males. This fact could be associated with the important role of males wing vibration during courtship (Koref-Santibañez, 1963). The same trait was shown to be the target of sexual selection in *D. melanogaster* (Partridge *et al.*, 1987). Also, the differences in head width were marginally significant, in which case the results agree with those obtained in the cactophilic species *D. buzzatii* (Norry *et al.*, 1995). These authors propose that this trait is associated with the size of the probosis and in turn with the capacity to transfer drops of yeast during courtship.

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Polytenization rate of some heterochromatic sequences in different tissues of *D. melanogaster otu*^{11} mutants.

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Introduction

The bulk of *D. melanogaster* heterochromatin consists of satellite DNA sequences (Bonaccorsi and Lohe, 1991; Lohe *et al.*, 1993). Transposable elements are also a constant component of mitotic heterochromatin (Pimpinelli *et al.*, 1995). Other repeated sequences from telomeres such as the He-T (Traverse and Pardue, 1989) and the Telomere-Associated Sequence (TAS) (Karpen and Spradling, 1992; Zhang and Spradling, 1995) have been found in heterochromatin as well. Furthermore, there are a number of vital genes, similar to "typical" euchromatic ones, in heterochromatin of the second (Hilliker, 1976) and the third chromosomes (Marchant and Holm, 1988). In the salivary gland (SG) polytene chromosomes of *Drosophila* larvae, the pericentric heterochromatin is strongly underpolytenized (Mulder *et al.*, 1968; Glaser *et al.*, 1992), but some unique and some repeated sequences form polytenized "islands" (Devlin *et al.*, 1990b; Le *et al.*, 1995; Zhang and Spradling, 1995).

It has been shown that true polytene chromosomes can appear in pseudonurse cells (PNCs) of *D. melanogaster otu* mutants (King *et al.*, 1981). Some parts of pericentric heterochromatin which do not polytenize in SG chromosomes, do polytenize in PNC chromosomes developing a specific morphological pattern which can be identified in every individual chromosome arm (Mal'ceva and Zhimulev, 1993).

Here we determined the level of polytenization sequences from various regions of pericentric heterochromatin in SG and PNCs polytene tissues.

Materials and Methods

Drosophila stocks: Fly stocks used are listed in Table 1.

Dot-hybridization analysis: Isolation and purification of genomic DNA were carried out by the standard methods (Sambrook *et al.*, 1989). For DNA isolation PNCs were manually purified from diploid oocyte and follicular cells covering egg chambers. DNA was isolated from heads and of adult flies and from 9-10 stages PNCs (see King, 1970), and from salivary glands of larvae was isolated. Both larvae and adults were homozygous for the *otu*^{11} allele. The salivary glands were isolated from third instar larvae and ovaries were isolated from four-days adult females, grown at 16°C.

The nylon filters for dot-hybridization were prepared and hybridized with ^{32}P labeled probe according to the procedure described by Kafatos *et al.* (1979). In the first dots of each filter, equal amount of DNA of every tissue were loaded. For the next dots, the amount of DNA was reduced to only 50% of the previous one. Hybridization intensity was measured on a liquid scintillation counter (1209 Rackbeta).

Southern-hybridization analysis: Genomic DNA was hydrolized by *Hin*dIII. The clones (Table 1) were labeled with α^{32}-P and hybridized after Sambrook *et al.* (1989). Two (*pltc35* and *rosy*) clones were hybridized simultaneously. For the hybridization signals to be of the same intensity, the specific activity of each probe was selected on an individual basis depending on which size and copy number in the genome. Hybridization intensity was measured on a spectrophotometer Hitachi 557. DNA representation in the clone under study was calculated as a ratio of hybridization intensity in salivary glands to that in adult heads after normalization on *rosy*.

Results

To determine level of polytenization, we used four sequences from different regions of pericentric heterochromatin. These sequences are located in mitotic chromosomes: 359bp satellite DNA in h31-32 region, AAGAC satellite DNA in h44 region and Y chromosome, *Rsp* repeat in h39 region, and cDNA clone of the *light* gene, localized in h35 region.

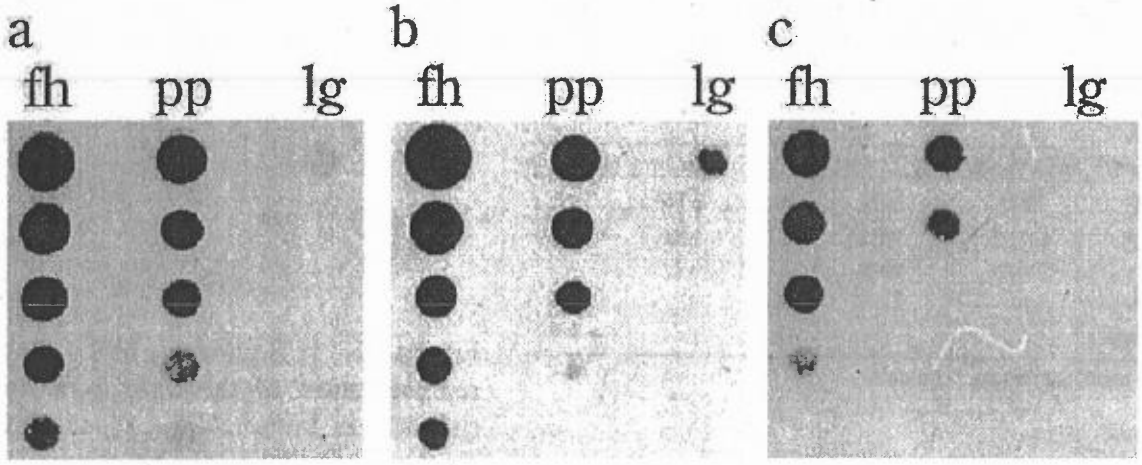

Figure 1. Dot-hybridization of *Rsp* DNA (H_0 clone) (a), 359bp satellite (b), and of AAGAC satellite DNA (1.686-198 clone) (c), with total DNA from fly heads (fh), purified PNCs (pp) and larval SGs (lg).

Table 1. Stocks and DNA clones used.

Stocks	Characteristics	Source
otu^{11}	Initial stock	R.C. King
sn^{3} *otu*^{11} / *FM3*	(1)	N.I. Mal'ceva
DNA clones	**Characteristics**	**Source**
pltc35	cDNA clone containing the *lt* gene, localized in h35 region (2,3)	B. Wakimoto
H_o	*Rsp* repeat, localized in h39 region (4,5)	T. Lyttle
1.686—198	AAGAC satellite DNA, located in h44 region and Y chromosome (6,7)	A. Lohe
aDm23-24	359 bp satellite DNA, located in h31-32 region (6,7)	A. Lohe
rosy	4.2 *EcoRI/HindIII* fragment of rosy gene (8)	A. Spradling

(1) Mal'ceva and Zhimulev 1993. (2) Dimitri 1991. (3) Devlin et al. 1990a. (4) Wu et al. 1988. (5) Pimpinelli and Dimitri 1989. (6) Lohe and Brulag 1986. (7) Lohe et al. 1993. (8) Rubin, Spradling 1986.

Table 2. Representation of 359bp satellite sequences in larval salivary gland cells and PNCs compared to that in adult head cells.

DNA amount	DNA Source				
	Adult heads	PNCs		Salivary glands	
(µg)	Cpm	Cpm	(%)	Cpm	(%)
1.35	40824	10202	25	1610	4
0.67	18048	5470	30	*	-
0.34	7358	2526	34	*	-
0.16	3246	1238	36	*	-
Average			31		4

Cpm – count per minute. *- no data

Table 3. Representation of AAGAC satellite sequences in larval salivary gland cells and PNCs compared to that in adult head cells.

DNA amount	DNA Source				
	Adult heads	PNCs		Salivary glands	
(µg)	Cpm	Cpm	(%)	Cpm	(%)
1.23	16123	7996	49	60	>0.5
0.61	10479	3998	38	*	-
0.31	6497	1799	28	*	-
Average			38		> 0.5

Cpm – count per minute. *- no data

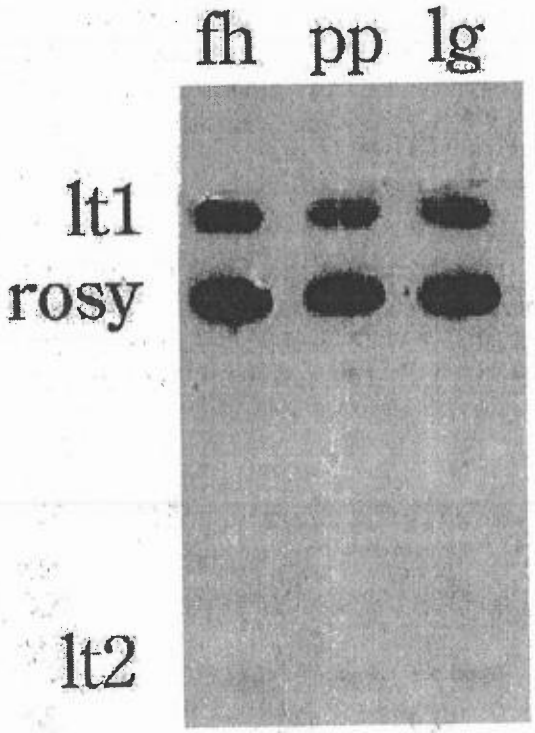

Figure 2. Southern blot analysis of representation of the *light* gene sequences (lt1, lt2) in larval SGs (lg) and PNCs (pp) compared to that in fly head cells (fh). The blot was hybridized with two probes containing DNA *light* (pltc35), and the *rosy* gene was used as a standard.

Table 4. Representation of *Rsp* repeated sequences in larval salivary gland cells and PNCs compared to that in adult head cells.

DNA amount (μg)	DNA Source: Adult heads Cpm	PNCs Cpm	PNCs (%)	Salivary glands Cpm	Salivary glands (%)
1.35	45677	16205	35	850	2
0.67	23420	9425	40	420	2
0.34	11434	4948	43	*	-
0.16	5400	2099	38	*	-
0.08	2912	987	35	*	-
Average			38		2

Cpm – count per minute. *- no data

Table 5. Representation of the *light* gene sequences in larval salivary gland cells and PNCs compared to that in adult head cells.

DNA source	Ratio of DNA quantities (%): *light (lt1) / rosy*	*light(lt2) / rosy*
Adult heads	0.67 (100)	0.13 (100)
PNCs	0.65 (100)	0.13 (100)
Salivary glands	0.63 (94)	0.13 (100)

The comparison between different tissues for the relative amount of repetitive sequences is shown in Figure 1. Quantitative data of hybridization are shown in Tables 2, 3 and 4. The rate of hybridization of these probes with genomic DNA from purified PNCs is close to that observed with mainly diploid DNA from fly heads and dramatically higher than that with DNA from SG cells.

In contrast, unique DNA sequence of the *light* gene mapped in polytene chromosomes of SG cells in eu-heterochromatin transition region 40E-F (Devlin *et al.*, 1990a) is fully represented in both polytene tissues (Figure 2; Table 5).

Data obtained showed that repetitive sequences are polytenized from 6 to 30 times more intensively in PNCs in comparison with salivary gland cells. These results coincide well with the data on replication patterns of heterochromatic sequences in normal nurse cells. It was found that, in the first four endocycles of normal oogenesis, heterochromatic DNA in these cells replicated completely (Dej and Spradling, 1995).

In contrast in salivary gland polytene chromosomes, most of the heterochromatic sequences were not replicated in the first cycle of polyenization in embryogenesis (Smith and Orr-Weaver, 1991).

These facts indicate that the degree of polytenization in the heterochromatic parts of chromosomes depends on type of tissue.

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Flight ability of the newly isolated three indirect flight muscle mutations in *Drosophila*.

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Abstract: Indirect flight muscle development is not well understood in *Drosophila*. We have generated a number of mutations to understand the genes involved in indirect flight muscle development. Here we report the flight ability of three new mutations. The analysis revealed that the drooping wing flies are either flightless or weak fliers with maximum sterility.

Introduction

The investigation of genes discovered by their homology to *Drosophila* genes is now one of the most successful approaches to understand the genetic control of vertebrate development. However, mechanisms in the fly are in several instances likely to be unique to this specialized organism (Nusslien-Volhard, 1996). In the fruit fly, *D. melanogaster*, muscle development takes place twice – in the embryo during the formation of the muscles of the larva and during pupal development when adult muscles are made (Bate, 1990; Fernandes *et al.*, 1991; Reedy and Beall, 1993; Baylies *et al.*, 1998). Muscle development in the embryo involves specification of the mesoderm (Thisse *et al.*, 1988; Azpiazu *et al.*, 1996; Riechmann *et al.*, 1997), the choice of a muscle founder cell (Bate, 1990; Ruchton *et al.*, 1995), and the consequent fusion of myoblasts to form a fibre which attaches to specific sites on the epidermis and gets correctly innervated (Bate, 1990; Broadie and Bate, 1993). During embryonic myogenesis, signals from the ectoderm and the mesoderm result in the selection and specification of a muscle progenitor cell (Carmena *et al.*, 1998) which divide asymmetrically to give rise to two daughter cells, one of which becomes an adult muscle progenitor (Baylies *et al.*, 1998). In the thoracic and head segments, these progenitor myoblasts associate with imaginal disc cells and proliferate during larval life (Poodry, 1980; Lawrence and Brower, 1982; Fernandes *et al.*, 1991; Fernandes and Vijay Raghavan, 1993; Roy and Vijay Roghavan, 1997, 1998).

The flight of insects such as *Drosophila* is powered by relatively large, striated indirect flight muscles (IFMs) that are similar in structure to vertebrate skeletal muscle (Sparrow, 1995). The IFMs, which are a bulk of muscle mass in the mesothorax, are divisible into sub-sets: the dorsal longitudinal muscle (DLMs) and dorso-ventral muscles (DVMs) which are structurally, physiologically, and biochemically identical. These two groups of IFMs have distinct development histories: the DLMs develop by fusion of disc myoblasts with the larval scaffold which serves as a template during metamorphosis while the DVMs develop *de novo* fusion of the imaginal disc myoblasts (Bate, 1993; Anant *et al.*, 1998).