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The origin of pattern duplications in segment polarity mutants of *Drosophila melanogaster*

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SUMMARY

Mutations of the segment polarity group in *Drosophila melanogaster* produce additional denticles with reversed polarity in every segment of the larval cuticle. We have investigated the effect of mutations in different elements of the bithorax complex on the segmental identity of these additional pattern elements. Our results suggest that they are derived, primarily, from the anterior compartment of each segment.

INTRODUCTION

The final body pattern of *Drosophila* depends, fundamentally, upon the activity of two classes of genes: the homoeotic genes, which control the pathway of differentiation followed by a given segment (Lewis 1978; Struhl 1982, 1983) and the 'segmentation genes', which together define the number, position and primary pattern of each segment (Nüsslein-Volhard & Wieschaus, 1980; Nüsslein-Volhard, Wieschaus & Jürgens, 1982). Normal development requires a precise and integrated pattern of expression of all these genes, though how this is achieved is unclear.

Several of the segmentation genes belong to the 'segment polarity' class. Mutations at these loci result in a homologous pattern deletion in every segment and a duplication of the remaining wild-type pattern, the duplicated pattern having reversed polarity (Nüsslein-Volhard & Wieschaus, 1980). To investigate the compartmental origin of the duplicated structures, we have exploited two properties of the elements of the bithorax gene complex (BX–C): (i) the activation of different elements of the BX–C within the developing embryo follows geographical coordinates along its anteroposterior axis (Lewis, 1978); and (ii) the realms of activation of different elements of the complex are defined by compartment boundaries (Garcia-Bellido, Ripoll & Morata, 1973; Minana & Garcia-Bellido, 1982; Morata & Kerridge, 1982; Struhl, 1984; Hayes, Sato & Denell, 1984).

Key words: Drosophila, pattern duplication, segment polarity mutations, polarity, mutants, homoeotic genes.

We have investigated the consequence of the absence of two BX–C elements, Ubx^+ and $abdA^+$, on the region of reversed polarity in some mutations of the segment polarity group with particular reference to the first abdominal segment (A1). In all the cases tested, the character of the region of reversed polarity of A1 is found to depend upon Ubx^+ but not $adbA^+$, suggesting that it is derived from the anterior compartment.

MATERIALS AND METHODS

Mutations employed

The alleles of the three segment polarity loci studied were kindly provided by C. Nüsslein-Volhard and were as follows: *gooseberry*, *Df(2R)IIX62 (zip, gsb)* and *Df(2R)SB1 (gsb,Kr)*; *hedgehog*, hh^{1J35}; and *cubitus interruptus dominant*, ci^D. These alleles have been described previously (Nüsslein-Volhard *et al.* 1984; Jürgens Wieschaus, Nüsslein-Volhard & Kluding, 1984; Wieschaus, Nüsslein-Volhard & Jürgens, 1984).

The BX–C mutations employed were: Ubx^1; $Df(3R)bxd^{100}$, which lacks the Ubx^+ and bxd^+ functions; Df(3R)P9, a deficiency for the entire BX–C; and $abd\text{–}A^{S1}$ a lethal allele of the *abdA* complementation group to the right of *Ubx* defined by the distal breakpoint of Df(3R)P10 (Sanchez-Herrero, Vernos, Marco & Morata, 1985; Tiong & Whittle, unpublished). Descriptions of the BX–C mutations other than $abd\text{–}A^{S1}$ may be found in Lewis (1978).

Double mutant combinations

Combinations of ci^D and *gsb* with BX–C mutations were generated by intercrossing animals doubly heterozygous for both the segment polarity and homoeotic mutations. In the case of *hh*, recombinant chromosomes of the constitutions $Ubx^1\ hh^{1J35}$ and $abd\text{–}A^{S1}\ hh^{1J35}$ were constructed.

Preparation and analysis of larval cuticle

Eggs were collected from appropriate crosses over 12 h periods and incubated for a further 24 h at 25 °C. Pharate larvae were collected, dechorionated, fixed and cleared according to the procedure of van der Meer (1977). They were then mounted in a 1:1 mixture of lactic acid and Hoyers medium. Preparations were examined using dark-field and phase-contrast optics. Double mutants were identified on the basis of their novel phenotype and frequency.

RESULTS

The three segment polarity mutants analysed display a similar but distinguishable phenotype: the posterior region of every segment is deleted and replaced by a duplication of the remaining anterior region (see Fig. 1). The deletion is very large in *hh* and smaller in ci^D and *gsb*. Absence of the Ubx^+ function results in the homoeotic transformation of various segments (Lewis 1978); the crucial observation for this study is that it causes the transformation of anterior A1 to anterior T2, but has no effect on posterior A1 in the first instar larva (Struhl, 1984 and Fig. 1). In contrast, in animals hemizygous for the $abd\text{–}A^{S1}$ mutation, anterior A1 develops normally whilst posterior A1 is transformed to posterior T3 (Tiong & Whittle, personal communication and Fig. 1).

In combinations of each of the three segment polarity mutations with $Dfbxd^{100}$ or Ubx^1, most of the denticle rows in A1, both with normal and altered polarity,

appear thoracic in character (Fig. 2). This result suggests that the denticles with reversed polarity are derived, primarily, from the anterior compartment. If they belonged to the posterior compartment, they might be expected to remain abdominal in character. In fact, in a few cases with each mutant, we observed some abdominal denticles with reversed polarity amongst the transformed denticles (Fig. 2). These denticles clearly belonged to A1 and appear separated from A2 by a region of naked cuticle.

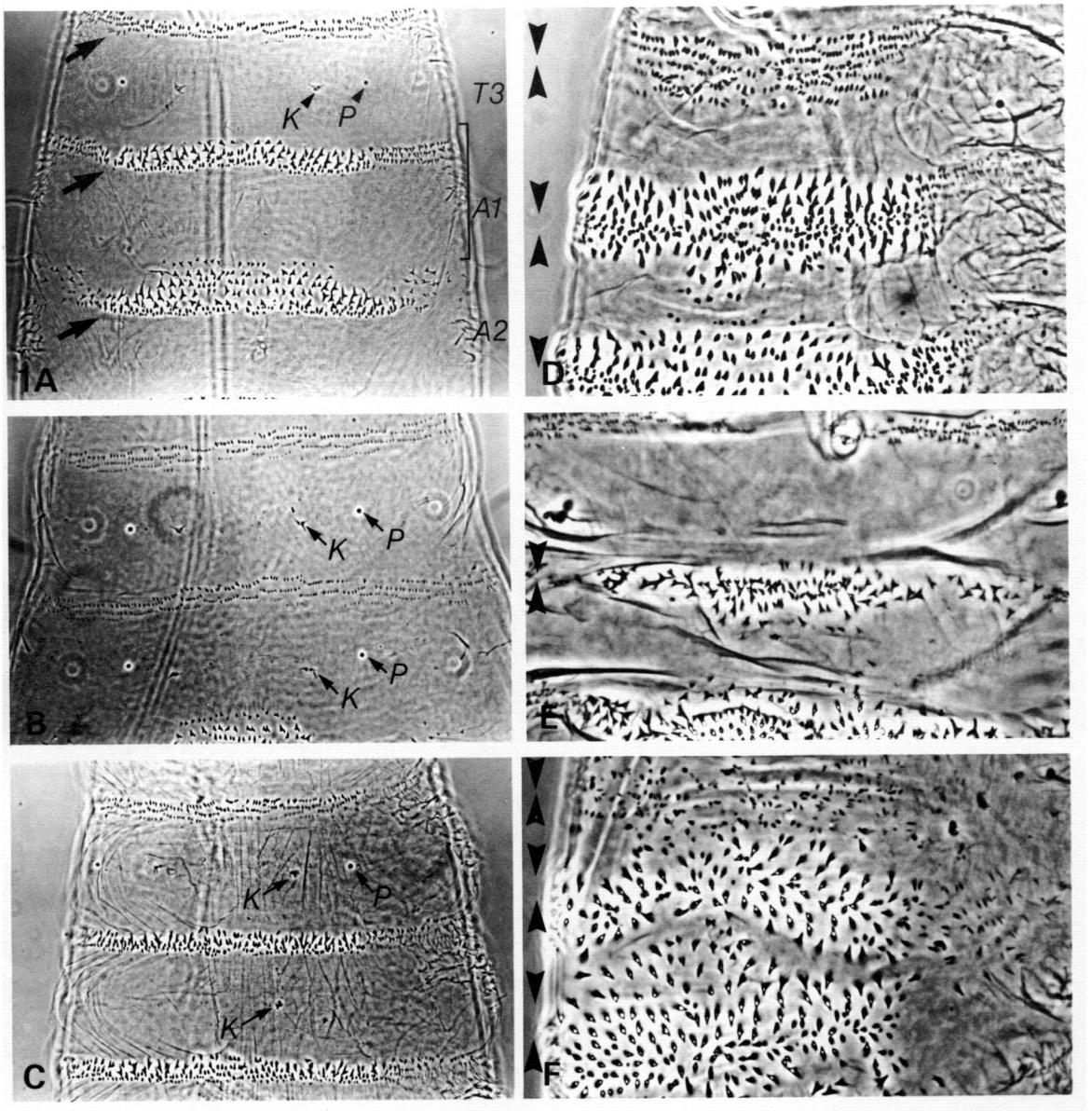

Fig. 1 for legend see p.133

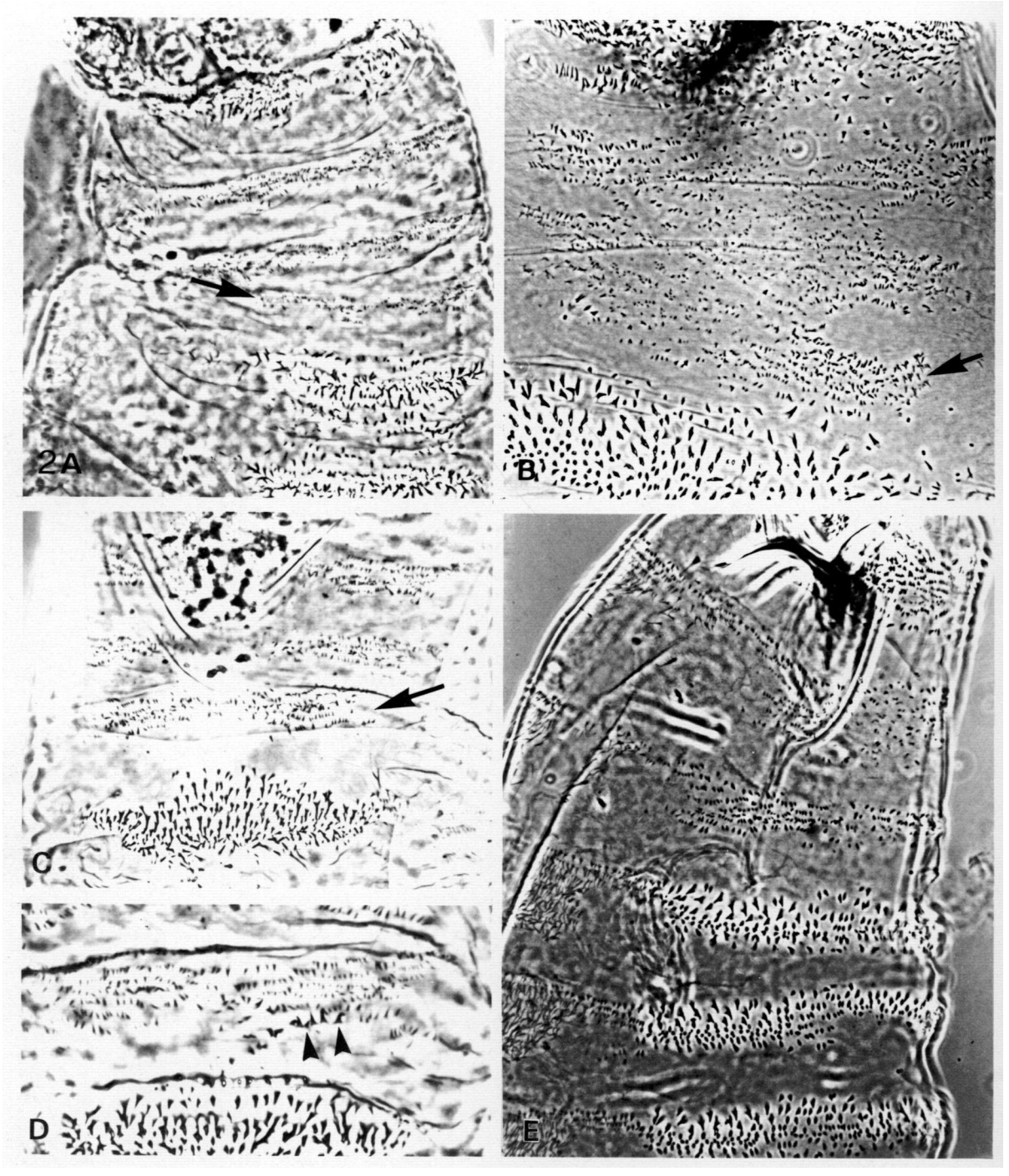

Fig. 2

Fig. 1. Ventral cuticle of first instar larvae viewed under phase-contrast illumination.
(A) Third thoracic (*T3*), first (*A1*) and second (*A2*) abdominal segments of a wild-type larva. The particular features important to this study are: 1) the denticles (arrowed) of the abdominal segments are considerably thicker than those of the thoracic segments. 2) the thoracic segments bear two specialized sensory structures viz. the ventral pit (*P*) and the Keilin organ (*K*); the latter is composed of three hairs, two of which apparantly derive from the anterior compartment of the segment whilst the third is a derivative of the posterior compartment (see Struhl, 1984). For a full description of the wild-type larval morphology see Lohs-Schardin *et al.*, 1979.
(B) Third thoracic and first abdominal segments of a $Ubx^{1}/Df(3R)bxd^{100}$ larva. The anterior part of the first abdominal segment is transformed towards T2 as evidenced by the change in size of the denticles in this segment, and also by the presence of ventral pits (*P*) and Keilin organs (*K*) usually, though not invariably, composed of only two hairs. The posterior compartment of this segment appears unaffected (as judged by the pattern of dorsal hairs, not visible here).
(C) Third thoracic and first abdominal segments of an $abd\text{-}A^{S1}$ hemizygote. The anterior part of A1 is essentially wild type; note however the presence of a partial Keilin organ, indicative of a transformation of posterior A1 to T3. This is confirmed by examination of the dorsal hair pattern of such larvae (Tiong & Whittle, personal communication and own observations). Note also that the anterior second abdominal segment is transformed to A1.
(D) Detail of the T3,A1,A2 region of a mutant *gsb* larva (genotypically *Df(2R)IIX62/ Df(2R)SB1* – see Materials and Methods). Note the presence of a normal denticle band and immediately posterior to it a complementary denticle band having reversed polarity and replacing most of the naked cuticle normally present in the wild-type segment (compare with (A)). The arrow heads indicate the polarity of each denticle band.
(E) Detail of T3,A1,A2 region of a homozygous ci^{D} larva. This exhibits the same phenomenon of reversal of polarity as *gsb* (see (D)). However, there is also some reduction in the size of the denticle bands and concomitantly more naked cuticle.
(F) Same region as in (D) and (E) of a homozygous hh^{1J35} larva. As in *gsb* and ci^{D} each segment bears an additional denticle band with reversed polarity. In addition there is a substantial deletion of pattern elements including the segment boundaries (Nusslein-Volhard & Wieschaus, 1980; Martinez-Arias, in preparation). This results in the juxtaposition of the denticle bands of each segment, which nevertheless retain their appropriate segmental identity.

Fig. 2. Ventral aspects of the larval cuticle in homoeotic and segment polarity double mutant combinations.
(A) Homozygous $Df(3R)bxd^{100};ci^{D}$. The denticles of the first abdominal segment have been completely transformed to the thoracic form (arrow). These transformed denticles show the polarity reversal characteristic of ci^{D} (Fig. 1E).
(B) Homozygous $Ubx^{1}hh^{1J35}$ larva. As in the case of ci^{D} all the denticles of the first abdominal segment are thoracic in character (arrow) irrespective of their polarity.
(C) Homozygous $Df(2R)gsb;\ Df(3R)bxd^{100}$. Both normal and reversed polarity A1 denticles are thoracic in character (arrow).
(D) Detail of a transformed first abdominal segment in an animal similar to that shown in (C). Notice that in this case there are some denticles with reversed polarity which have abdominal character despite being in the transformed segment A1 (arrowheads). This may mean that, occasionally, cells from the posterior compartment of A1 contribute to the reversed polarity pattern in *gsb*. Alternatively, they may derive from anterior A2. This feature was observed in, approximately, 10% of the double mutants.
(E) $Df(2R)gsb;\ abd\text{-}A^{S1}/Df(3R)P9$. Notice that the denticles with reversed polarity in A1 are clearly abdominal in character.

In combinations of each of the three mutations with *abd–A*^{S1}, all the denticles of A1 are abdominal in character (Fig. 2). This observation supports the notion that the region of reversed polarity is of anterior origin, since posterior A1 is transformed to T3 in *abd–A*^{S1} mutants. If this region were derived from the posterior compartment, it would be expected to become thoracic in the double mutant.

DISCUSSION

Our results suggest that the regions of reversed polarity in the segment polarity mutations are derived from the anterior compartment of each segment. An alternative interpretation is to suppose that each mutation causes the transformation of the posterior compartment to anterior. Since in A1 the state of BX–C expression differs between compartments, (the anterior compartment requires *Ubx*$^+$ while the posterior compartment does not), such a transformation could change the BX–C state of cells in posterior A1 to that of anterior A1. If this were the case, complete absence of *Ubx*$^+$ function should result in all the denticles in the reversed polarity region of A1 being thoracic in character. Yet in each mutation studied we have observed cases of abdominal denticles in this region (for each genotype, a few deticles in one of ten double mutants). Alternatively, the level of BX–C expression in the embryo may be independent of the genetic specification of compartments. In this case, if the region of reversed polarity was derived from posterior A1 it would differentiate an anterior pattern appropriate to its BX–C state (*abdA*$^+$), namely that of anterior A2. In this case, absence of *Ubx*$^+$ should have no effect on the reversed polarity region; however, we find that in the majority of the cases all the denticles of A1 are transformed. The fact that in the absence of *Ubx*$^+$ each mutant occasionally develops a few abdominal denticles suggests that some cells of posterior provenance can contribute to the region of reversed polarity and autonomously express their appropriate state of BX–C activity.

Since the majority of the reversed polarity region of A1 requires *Ubx*$^+$ but not *abdA*$^+$ we conclude that it is derived from the anterior compartment of this segment. This conclusion can be extended to the other segments and is supported by the results of Garcia-Bellido & Santamaria (1972) which show that the activation of elements of the BX–C is not altered by a mutation like *engrailed*, which causes a partial posterior to anterior transformation. A corollary of our observations is that in the segment polarity mutants studied, the posterior compartment of each segment is either absent (*hh*) or greatly reduced in size (*gsb* and *ci*^{D}).

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REFERENCES

GARCIA-BELLIDO, A. & SANTAMARIA, P. (1972). Developmental analysis of the wing disc in the mutant engrailed of Drosophila melanogaster. *Genetics* **72**, 87–104.

GARCIA-BELLIDO, A., RIPOLL, P. & MORATA, G. (1973). Developmental compartmentalisation of the wing disk of *Drosophila*. *Nature* **245**, 251–253.

HAYES, P. H., SATO, T. & DENELL, R. E. (1984). Homoeosis in *Drosophila*: the *Ubx* syndrome. *Proc. natn. Acad. Sci., U.S.A.* **81**, 545–549.

JÜRGENS, G., WIESCHAUS, E., NÜSSLEIN-VOLHARD, C. & KLUDING, H. (1984). Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster*. II. Zygotic loci on the third chromosome. *W. Roux' Arch. devl Biol.* **193**, 283–295.

LEWIS, E. B. (1978). A gene complex controlling segmentation in Drosophila. *Nature* **276**, 565–570.

LOHS-SCHARDIN, M., CREMER, C. & NÜSSLEIN-VOLHARD, C. (1979). A fate map for the larval epidermis of *Drosophila melanogaster*. *Devl Biol.* **73**, 239–255.

MINANA, F. J. & GARCIA-BELLIDO, A. (1982). Preblastoderm mosaics of mutants of the BX-C. *W. Roux' Arch. devl Biol.* **191**, 331–334.

MORATA, G. & KERRIDGE, S. (1981). Sequential functions of the bithorax complex of *Drosophila*. *Nature* **290**, 778–781.

NÜSSLEIN-VOLHARD, C. & WIESCHAUS, E. (1980). Mutations affecting segment number and polarity in *Drosophila*. *Nature* **287**, 795–801.

NÜSSLEIN-VOLHARD, C., WIESCHAUS, E. & JÜRGENS, G. (1982). Segmentierung bei *Drosophila* – Eine genetische Analyse. *Verh. Dtsch. Zool. Ges.* **000**, 91–104.

NÜSSLEIN-VOLHARD, C., WIESCHAUS, E. & KLUDING, H. (1984). Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster*. I Zygotic loci on the second chromosome. *W. Roux' Arch. devl Biol.*, **193**, 267–282.

SANCHEZ-HERRERO, E., VERNOS, I., MARCO, R. & MORATA, G. (1985). Genetic organization of *Drosophila* bithorax complex. *Nature* **313**, 108–113.

STRUHL, G. (1982). Genes controlling segmental specification in the Drosophila thorax. *Proc. natn. Acad. Sci., U.S.A.* **79**, 7380–7384.

STRUHL, G. (1983). Role of the *esc*$^{+}$ gene product in ensuring the selective expression of segment specific homoeotic genes in *Drosophila*. *J. Embryol. exp. Morph.* **36**, 297–331.

STRUHL, G. (1984). Splitting the bithorax complex of *Drosophila*. *Nature* **308**, 454–457.

VAN DER MEER, J. M. (1977). Optically clean and permanent whole mount preparation for phase contrast microscopy and cuticular structures of insect larvae. *Drosoph. Inf. Serve.* **12**, 160.

WIESCHAUS, E., NÜSSLEIN-VOLHARD, C. & JÜRGENS, G. (1984). Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster*. III Zygotic loci on the X chromosome. *W. Roux's Arch. devl Biol.*, **193**, 296–307.

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