is suggested that functional significance should be sought in the cell cycle which gives rise to polyteny. (It will be realized that polytene chromosomes become apparent only well after the introduction of a polytene cycle, and that this appearance may not therefore correspond temporally with the factors to which the cycle relates.)

Both polyteny and endopolyploidy arise during development in the differentiation of specific cell types, and represent end-products of that cell lineage. The metamorphosis of the midgut of *Aedes* is the only known case of polytene cells giving rise to further cell lines for redifferentiation. In other systems this is not so. For example, the polytene epidermal cells of *Calliphora* appear incapable of reactivation on wounding; the giant trophoblast cells of early mouse embryos are unable to proliferate when transplanted into a host uterus without inner cell mass (ICM), whereas minimal ICM fragments with (diploid) polar trophoblasts form a normal embryonic vesicle including numerous giant trophoblast cells (Gardner, 1972).

It has been claimed on the basis of studies of the differentiation of vertebrate cells (Holtzer, 1968) that cell division is a prerequisite for a new differentiative step by a cell. Cooke (1973), on the other hand, has clearly shown that embryonic cells of *Xenopus* can differentiate in the absence of any cell division, and moreover, do so
after experimental transplants, resulting in morphogenesis consonant with their new cell relations and with new positional value. These cells are, by definition, undetermined at the early gastrula stage when these operations were performed: the cells which are recruited into a new developmental field on the transplant of an organizer pass from a labile pluripotent state to one of determination. The evidence of experiments in pattern homeostasis in an insect segmental epidermis (Lawrence, Crick & Munro, 1972), and the determination of bristles in *Rhodnius* (Wigglesworth, 1940; Lawrence, 1966) indicates that positional evaluation and/or determination are effected only with the traverse of some particular phase of the cell cycle, and Gurdon (1969) has suggested that it is a transient breakdown of the nuclear membrane, normally associated with mitosis, which is necessary for such reprogramming in a cell lineage. It is of interest that the only known case of redifferentiation of dipteran adult tissues from polytene larval cells rather than from a separate stock of diploid imaginal cells should involve a unique prior mitotic reduction. It may be that some such process is a prerequisite of reprogramming.

While such observations may help to explain the case that polytene cells are almost always terminally differentiated, they give no indication why a polytene cell cycle should be regularized in any tissue, and in dipteran larval tissues particularly, in the first place. In many polytene systems, enormous and rapid growth is the characteristic feature of cells and tissues already functionally differentiated. In the case of Diptera, this growth habit dominates the biology of the aceanphal larva, and the extreme development of the salivary glands in particular can be correlated with a continuous feeding habit on (partially) externally digested food. In trophoblast giant cells too, an early functional differentiation as trophic intermediary tissue accords with the rapid growth of the trophoblastic vesicle through a polytene cycle. In such cases where cells are early differentiated for a specific function, and where the same function is intimately connected with a high growth rate, a continuous separation of cytoplasmic and nuclear phases in the absence of division allows uninterrupted physiological activity of the cell simultaneous with rapid continuous cycling. In the abdominal epidermal cells of *Calliphora* larvae during maximum growth, it was found that the S-phase occupied over 50% of a cell cycle lasting only 3 h (Pearson, 1974). In the case of angiosperm ovular nuclei and the nurse cells of Diptera, a functional emphasis on terminal degeneration, with the transfer of large reserves to the rapidly growing embryo or egg, demands no further morphogenetic capacities and allows the achievement of cell growth as quickly as possible through a polytene cycle.

How significantly does polyteny differ from endopolyploidy? The omission of the M-phase in a polytene cell means that interphase transcription is possible for all but the brief replication event; the evidence, however, of last-instar *Chironomus* salivary glands shows that in these cells protein synthesis is not continuously monitored at the gene level: m-RNA is long-lived. The other difference afforded by polyteny over an endopolyploid cycle is simply the reduction of the cycle time, and hence faster growth. It is therefore significant that, while polyploidy is known both among hemimetabolous (Geitler, 1953) and holometabolous (Romer, 1964) insects, polyteny is the rule among the specialized cells of larval and adult Diptera, where rapid growth is so character-
istic. Among angiosperms, where polyploidy is relatively common, polyteny is clearly
associated with the rapid development of specialized embryonic cells; and in the case
of suspensor cells it is apparently this growth itself which is their function.

The term 'polytene' was apparently first introduced by Darlington and his associ-
ates to describe the salivary gland nuclei containing giant chromosomes (Koller, 1935)
and the multistranded structure of those chromosomes (Darlington, 1937). 'Polyteny'
describes this state of chromosomal organization, but has also been applied to the cell
cycle which leads to these structures. There are other cases, however, where cell
cycles entailing replication without sign of M-phase, i.e. endoreduplication (Barlow &
Sherman, 1972), give rise to a nuclear condition in which discrete banded chromosomes
or chromosomal regions are not seen. Such nuclei have been referred to as 'polyneme',
or often, incorrectly, 'polyploid'. Since there is clearly a range of morphology in
chromosomal organization associated with the same cell cycle; and since in more than
one case this morphology is directly affected by temperature, it may be preferable to
use the term 'polytene' in its original descriptive sense – multistranded – and to refer
to such cell cycles, regardless of nuclear morphology and in contradistinction to the
endopolyploid cycle, as 'polymeric'. It would then be preferable to refer to all such
nuclei as polymeric until it is ascertained whether (a) the derivative chromonemata of
an originally mitotic chromosome remain closely associated, at least at the centromeric
regions as in trophoblast nuclei (Barlow & Sherman, 1974); that is, the polytene condi-
tion referable to single chromosomes; or (b) whether numerous independent and
uncondensed chromosomal threads properly constitute a polyneme condition, as in
larval cells of Aedes midgut. It is remarkable, however, in both the case of Calliphora
nurse cells (Bier, 1957) and Aedes midgut (Berger, 1938), that cells which have
omitted an M-phase are yet capable of changing their cycle by the reintroduction of
M-phase (though only partially in the former case). These instances suggest that
polymeric cycles do not become established merely by an accidental loss of ability
to undergo mitosis, but are under active control; and it is this suggestion which both
introduces and underlies the question of the functional significance of polyteny, and
of the polytene, or polymeric, cell cycle.

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Figs. 2–4. These illustrate the range of chromosomal morphology observed in squash preparations of third instar epidermal cells of Calliphora erythrocephala.

Fig. 2. Polytene chromosomes are evident, with characteristic pattern of bands and interbands, although separate chromosomes cannot be followed throughout the preparation.

Fig. 3. There is a greater breakdown of chromosomes into separate homologues and oligotene fibrils.

Fig. 4. A reticular oligotene nucleus contains short banded polytene regions (arrowed).

Figs. 5–7. Loss of polytene banding and the transition from polytene to oligotene organization of chromosomes. Polytene regions (arrows) pass into more diffuse, fibrillar oligotene organization.

Fig. 8. The nuclear pattern resulting from this disintegration of polytene chromosomes in a squash preparation.

Fig. 9. Similar pattern in a squash of Schistocerca oenocytes. Banded polytene chromatin regions are arrowed.
Figs. 10–13. Squash preparations of mouse giant trophoblast cells showing reticular chromatin and chromatin centres. Short banded regions, often associated with chromocentres, are arrowed.

Figs. 14, 15. Whole squash preparations of oenocytes from mid-fifth-instar Schistocerca. Although less distinctly, the same nuclear organization of short-banded polytene chromosomal regions (arrows) separated by chromatin fibrils which give a reticular pattern in squashes, is apparent as in Calliphora epidermal nuclei. Estimates of nuclear volume indicate 5 or 6 cycles of endoreduplication which have resulted in this condition, whereas the larger nuclei in Calliphora epidermis have undergone 7 such cycles (Pearson, 1974).
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Fig. 16. *Schistocerca* oenocyte nucleus of a low level of endoreduplication (20 \( \mu m \) diameter), showing evidence of polytene chromosomes in the intact nucleus.

Figs. 17-20. Squash preparations showing banded chromosomes in the nuclei of *Schistocerca* oenocytes. The chromosomes dissociate into constituent fibrillar chromatin and reassociate in reticular fashion. In Fig. 19, banded chromosomes apparently converge upon a chromocentre (ch).
Functional significance of polyteny