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Characterization of a region of the X chromosome of *Drosophila* including *multi sex combs* (*mxc*), a *Polycomb* group gene which also functions as a tumour suppressor

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Abstract Genetic analysis of the 8D3;8D8-9 segment of the *Drosophila melanogaster* X chromosome has assigned seven complementation groups to this region, three of which are new. A *Polycomb* group (*Pc-G*) gene, *multi sex combs* (*mxc*), is characterized and mutant alleles are described. Besides common homeotic transformations characteristic of *Pc-G* mutants that mimic the ectopic gain of function of *BX-C* and *ANT-C* genes, *mxc* mutants show other phenotypes: they zygotically mimic, in males and females, the characteristic lack of germ line seen in progeny of some maternal effect mutants of the so-called posterior group (the grandchildless phenotype). Loss of normal *mxc* function can promote uncontrolled malignant growth which indicates a possible relationship between *Pc-G* genes and tumour suppressor genes. We propose that gain-of-function of genes normally repressed by the wild-type *mxc* product could, in *mxc* mutants, give rise to an incoherent signal which would be devoid of meaning in normal development. Such a signal could divert somatic and germ line developmental pathways, provoke the loss of cell affinities, but allow or promote growth.

Key words *Drosophila melanogaster*
Polycomb group genes · Tumour suppressor genes
Homeosis

Introduction

Botas (1985) has described a single allele of a *Pc-G* gene named *multi sex combs* (*mxc*). To characterize this locus further, we localized *mxc* to $Df(1)lz^{10-70d}$ and carried out a search for lethal mutations uncovered by this deficiency. This allowed us to isolate three new complementation groups required during development, and to localize *amx* within $Df(1)lz^{10-70d}$. Four new *mxc* alleles were also obtained. The group of genes known as the *Polycomb* group (*Pc-G*; Jürgens 1985) is required in most segments of *Drosophila* for correct differentiation (Paro 1990). Mutations in these loci cause pleiotropic homeotic transformations that mimic gain-of-function mutations of selector genes, especially, but not exclusively, those of the *bithorax* and *Antennapedia* gene complexes (*BX-C* and *ANT-C*). After an initially correct expression pattern, transcripts of selector genes are ectopically expressed in *Pc-G* mutants (Dura and Ingham 1988; Busturia and Morata 1988; McKeon and Brock 1991; Simon et al. 1992), suggesting that normal *Pc-G* products are needed to maintain the repressed state of the genes they regulate. Paro and Hogness (1991) proposed that the *Pc-G* genes cooperate to organize the chromatin structure of their targets in such a way that these genes are maintained in a repressed state throughout development, a phenomenon functionally similar to imprinting (Paro 1990). Alternatively, *Pc-G* genes could leave the chromatin fibre unaltered, but help assign the genes to be repressed to a nuclear compartment, to which not all transcription factors have access (Schlossherr et al. 1994). The products of two *Pc-G* genes, *polyhomeotic* (*ph*) and *Polycomb* (*Pc*), have been shown to associate in a large protein complex (Franke et al. 1992). PC and PH proteins bind to the same set of about 100 sites on polytene chromosomes (Zink and Paro 1989; De Camillis et al. 1992; Franke et al. 1992). The product of another *Pc-G* gene, *Posterior sex combs*, *Psc*, and that of *Suppressor 2 of zeste*, $Su(z)^2$, share many binding sites on polytene chromosomes with PH and PC (Martin and Adler 1993). All these *Pc-G* gene products share chromosomal binding sites with the product of *zeste*, another gene involved in regulation of *BX-C* and *ANT-C* (Rastelli et al. 1993). As the selector genes downstream of *Pc-G* genes are autonomously required through development, the developmental identity of a cell at any moment is given by the combination of selector genes that are acti-

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vated or repressed within this cell, corresponding to chromatin segments that are open or closed at that specific moment. Maintenance of the determined state is accordingly as important as its initiation. Here we characterize the *Pc-G* gene *multi sex combs* (*mxc*) and its adjacent complementation groups. The phenotypes of *mxc* mutant alleles, some common to other *Pc-G* mutants, others specific to this locus, could lead to a better understanding of the *Pc-G* group. As many selector genes have been conserved through evolution, and maintenance of the determined state is a process required in every organism undergoing development, knowledge gained from the study of *Pc-G* in *Drosophila* will probably be of general interest.

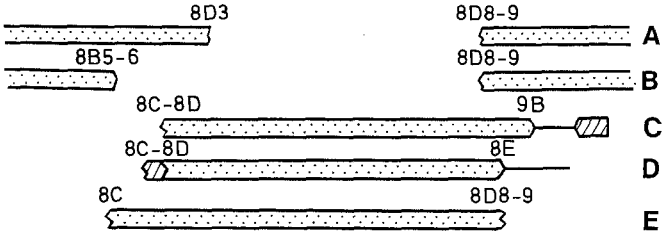

Fig. 1 Map of chromosomal rearrangements affecting the *mxc* region. Deficiencies and duplications uncovering *mxc* are represented. *Dotted bars* indicate material in the 8 region on the rearranged chromosomes; *diagonally striped bars* correspond to material from the tip of chromosome X. (*A*: $Df(1)lz^{10-70d}$, *B*: $Df(1)lz^{90b-24}$, *C*: $Dp(1;Y)FF1,y^+ac^+$, *D*: $Dp(1;Y)lz^+dr^-$, *E*: $Dp(1;4)A17$)

Materials and methods

Stocks

Deficiency $Df(1)lz^{10-70d}$ was induced by Green and Lefevre (1972). It has been named $Df(1)^{10-70d}$ and $Df(1)lz^{10-5}$ in different stock lists. Bands 8D3 to 8D8-9 are missing (Green and Lefevre 1972). Besides *lozenge* (*lz*), $Df(1)^{10-70d}$ includes at least six other genes (this work). $Df(1)lz^{90b-24}$ was generated as an X-ray-induced *lz* allele by M. M. Green, and determined to be a deletion of 8B5-8;8D8-9 by Drisdale et al. (1993). $Dp(1;Y)z^+dvr^-$, which rescues the lethality of $Df(1)lz^{10-70d}$ but not that of $Df(1)^{90b-24}$, was obtained from the University of California at Davies. It is probably $Dp(1;Y)y^+lz^+$ of Schalet (Lindsley and Zimm 1992) that has lost y^+ (but is still ac^+). *Dp(1;Y)FF1* was induced in our laboratory (by M. Masson) by irradiating *Dp(1;Y)B168* of J. Merriam (UC, Los Angeles). It behaves like $Dp(1;Y)lz^+$ but is usefully marked with y^+ac^+ (see Fig. 1). *Dp(1;4)A17* was obtained from E. Gateff (Mainz). *Dsor* mutants were provided by Y. Nishida. Other mutants and strains are described in Lindsley and Zimm (1992).

Mutagenesis

The $y^1ac^1z^1$ strain was used for mutagenesis. Ethyl methane sulfonate (EMS) mutagenesis was carried out following the procedure of Lewis and Bacher (1968). Mutagenized $y^1ac^1z^1$/*Dp(1;Y)FF1* males were crossed with virgin *C(1)DX*, *y w f*/*Dp(1;Y)FF1* females. In the next generation, individual $y^1ac^1z^1$/*Dp(1;Y)FF1* males were crossed with $Df(1)lz^{10-70d}$, z^1sn^3/*FM7c* females, and their progeny was scored for the absence or sublethality of $Df(1)lz^{10-70d}$, $z^1sn^3/y^1ac^1z^1$ females. Mutant stocks were established from $y^1ac^1z^1$/*FM7c* siblings crossed with *FM7c*/*Dp(1;Y)FF1* males.

Gynandromorphs

Ring chromosome *R(1)2* was used in attempts to induce mosaic gynandromorphs for all lethal mutants.

Results

Screening

A search for mutants in $Df(1)lz^{10-70d}$ was carried out as described in Materials and methods. In all, 5200 chromosomes were checked for the presence of lethals over $Df(1)lz^{10-70d}$. Besides *mxc* and the other complementation groups described below, three mutants appeared which were lethal over $Df(1)lz^{10-70d}$ and dominant sterile in more than 40 sibling *FM7c*/* heterozygous females. Thus, these latter were lost. This points to the existence of a locus, which may be included among the complementation groups described below, that can mutate to produce dominant female sterility. $Df(1)lz^{10-70d}$ includes the following complementation groups:

1. *almondex* (*amx*). We found that *amx*, localized in the 8D region (Lindsley and Zimm 1992), is included in $Df(1)lz^{10-70d}$. Embryos from $Df(1)lz^{10-70d}/amx^1$ females die and show the hypertrophy of the central nervous system characteristic of amx^1/amx^1 progeny (results not shown). amx^+ is included in $Dp(1;Y)lz^+dvr^-$ and in *Dp(1;Y)FF1*, as $amx^1/amx^1/Dp(1;Y)FF1$ females are fertile and give normal adult progeny. All mutants obtained in the present screen complement amx^1, an X-ray-induced allele.
2. *abortex* (*atx*). A new maternally required gene for which only one allele was obtained. The homozygous and hemizygous flies are viable. Progeny of atx^{G1}/atx^{G1} females die after germ band retraction. Embryos lack ventral epidermis. The mutant phenotype can be partially rescued (2%) by wild-type sperm. This mutant, atx^{G1}, is in many aspects similar to $almondex^1$ but complements it.
3. *lozenge* (*lz*). Four new alleles were found. Two are lethal over $Df(1)lz^{10-70d}$, while two others allow escapers to live. None of the four complement *spectacle*, *lozenge*, *glossy* or *K* mutations but they all complement lz^{50e}. All flies have reduced antennae. The eyes are rough and reduced. Claws are absent or small. The adults show reduced fertility, although the two spermathecae and parovaria (female accessory glands) are present.
4. *Downstream of raf 1* (*Dsor1*). This locus has been described and cloned by Tsuda et al. (1993) as a ***d***own**s**tream **o**f **r**af suppressor. *Dsor* encodes a MAP kinase kinase. Eight *Dsor* alleles obtained by the Nishida group rescue weak *D-raf* mutations. We obtained six new *Dsor1* alleles. Three were lethal in males and over $Df(1)lz^{10-70d}$ or $Dsor1^{r1}$. The other three were hypomorphs: they gave rare male escapers or *Dsor1*/

Table 1 Characteristics of mutant *mxc* alleles. Homeotic transformations were rated in adult mutant males and females; for semi-viable alleles, phenotype descriptions are based on analysis of dissected pharate adults

Allele	Viability	Fertility	Suppression of *zeste*[1]	Tumorigen	Homeotic transformations					
					Dfd-like[a]	T2 to T1[b]	L2 to L1[c]	L3 to L1[d]	W to H[e]	A4 to A5[f]
mxc^{G9}	Viable	Reduced over $Df(1)lz^{10-70d}$								
mxc^{M1}	Viable	Reduced			A few eyes slightly reduced	3%	16%	1%		55%
mxc^{G46}	Viable	Very reduced	Yes		27%	29%	29%	3%		80%
mxc^{G43}	Semi-lethal	Larvae have atrophic gonads adults devoid of germ line	Yes	Occasional floating tumours	55%	40%	65%	20%	Yes	Yes
mbn^{1} mbn^{SO}	Lethal in third instar larvae, very rare pharate adults	*mbn*/G46 and *mbn*/G43 females devoid of germ line		Invasive blood cell tumours in larvae	Yes	Yes	Yes	Yes	Yes	Yes
mxc^{G48}	Lethal in egg-first instar larvae		z^{1} is suppressed in G46/G48 females	Most G48/*mbn* die in third instar with blood cell tumours						

[a] *Dfd*-like: mutant eye phenotype similar to gain of function of Dfd^{D}
[b] T2 to T1: presence of first tarsus-specific sex combs on second tarsus of prothoracic legs
[c] L2 to L1: presence of first leg-specific sex combs on second leg
[d] L3 to L1: presence of first leg-specific sex combs on third leg
[e] W to H: partial transformation of wing into haltere
[f] A4 to A5: partial transformation of abdominal segment A4 towards abdominal segment A5

Df(1)lz^{10-70d} females. The strongest alleles are not viable in gynandromorphs.

5. *rotated genitalia* (*rtg*). Among three alleles of this new gene, two hypomorphs showed some escapers or died as pharate adults. In these two mutants, the normal morphogenetic rotation of the genitalia is prevented, and sixth tergite malformations were occasionally observed. The lethal allele survives in gynandromorphs, but shows poor cuticular differentiation and swollen aristae.

6. *elongatus* (*elg*). Two alleles were obtained for this new complementation group. Most imagoes die as very elongated pupae due to lack of retraction of the third instar larvae at pupariation. Mosaics in gynandromorphs survive and show weak abnormal pigmentation.

7. *multi sex combs* (*mxc*). The first allele of this group, *mxc*^{M1}, was discovered by Botas and Garcia Bellido (Botas 1985), and identified by them as a *Pc-G* gene. We localized it by recombination to be less than 1 cM from *lz*. When checked over deficiencies of the region we found that *Df(1)lz*$^{10-70d}$/*mxc*^{M1} females were viable but sterile. Both *Dp(1;Y)lz*$^{+}$ and *Dp(1;Y)FF1*, which cover the lethality of *Df(1)lz*$^{10-70d}$ (see Fig. 1) also rescue the homeotic transformation phenotypes of *mxc*^{M1} males . Four new *mxc* alleles were isolated in this screen: *mxc*^{G9}, *mxc*^{G43}, *mxc*^{G46} and *mxc*^{G48}. In addition to this, we found that two other mutant alleles could be assigned to the *mxc* complementation group: *lethal (1) malignant blood neoplasm* (*l(1)mbn*^{1} and *l(1)mbn*^{SO}) alleles isolated by Gateff (1974) turned out to be alleles of *mxc*. The two alleles will be referred to as *mxc*^{mbn1} and *mxc*^{mbnSO}. The order of severity of all seven alleles is *mxc*^{G9}, *mxc*^{M1}, *mxc*^{G46}, *mxc*^{G43}, *mxc*^{mbnSO}, *mxc*^{mbn1} and *mxc*^{G48} (Table 1). The three last are lethals: both *mxc*^{mbn} alleles in third instar larvae, and *mxc*^{G48} in the embryo-first larval instar. *mxc*^{G48} is probably an amorph allele. *mxc*^{G43} is semi-lethal, the others are viable. The study of these seven alleles (from here on named by their exponent) allowed an extensive characterization of *mxc* (see Table 1).

Homeotic transformations and interaction of *mxc* with *zeste* mutants

Adult *mxc* flies show homeotic transformations like those observed in other *Pc-G* mutants (Table 1). All homeotic transformations are patchy with variable penetrance. In hemizygous M1, G46 or G43 mutant males, the second and third legs are partially transformed into the first (Table 1, Fig. 2B), and the fourth abdominal segment is partially transformed into the fifth. The third segment of the antenna can be reduced (100% in G43). The wings are sometimes scalloped, or transformed towards halteres (85% in G43). In addition, M1, G46 and G43 occasionally show a reduction of the eye often coupled with the appearance of extra bristles (Fig. 3). An identical phenotype has been described by McGinnis et al. (1990) for a gain-of-function allele of *Deformed* (*Dfd*^{D}) and interpreted as a homeotic transformation of the eye towards rostral membrane. As in some other *Pc-G* mutant males (*Pc, ph, polycombeotic*), an additional sex comb sometimes differentiates on the second tarsus of the first leg of M1, G46 and G43 (see Fig. 2A). These phenotypes are enhanced by other *Pc-G* mutants: most *trans*-heterozygous males of *mxc*^{G46} with *Psc*, *Pc*, or *Polycomblike* die as pharate adults with extreme homeotic transformations (100% of second and third legs transformed toward first), while *trans*-heterozygotes for M1 and *Pc* or *Pcl* are also very transformed, but viable. Loss of function of *ANT-C* and *BX-C* genes can modify the mutant *mxc* phenotypes. For instance, *mxc*^{G43};*Scr*^{4}/+ males are viable and do not show extra sex combs in the second tarsus of the first leg.

Some alleles (G43 and G46) can act as recessive suppressors of *zeste*^{1} eye colour which becomes orange-maroon. When observed, this suppression is mainly restricted to the most posterior-ventral part of the eye (Fig. 4).

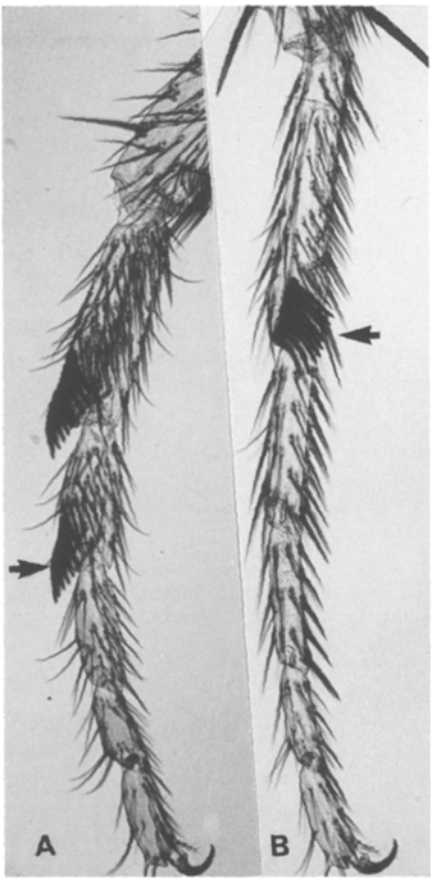

Fig. 2A, B Homeotic transformation of legs observed in *mxc* mutant males. **A** Prothoracic leg of *mxc*^{43} male that carries an additional sex comb on the second tarsus (*arrow*). **B** *Arrow* points to first tarsus of mesothoracic leg of an *mxc*^{43} male that presents a first leg-specific sex comb, as seen in *ANT-C* gene *Src* gain-of-function mutants

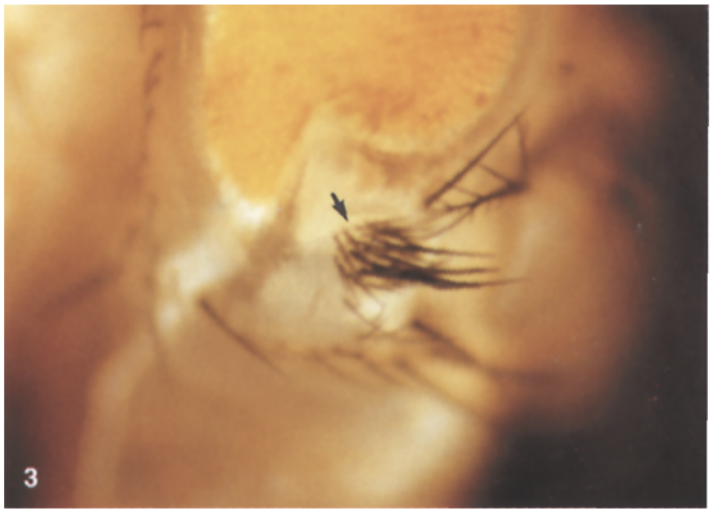

Fig. 3 Head phenotype of mutant *mxc* flies. Head of homozygous mxc^{G43} female showing a reduced eye, and presence of additional bristles (*arrow*)

Fig. 4 Suppression of z^1 by *mxc*. Eye of a $z^1mxc^{G43}/z^1mxc^{G43}$ female showing the basic yellow eye colour, and the patchy maroon-orange spots in the posterior-ventral region, typical for the suppression of z^1 by mcx^{G43}

Sterility of *mxc* mutants

The fertility of males and females of all *mxc* mutants is affected. All eggs laid by $Df(1)lz^{10-70d}/+$ or $mxc^{G48}/+$ females differentiate pole cells. During gastrulation, these cells are carried into the posterior midgut primordium as usual. From this point onwards, every step of gonadogenesis and gametogenesis can be affected depending on the mutant combination (see Table 1). M1 or G46 males are almost devoid of motile sperm, and less than 0.5% give progeny. Larvae of G43, mbn^1 or mbn^{SO} have atrophic gonads of half the normal size, especially conspicuous in male larvae. Rare male escapers of G43 have only the somatic component of the gonad, devoid of germ line (Fig. 5D). This male gonad is rudimentary and identical to that of the male offspring of females homozygous for the thermosensitive osk^{301} allele of the posterior group gene *oskar*, whose product is maternally required for pole cell formation (Lehmann and Nüsslein-Volhard 1986).

Females G46/*mbn* or G43/*mbn*, or homozygous for M1, G46 or G43 are sterile. Egg chambers with 16 cells are formed in M1/M1 females. Most degenerate before egg-laying. Most G46 and all G43, G46/*mbn* and G43/*mbn* females have extremely reduced ovaries, devoid of germ line and identical to those of osk^{301}/osk^{301} offspring (Fig. 5B). Females G9/G48 show reduced fertility. To induce germ line clones of homozygous mutant cells, females heterozygous for mxc^{M1}, mxc^{G43}, or mxc^{G48}; and the dominant female-sterile ovo^{D1} mutation were irradiated as first instar larvae (Docquier et al., in preparation). They never produced *mxc* mutant offspring.

Neoplasic effects of *mxc* mutants

We checked allelism with known mutants from the 8D region and found that *l(1) malignant blood neoplasm*, *l(1)mbn* (Gateff 1974) is a *mxc* allele, which is lethal over $Df(1)lz^{10-70d}$ and over mxc^{G48}. It is covered by the duplications that cover *mxc*, and complements mutations of all other loci in $Df(1)lz^{10-70d}$. The two alleles of *l(1)mbn*, $l(1)mbn^1$ and $l(1)mbn^{SO}$, have been extensively studied (Gateff 1974, 1978, 1982; Sherestha and Gateff 1982; Gateff and Mechler 1989). These authors showed that differentiated blood cells are abnormally present in the hematopoietic organs which are enlarged. The hemocytes increase in number by a factor of 15 to 20, and the plasmatocytes seem unable to recognize "self" from "non-self". They encapsulate and invade many larval tissues. Plasmatocytes transplanted into wild-type larvae kill the host. We found that $l(1)mbn^{SO}$ individuals can very occasionally survive to pharate adulthood, and then show the same homeotic leg transformations as those described for other *mxc* mutants. $l(1)mbn^1$ and $l(1)mbn^{SO}$ are both viable over mxc^{G46} or mxc^{M1} but semi-lethal over mxc^{G43}. All these females are sterile and devoid of germ line. Females $G48/mbn^{SO}$ die as pharate adults, present the Dfd^D phenotype, and lack most tergites. mxc^{G43} shares the hemocyte characteristics of $l(1)mbn^1$ and $l(1)mbn^{SO}$ but to a lesser extent. Heterozygous G43/$l(1)mbn^1$ or G48/$l(1)mbn^1$ larvae have much enlarged hematopoietic organs and the characteristic encapsulation and melanization of various larval organs. The few surviving G43/*mbn* females are short lived. They die with floating melanotic aggregates of cells in various parts of the body.

Discussion

We have described seven complementation groups included in the chromosomal region 8D3;8D8-9. Those previously known were: *almondex* (*amx*), *Downstream of*

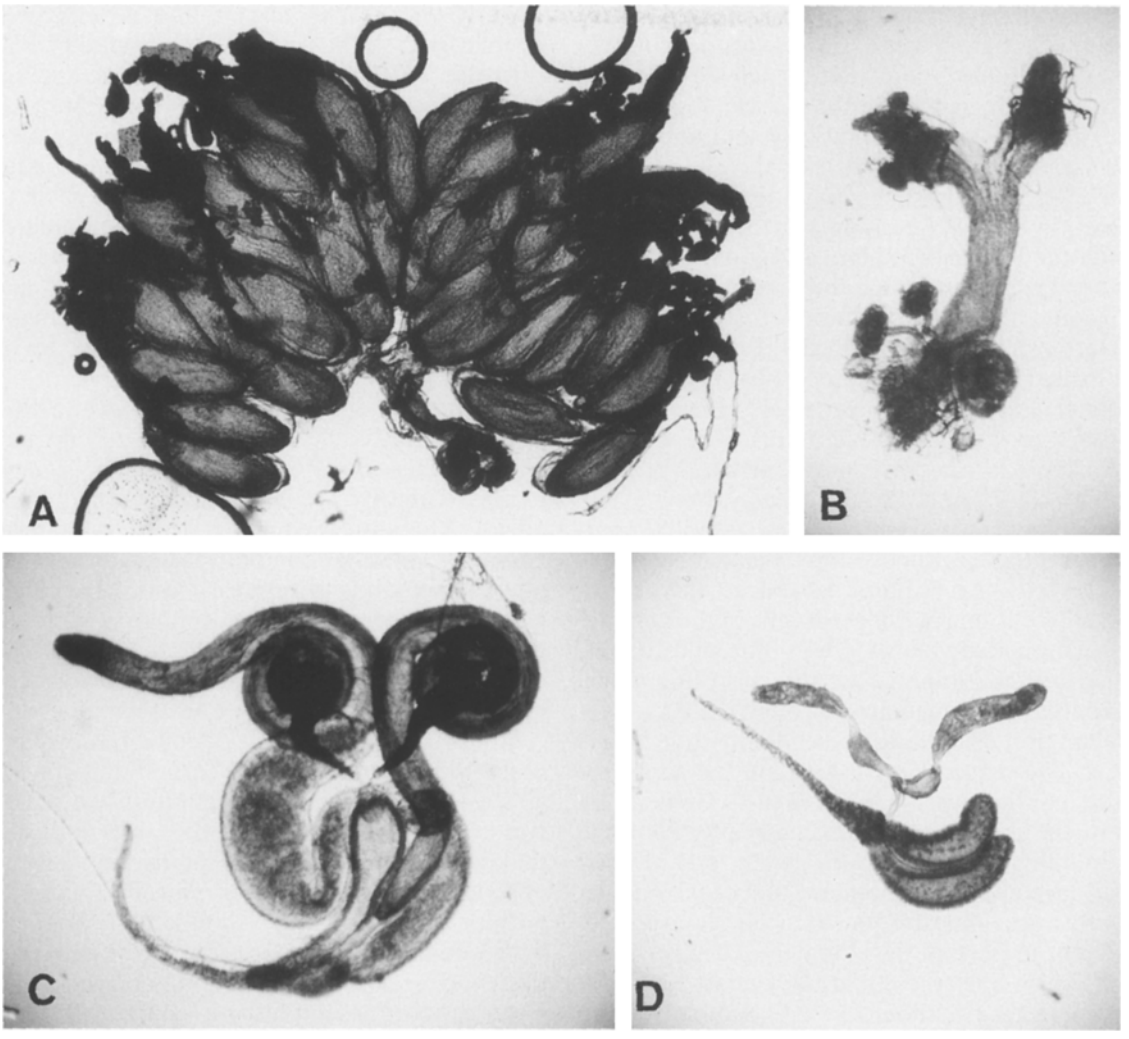

Fig. 5A–D Gonads from *mxc* mutant adult flies. **A** Ovaries from a wild-type female. **B** Strongly reduced, rudimentary ovaries from an mxc^{G46}/mxc^{G46} female. **C** Testis from a wild-type male. **D** Testis from a G43 mutant male

raf 1 (*Dsor1*), *lozenge* (*lz*) and *multi sex combs* (*mxc*). The new ones are *abortex* (*atx*), *rotated genitalia* (*rtg*) and *elongatus* (*elg*).

We did not find any new alleles of *amx*, probably because fertility was not systematically checked in the screening. Most of the normal product of the *amx* gene is maternally provided and zygotically required (Shannon 1973; Lehmann et al. 1983; Germeraad and Disano 1984; LaBonne and Mahowald 1985). Heterozygous $amx^1/+$ mothers can provide enough wild-type product to cover the hemizygous amx^1 mutant zygotic requirement. Thus any amorphic mutant induced in this screen would probably have been maternally rescued. The mutation amx^1 does not seem to be an amorph, as progeny of $Df(1)lz^{10-70d}/amx^1$ females cannot be rescued by the father, while amx^1/amx^1 progeny can. The reduction of the eye and the sterility of amx^1 are complemented by all other mutants uncovered by $Df(1)lz^{10-70d}$.

Some alleles of *lozenge* produce phenotypes superficially similar to those of *mxc* mutants: they can reduce the size of the eyes, cause sterility and affect the legs. Yet *lz* alleles are complemented by all *mxc* alleles. The presence of three spermathecae in some *lz* mutants is similar to a transformation described for two *Pc-G* gene mutants: *Polycomblike* and *pleiohomeotic* (Duncan 1982; Girton and Jeon 1994). The presence of three spermathecae is a plesiomorphic character of most Muscomorpha, whilst the Syringogastridae have four (McAlpine 1988). Throckmorton (1962) suggested that this could be considered as a homeotic transformation. We have never observed this phenotype in *mxc* mutants.

The *Dsor* locus was the most easily mutated. *Dsor* interacts with the proto-oncogene *D-raf*, but none of the alleles we isolated showed any interaction with *mxc* mutants.

Analysis of the locus *multi sex combs* could help to gain new insight into the roles of the *Pc-G* genes. First of all, the *mxc* mutations affect not only the soma but also the germ line. Both cell lineages have determined states that require the mxc^+ product. That certain *Pc-G* gene products might be needed during oogenesis was known since Paro and Zink (1992) found strong expression of

PC protein in follicle cells and nurse cells during oogenesis, while another *Pc-G* gene, *polycombeotic* can provoke sterility in homozygous mutant females (Phillips and Shearn 1990). In *mxc* mutants, the somatic component of the gonad is well differentiated and apparently normal, while, depending on the *mxc* allele considered, all steps in development of the germ line can be affected in both sexes. In normal development, the pole cells are swept inside the posterior midgut invagination during germ band extension, and must then move through the gut wall to contact the gonadal mesoderm (Campos-Ortega and Hartenstein 1985). Primordial germ cell migration is controlled by somatic tissue, probably by the gut primordium (Jaglarz and Howard 1994). However, this tissue appears normal in *mxc* mutants. The gonadal mesoderm appears to originate specifically from parasegments 10, 11 and 12 (Brookman et al. 1992). Even in the absence of pole cells the mesodermal components differentiate normally into agametic gonads. The *BX-C* gene *iab-4* is required for correct differentiation in somatic cells but not in germ cells of the gonadal primordia (Cumberledge et al. 1992). But since the effects of *mxc* mutants impinge on the germ line alone, they are apparently not mediated through the *BX-C*-dependent regulation of somatic tissue identity during gonadogenesis. The requirement for *mxc* in the germ line seems to be continuous all through development, as suggested by the stages of gonadogenesis affected in the different mutants. Other *Pc-G* genes are needed throughout development in somatic tissues (Capdevila et al. 1986; Busturia and Morata 1988; Santamaría et al. 1989). Different important developmental decisions are taken by the germ line, and could be regulated by *mxc*. In the same way as a number of *Pc-G* mutants can be affected by duplications and deficiencies of *BX-C* and *ANT-C*, and thus point to the target of *Pc-G* products, we hope that the study of the targets of *mxc* may offer new insights into the loci involved in germ line determination.

The proximodistal transformation of the second tarsal segment of prothoracic legs towards the first has been reported not only in *Pc-G* mutants, such as *Polycomb* (Capdevila et al. 1986), *polycombeotic* (Jones and Gelbart 1990), *pleiohomeotic* (Girton and Jeon 1994), and *polyhomeotic* (N. B. Randsholt, unpublished results), but also in other pleiotropic mutants like *bric a brac*, (Godt et al. 1993), *cramped* (Lindsley and Zim 1992), or *Montium-like* (P. Santamaría, unpublished results). A common target gene could possibly be affected by all these mutations. For example, a gain of function of *proboscipedia* can induce the same transformation (D. Cribbs, personal communication). Genes that control the proximodistal axis, such as *rotund* (Kerridge and Thomas-Cavallin 1988) or *Distalless* (Cohen and Jürgens 1989), or more indirectly *Deformed* (O'Hara et al. 1993), could also be affected by the absence of mxc^+ product.

Products of several *Pc-G* mutants have been reported to affect *zeste* expression: $Psc\text{-}Su(z)^2$, $polycombeotic\text{-}Su(z)^{301}\text{-}E(z)$, $Scm\text{-}Su(z)^{302}$ and now *mxc*. The *zeste* protein is thought to facilitate regulatory interactions (Pirrotta 1991) by forming multimeric aggregates that bind different sites within target genes. It has been suggested that *z* promotes the formation of DNA loops that serve to bring regulatory sites near the promoters. The *Pc-G* products can either repress their targets by making multimeric complexes or "aggregulates" (Jacob 1993; Santamaría 1993) that fix DNA in a closed configuration (Paro 1990). Thus, *Pc-G* genes and *z* are all involved in establishing chromatin structure, so allele-specific interactions between z^1 and a certain number of the *Pc-G* genes are not surprising.

As the only complementation group in $Df(1)lz^{10\text{-}70d}$ that does not complement *l(1)mbn* is *mxc*, and since heterozygous $mxc^{G43}/l(1)mbn^{90}$ or $mxc^{G46}/(1)mbn^{90}$ flies manifest sterility, we concluded that *mxc* and *mbn* are alleles. This appeared to us an exciting result which could shed new light on both the function of *Pc-G* genes and certain kinds of tumorigenesis. Molecular analysis of *Psc* has shown sequence similarity with two mouse genes, *bmi-1* and *mel-18*, that can bind DNA and are involved in cell proliferation and tumorigenesis (Brunk et al. 1991; van Lohuizen et al. 1991; Goebl 1991). Recently, the wild-type *bmi-1* product was shown to be required for correct pattern formation (van der Lugt et al. 1994), since mice null mutant for *bmi-1* present posterior transformations along the anterio-posterior axis that could be compared to homeotic transformations observed in *Psc* mutant flies. Thus *bmi-1* and *mxc* could both be considered as developmentally important genes with wild-type functions necessary for pattern specification, and which, by maintaining correct cell identities, have a tumour-suppressing effect. *l(2)giant larvae*, *l(2)gl*, is another *Drosophila* tumour-suppressor locus whose wild-type product is specifically required during normal oogenesis (Szabad et al. 1991), while the mouse homologue of *l(2)gl* is controlled in vivo by the murine homeotic gene complex locus Hox-C8 (Tomotsune et al. 1993). All these data point towards possible connections between developmentally required loci and tumour suppressor genes.

Cell physiology or aspects of housekeeping metabolism are probably not affected in *Pc-G* mutants. The study of ph^o clones (Santamaría et al. 1989) showed that cells without ph^+ product appear to die after losing cell affinities. ph^o cells can divide two or three times before sorting out and make vesicles, which suggests that they die because they have lost cell identity and not because of impaired housekeeping functions. The same appears to happen in mxc^- clones (O. Saget and P. Santamaría, in preparation). Another important effect of ph^o clones was the induction of abnormal patterns (such as triplications) formed by adjacent wild-type cells. Similar results of abnormal pattern formation were observed in *pleiohomeotic* mutants (Girton and Jeon 1994). A possible explanation of the origin of these abnormalities could be that mutant cells which have lost their identity cannot provide correct positional information for their

wild-type neighbours, which in turn respond by abnormal pattern formation. Possible candidates for the proteins involved are the secreted products of *hedgehog* (*hh*), *wingless* (*wg*) or *decapentaplegic* (*dpp*), regulated by homeodomain genes (*BX-C*, *ANT-C*, *apterous*, *engrailed*) which in turn could be, or are, controlled by the *Pc-G* genes (see Lawrence and Morata 1994).

The role of *Pc-G* genes seems to be to imprint (Paro 1990) chromosomal structure to keep a number of important genes, the selector genes (McKeon and Brock 1991; Simon et al. 1992) repressed, or to silence them by assigning them to a compartment within the nucleus to which not all transcription factors have access (Schlossherr et al. 1994). Errors in the transmission of the chromatine states at cell division have been proposed to account for shifts in the fates of cells (Paro 1990). When *Pc-G* genes are mutated, all their target genes are more or less derepressed. Normally, the identity of a cell is given at any time and place by the specific combination of selector genes expressed within it. In a *Pc-G* gene mutant cell, all these selectors could be expressed at the same time, providing too many signals at once for the cell to make sense of them. Since houskeeping genes are still supposed to be functional in *Pc-G* mutant cells, they can continue to divide. In hypomorphic mutants, cells can still differentiate but with homeotic transformations, while in stronger mutants, cells can survive and divide, but as they have lost their identity and cell affinities, they could as a consequence become invasive.

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