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Original Article

Modeling the regulation of the bithorax complex in Drosophila melanogaster: the phenotypic effects of Ubx , abd-A and Abd-B heterozygotic larvae, and a homozygous Ubx- abd A hybrid gene

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ABSTRACT As an intermediate step in the development of a defined quantitative model of pattern formation during Drosophila segmentation, we present here a model capable of predicting the experimentally determined levels of gene activity and their phenotypic consequences. In its present form, the model includes only four genes: the three genes of the bithorax complex (Ubx, abd-A and Abd-B) and Antennapedia. It is shown that the model is quite robust, predicting many properties in the behavior of these genes. A previously undescribed property is that all of these genes should phenotypically exhibit some kind of haploinsufficiency when present in only a single dose in the genetic background of the animal. This is shown both by the model and by a new method of quantitatively analyzing the differences in the more obvious cuticular features of the larvae, i.e., the patterns in the ventral denticle belts. The model is also capable of dealing with a complicated genetic situation, a hybrid gene of Ubx and abd-A produced by the C1 deletion.

KEY WORDS: *Drosophila, bithorax, Antennapedia, modeling*

Introduction

As reported in a previous paper (Carratalá *et al.*, 1989), we are developing a quantitative model which will account for the effect of homeotic genes on the specification of segmental identities along the anterioposterior axis of *Drosophila*. As an initial step in this development, we proposed a minimal crossregulatory network of four genes: *Antp, Ubx, abd-A,* and *Abd-B,* which was able to predict the spatial distribution of the transcripts and/or proteins in a series of mutants (Struhl and White 1985; Carroll *et al.*, 1986; Weeden *et al.*, 1986) and of ventral denticle belt pattern diversity (Carratalá *et al.*, 1989). This model is schematized in Fig. 1.

In the model, the four genes considered (*Abd-B, abd-A, Ubx* and *Antp*) are repressed by a general repressor system which decreases from anterior toward posterior segments (GRS, r in the equations) and shows variable affinity for the genes, with greatest affinity for the genes that are expressed more posteriorly (Fig. 1A). This repressor system would be spatially distributed in a decreasing gradient along the anterioposterior direction as shown in Fig. 1B.

A second regulatory component, the posterior repressor element (PRE, r_p in the equations), in principle with affinity only for the *Antp* gene (Fig. 1A) and required only in the A8-a compartment (Fig. 1B), is necessary there to prevent *Antp* expression in this compartment in our simulations, particularly in the case of the absence of *Bithorax* genes, as reported experimentally by Carroll *et al.*, 1986.

In addition, each gene represses the more anteriorly expressed genes with increasing affinity (Fig. 1A).

From these assumptions it is possible to derive equations (Fig. 1C) to obtain the predicted level of expression of

Abbreviations used in this paper: GRS, general repressor system; PRE, posterior repressor system; r, level of general repressor system; r_p, level of posterior repressor element; b, Abd-B gene expression level; a, abd-A gene expression level; u, Ubx gene expression level; t, Antp gene expression level; h, abd-A, Ubx hybrid gene expression level; P_b, number of Abd-B promoters; P_a, number of Abd-A promoters; P_u, number of Ubx promoters; P_t, number of Antp promoters; P_h, number of abd-A, Ubx hybrid promoters; K_{br}, K_{ar}, K_{ur}, K_{tr}, K_{hr}, affinity constants of GRS for different genes; K_{ab}, K_{ub}, K_{tb}, affinity constants of Abd-B product for different genes; (ab, abd-A; ub, Ubx and tb, Antp); K_{au}, K_{at}, affinity constants of abd-A product for different genes (ua, Ubx and ta, Antp); K_{tu}, affinity constant of Ubx product Antp; K_{trp}, affinity constant of Ubx product Antp.

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each of the four genes in each segment (a complete description of this set of equations is presented in Carratalá *et al.*, 1989). The expression level of each gene depends on the levels of the more posteriorly expressed genes and of the repressor system. In addition to the affinity constants (K), a second class of constants (P series) also appears in the equations. The values of these constants depend, among other things, on the number of promoters available for transcription. The constant values have been made equal to 100 in the wild type case, where two promoters are present (one for each copy of the wild type gene), but in heterozygotic mutants the values become equal to 50 and in homozygous mutants are equal to zero.

From these equations and using the values for the constants described above, the predicted gene expression levels for wild type (Fig. 1D) and for any type of mutant can be obtained. They are compared with the spatial distributions of transcripts and gene products experimentally detected (Akam and Martinez-Arias, 1985; Struhl and White, 1985; White and Wilcox, 1985; Carroll *et al.*, 1986; Weeden *et al.*, 1986). An example of these data is presented in Fig. 1E (data modified from Weeden *et al.*, 1986).

In the model we try to address the problem of the connection between phenotype and genotype. We have done this by trying to relate the pattern of larval denticle belts to the level of homeotic gene expression in each segment. A true picture of the relative difference in the spatial patterns can only be obtained by employing a quantitative measure. For this purpose, we have used the method of comparison of ventral denticle belt patterns schematized in Fig. 2 (Carratalá *et al.*, 1989). As a result of this analysis, an empirical measurement of the difference between patterns, the *empirical distance*, is obtained. This is related by a linear approximation to the level of gene expression that results in a second measure of distance called the *estimated distance*. The estimated distances and the estimated gene expression levels are obtained by an iterative process shown in Fig. 2 and further described in the experimental section.

Although a satisfactory agreement between the model and the experimental data included in the previous paper (Carratalá *et al.*, 1989) was achieved, the robustness of any model has to be tested by extending the application of its predictions to additional experimental situations.

In the current paper, we have extended our work to a set of new experimental situations not initially included in our analysis. First, one prediction of a model in which the segmental identity is directed by a linear combination of the levels of activity of four main genes is that not only homozygous but also heterozygous hemideficient animals should show phenotypic alterations, i.e, some type of haploinsufficiency. To detect such potential alterations we have used the quantitative method of comparison between ventral denticle patterns of the larva, which we show here is able to detect slightly homeotic transformations. In these patterns in fact, a homeotic transformation in the A2 segment of the heterozygotic *abd-A* larva and in the A8 segment of the heterozygotic *Abd-B* larva can be found. Both alterations are predicted by our model. Secondly, the availability of an unusual experimental situation, a hybrid gene, made up of the 3' end of *Ubx* and the 5' end of *abd-A* (Casanova *et al.*, 1988; Rowe and Akam, 1988) allowed us to test how the quantitative model could also deal with this situation.

Results

Heterozygous larvae

As already mentioned, the model proposed (Carratalá *et al.*, 1989), predicts that the spatial pattern distribution of the gene products of the Bithorax and *Antp* Complexes will also be altered when a variation in the genetic dose occurs. For instance, in the case of a heterozygous mutant, a single dose of a certain gene product will generate a weak increase, in posterior segments, of one or several of the genes operating more anteriorly in the animal. This is because in the model these genes are regulated by the more posterior ones. There is a large body of experimental evidence supporting this type of regulation by more posterior genes in the BX-Complex (Hafen *et al.*, 1984; Harding *et al.*, 1985; Struhl and White, 1985), but not for effects of hemizygosity on the larval cuticle.

To test this prediction, we have studied the ventral denticles belts of *Ubx⁻/+*, *abd-A⁻/+* and *Abd-B⁻/+* (shown in Fig. 3B-D) using the method of comparison (Carratalá *et al.*, 1989) described above (see also the Materials and Methods section in this paper).

abd-A⁻/+ larvae

The profiles corresponding to the predicted and estimated gene expression levels for the *abd-A⁻/+* larvae are shown in Fig. 4A and B, respectively, together with the homologous wild type and *abd-A⁻/abd-A⁻* profiles. The predicted values for *Abd-B* expression levels are identical in the three types of larvae (data not shown) the levels of *abd-A* in the heterozygote are half the corresponding levels in wild type (data not shown). As a consequence, the levels of the *Ubx* and *Antp* expressions in *abd-A⁻/+* are higher than in wild type but lower than in *abd-A⁻/abd-A⁻*. We have tested to see whether the relative variations in the predicted gene expression levels presented here can explain the empirical distances obtained by digitizing the denticle belt patterns of this type of larvae. The majority of the empirical distances from heterozygous vs wild type larvae (Fig. 4C) present a distribution not significantly different to that found in the comparison of wild type vs wild type larvae, except in the interesting case of the heterozygous A2 segment whose distances to wild type T3 and A1 segments (Fig. 4C, arrows) is intermediate and significantly different from those obtained in the wild type vs wild type and homozygous vs wild type sets of comparisons. In fact this slightly homeotic transformation of the A2 segment in the heterozygotic mutant can be found directly in the pictures of the segments, where the intermediate phenotype of the A2 segment between the phenotypes of the A1 and A2 segments of a wild type larva can be seen (Fig. 3). Such differences are also reproduced by the estimated distances (filled squares in Fig. 4C) .

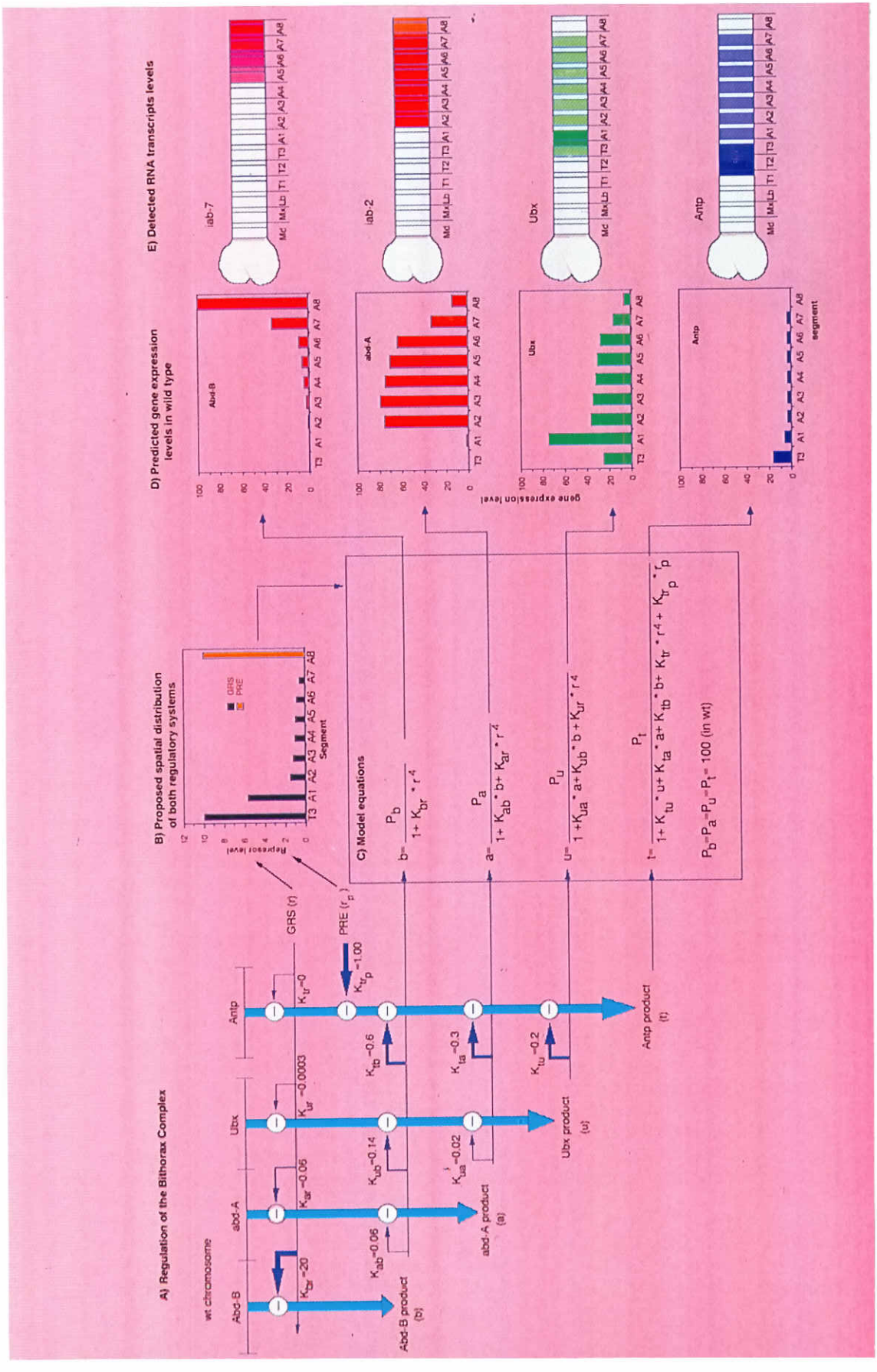

Fig. 1. Model proposed for the regulation of *Antennapedia* and the bithorax complex genes. (A) *Network gene model: the expression of the four genes Abd-B, abd-A, Ubx and Antp would be repressed by a general repressor system (GRS) with different affinities, greater when the gene is being expressed more posteriorly in the animal. A second repressor element, the posterior repressor element (PRE) would affect only Antp expression and in principle would be expressed only in the A8-a compartment in the case of the modeling attempted here. Each of the gene products represses the more anteriorly expressed genes with different affinities, greater when the target gene is expressed more anteriorly. As explained in our previous work (Carratalá* et al., *1989), the affinity constants (k series) are obtained by fitting the equations to the experimental results of transcripts and gene product levels.* **(B)** *Proposed spatial distribution of both repressor systems. The GRS levels would be in a decreasing gradient along the anterioposterior direction while the PRE would be only present in the A8-a compartment. (The units used in the plot are arbitrary).* **(C)** *Equations derived from the network schematized in A) for each of the four gene products.* **(D)** *Predicted profiles (in arbitrary units) of gene expression for wild type segments obtained by substituting the values of the affinity constants and the repressor levels for each segment in the equations shown in (C).* **(E)** *Example of transcript spatial distributions detected by in situ hybridization (data taken from Weeden* et al., *1986 presented in a slightly modified form).*

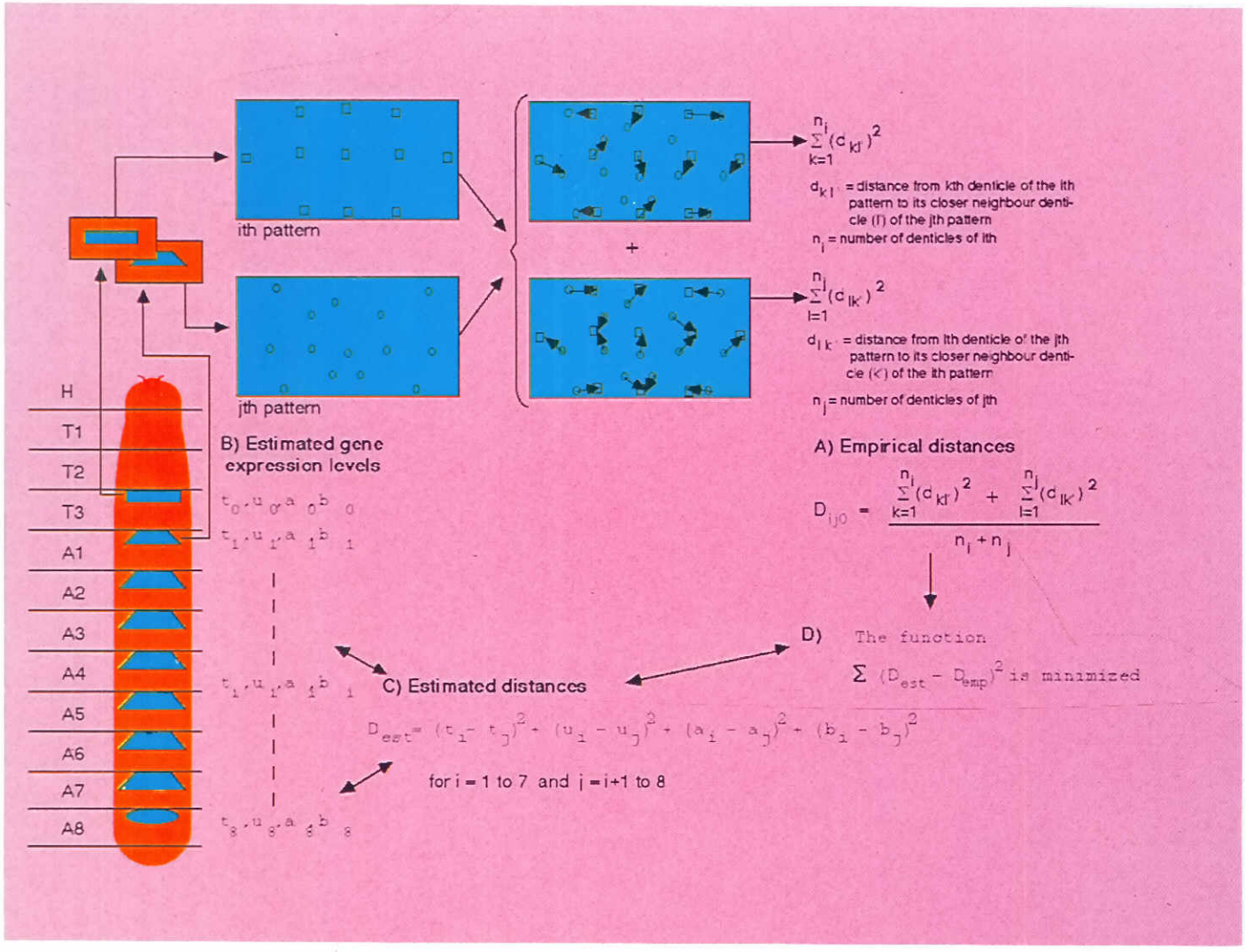

Fig. 2. Diagram of the method of comparison and correlation between the results and the levels of expression of *Abd-B*, *abd-A*, *Ubx* and *Antp* in each segment. (A) *Each segment is digitized and superimposed on the rest of segments as explained in the experimental section. The* empirical distance *is calculated by the sum of the square distances from each denticle of each segment to the nearest denticle of the other segment and this sum is divided by the total number of denticles in the two segments. The two segments compared can be from the same larva or from different larvae.* **(B)** Estimated expression levels. *A value for the level of each of the four genes* Abd-B (b), abd-A (a), Ubx (u) *and* Antp (t) *in each segment is initially arbitrarily chosen.* **(C)** Estimated distances. *A second type of distance, the estimated distance, is determined between two segments by the sum of square differences between the four estimated expression levels of the genes in these two segments. By an iterative process the initial levels of estimated gene expression are modified to obtain the best fit by least squares between* estimated *and* empirical distances.

Moreover, opposite trends in the comparison can be identified. Namely, while the values for heterozygous larvae are intermediate to those of wild type vs wild type and homozygous vs wild type, in the comparisons with the more anterior segments, the values for the homozygous distances are the lowest, while in the comparisons with the posterior segments the homozygous distances are the highest. These trends are also reproduced by the estimated distances (filled squares in Fig. 4C).

Thus, in the *Abd-A*$^{-}$/+ heterozygous mutants there is a 50% decrease in *abd-A* expression and corresponding increases in the *Antp* and *Ubx* expressions predicted by the model, which can lead to detectable homeotic transformations. The heterozygous segments, especially A2, are more similar to the more anterior wild type segments than in the case of the corresponding wild type segments.

Ubx^{-}/+ *larvae*

In this type of heterozygous mutant, the presence of only half a dose of *Ubx* product leads to a smooth increase in the *Antp* expression only detectable in segments T3 and A1 (data not shown). The *Antp* product levels in all the heterozygous segments are slightly higher than in wild type but lower than in the homozygous *Ubx* case. The opposite is true for the *Ubx* product. No differences in the levels of *abd-A* or *Abd-B* are predicted by the model.

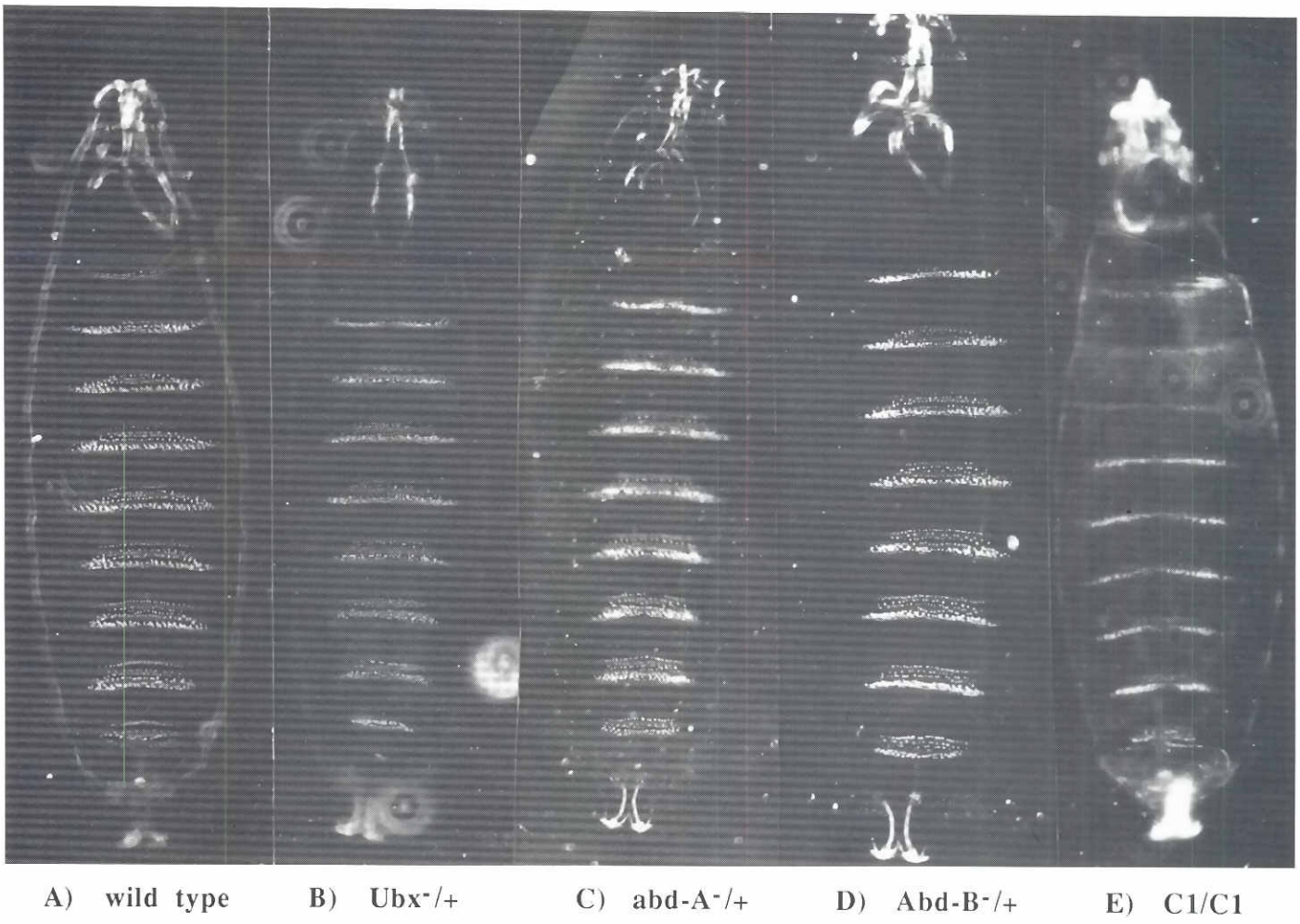

Fig. 3. Examples of the denticle belt patterns of (A)*wt/wt*, (B)*Ubx*$^{-}$/+, (C)*abd-A*$^{-}$/+, (D)*Abd-B*$^{-}$/+, and (E)*C1/C1* larvae used in this work.

Although the difference in *Antp* and *Ubx* levels is small, we have tried to detect the alteration in phenotype by using the comparison method described above. In this case, no difference between the wild type and the heterozygous phenotype was observed with the number of cuticle samples studied.

Nevertheless, the general trend of variations in the estimated expression levels agree with the trend of the variations in the predicted expression levels, although there are differences in the quantitative values. This general agreement can be summarized as follows: the *Ubx* level in *Ubx*/+ is 50% of the wild type *Ubx* level and decreases toward the more posterior segments, the *Antp* level is increased, while the *abd-A* and *Abd-B* levels remain similar both for the estimated and predicted expression levels (data not shown).

Abd-B^{-}/+ *larvae*

The predicted and estimated expression levels for the *Abd-B*$^{-}$ heterozygous and homozygous mutants show a repression of *abd-A* and *Ubx* genes particularly in the more posterior segments.

As already indicated (Carratalá *et al.*,1989), the absence of *Abd-B* expression in homozygous larvae leads to alterations only clearly detectable in the A8 segment. The intermediate expression level of this gene in heterozygotes has a phenotypic response, already implied in its dominant phenotype in adult flies (Sánchez-Herrero *et al.*, 1985), which does not seem to be reflected in significant changes in other parts of the organism. The distances from the heterozygotic A8 segment to A3, A4 and A5 wild type segments (Fig. 5, arrows), however, look significantly different of those from the wild type A8 segment. Thus, a slight homeotic transformation is also detected in heterozygous *Abd-B*$^{-}$/+ larva.

There is also a weaker trend in the pattern of estimated and empirical distances (Fig. 5) similar to that described in the case of *abd-A*$^{-}$ larvae which is also reproduced by the changes in gene expression levels predicted by the model.

In conclusion, the model of Bithorax complex gene expression regulation by varying affinities predicts, in principle, slight homeotic transformations when a single dose of one gene of the complex is present. Our method of analysis of differences between segments allows detection of

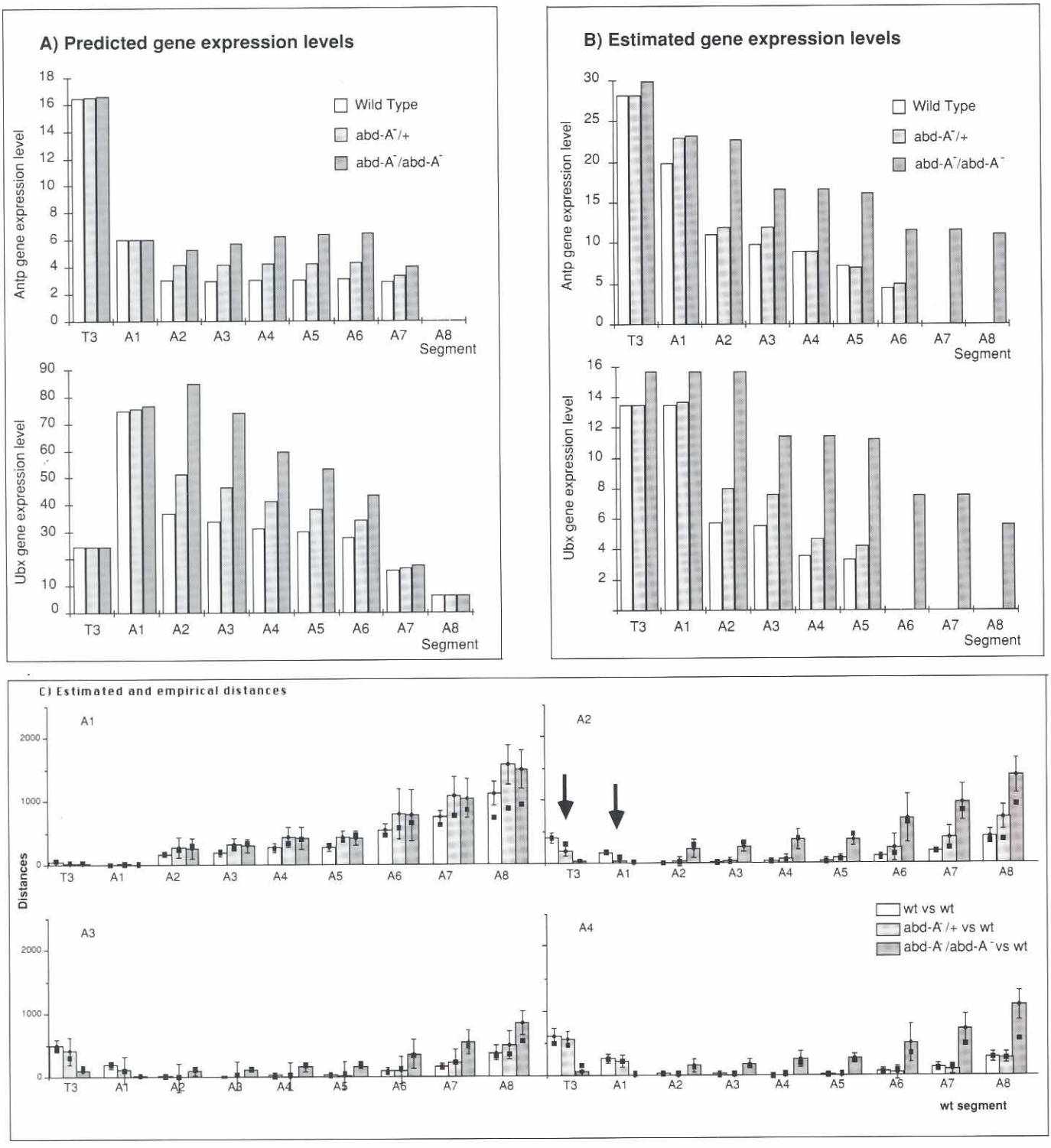

Fig. 4. (A) Predicted gene expression levels *in wild type,* abd-A $^{-}$/+ *and* abd-A /abd-A $^{-}$ *larvae obtained from the model equations, introducing the appropriate values for the mutants, as explained in the text.* **(B)** *Estimated gene expression levels in the same larvae. The values corresponding to the mutants are obtained by introducing the changes predicted by the model equations with the condition that the* Abd-B *levels are the same in all conditions while* abd-A *is 50% of the wild type value for the heterozygous condition and 0% for the homozygous state. From these values the corresponding estimated distances between segments are obtained, plotted as filled squares in Fig. 5C. All values are in arbitrary units.* **(C)** *Empirical and estimated distances from each segment of the wild type,* abd-A $^{-}$/+ *and* abd-A^{-}/abd-A^{-} *larvae to the nine wild type segments studied. Empirical distances are plotted as bars together with their standard deviation intervals (the units are* 10^{4} *in*^{2}*/point). As explained above, filled squares are the estimated distances in arbitrary units.*

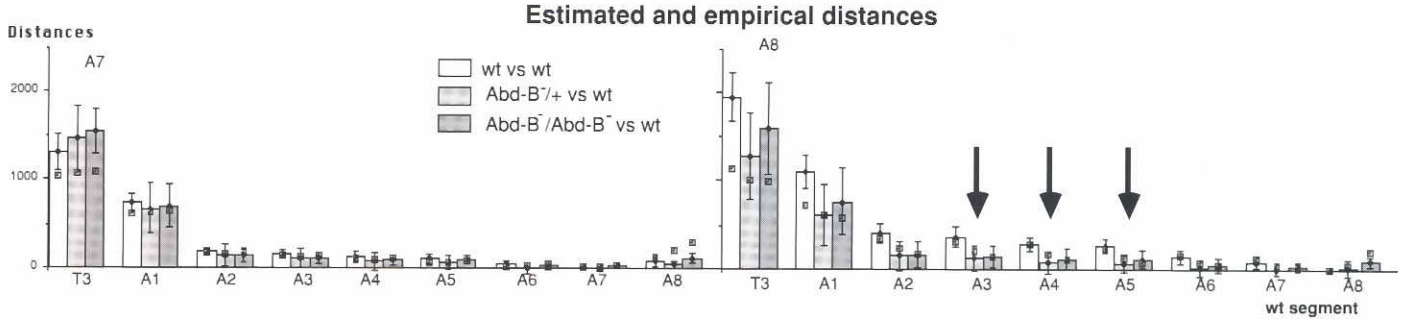

Fig. 5. Estimated and empirical distances *from segments A7 and A8 of the wild type,* Abd-B⁻/+ *and* Abd-B⁻/Abd-B⁻ *larvae to the nine wild type segments studied. Empirical distances are plotted as bars together with their standard deviation intervals (the units are* 10^{-4} *in²/point). Filled squares are the estimated distances in arbitrary units.*

such types of alteration. Although more larvae should be analysed to increase the statistical significance of the results, the alterations are clearly observed in the cases of *abd-A*⁻/+ and *Abd-B*⁻/+.

The C1 deletion chromosome

The molecular analysis of this deletion (Rowe and Akam, 1988) indicates that major portions of both the *Ubx* and *abd-A* protein coding regions are missing, giving rise to a set of novel fusion transcripts that may encode hybrid *abd-A-Ubx* proteins, which become regulated by the *abd-A* promoter. The denticle belt pattern of this mutant is shown in Fig. 3E.

The spatial distribution of these hybrid products observed by Rowe and Akam by *in situ* hybridization extends from parasegment 5 (anterior T3) to parasegment 13 (anterior A8). This result would be predicted by our model, if the hybrid production did not conserve the regulation of both genes, *Ubx* and *abd-A*. As shown in Fig. 6 and presumably as a consequence of the lack of 3' *abd-A* and 5' *Ubx* sequences, the regulation by *Abd-B* is missing or strongly reduced in the case of the hybrid, thus explaining its expression in parasegment 13. Furthermore, the affinity by the repressor system will also be decreased below that of intact *Ubx* explaining the expression in parasegment 5. Obviously, the repression by *abd-A* of the *Ubx* part of the gene will also be missing.

With these qualifications, the equation for the steady state of hybrid production as shown in Fig. 6 would be:

$$[\text{hybrid}]=100/(1 + K_{hr} * r^4)$$

Using a value for $K_{hr}= 10^{-5}$ in the network gene model equations (lower than for *Ubx* in the original proposal shown in Fig. 1A), the spatial distribution for the predicted levels of hybrid expression larvae is shown in Fig. 7A, where it can be seen that the hybrid expression extends through segments T3-A8 (parasegments 5-13) as detected by Rowe and Akam using *in situ* hybridization experiments.

Another difference with the levels observed in wild type is that the level of *Antp* expression should increase along the abdomen due to the assumed incapability of the new hybrid product to regulate *Antp*. This assumption (not yet verified experimentally), supports explanations for the larval phenotypes observed in animals carrying this deletion, as can be seen below.

The results of genetic studies carried out by Casanova *et al.* (1988) are also predicted by the model using the few assumptions indicated above.

For the *C1/abd-A* data, the equations corresponding to the four genes would be as follows in terms of the model:

Abd-B would be as in wt:

$$b=100 /(1 + K_{br}*r^4)$$

The phenotypic effect of the *abd-A* gene would be mimicked by the single dose of the hybrid protein :

$$h=50/(1 + K_{hr} * r^4)$$

Ubx would have half a normal dose from the wild type chromosome

$$u=50/(1 + K_{ub}*b + K_{ur}*r^4)$$

plus the phenotypic contribution from the hybrid *C1* chromosome:

$$h=50/(1 + K_{hr} * r^4)$$

Finally *Antp* would be regulated by the *Ubx* normal product, *Abd-B* and the posterior regulator gene, PRE:

$$t=100/(1 + K_{tu}*u + K_{tb}*b + K_{trp}[r_p])$$

The predicted levels of the partial *abd-A* due to the hybrid product are lower than the levels of *abd-A* in wild type in the segments A2-A6 but higher, obviously, than in *abd-A*⁻ homozygotes, thus explaining the experimental observation of a weak phenotypic transformation towards parasegment 6 and the presence of monohairs (Casanova *et al.*, 1988). In addition, the product levels obtained adding the normal product plus the partial *Ubx* hybrid contribution are higher even than the wild type ones along every segment, descending towards A8, explaining that the transformation is only towards parasegment 6 and not towards parasegment 4.

In *C1/DfP9*, the corresponding equations of the model for this case would be:

$$b=50/(1 + K_{br}*r^4)$$
$$a=h=50/(1 + K_{hr} * r^4)$$
$$u=h=50/(1 + K_{hr} * r^4)$$
$$t=100/(1 + K_{tb}*b + K_{trp}[r_p])$$

The intermediate partial *abd-A* levels due to the hybrid product may explain the presence of abdominal elements in the pattern, whereas the extension of *Antp* expression along the abdomen, due to the lack of regulation by both

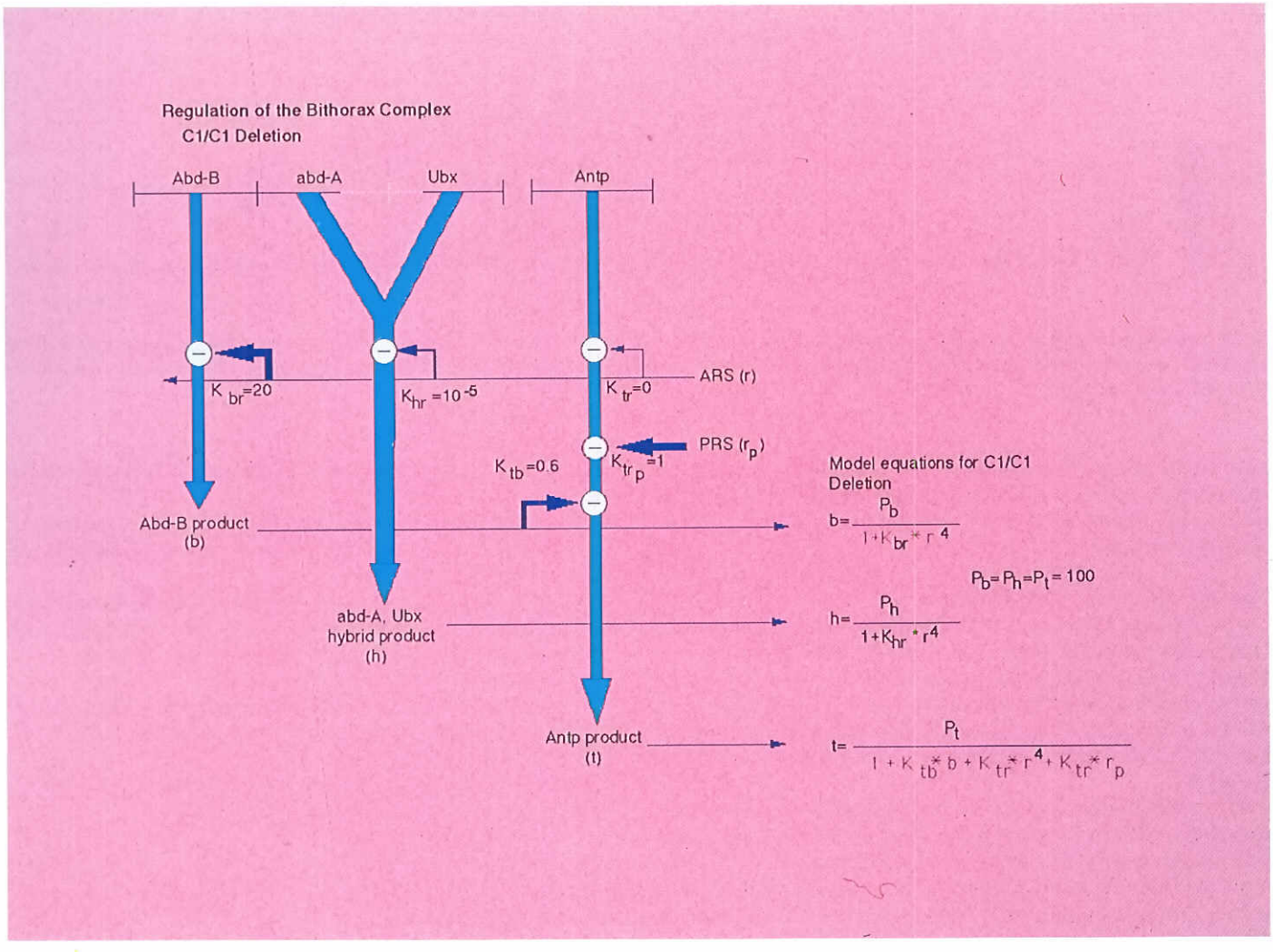

Fig. 6. Model of regulation in the case of the C1/C1 deletion. *A hybrid product is coded by the fusion of the remaining 5'* abd-A *and 3'* Ubx *sequences. This hybrid product would be regulated only by the GRS and with an affinity less than that of* Ubx *or* abd-A *for the same repressor. After several attempts we concluded that the best fit corresponds to the case where the hybrid would not present affinity for* Antp. *The affinity constants and the corresponding model equations for this particular case are shown.*

Ubx and *Abd-A* product, may explain the appearance of thoracic pattern elements (Casanova *et al.*, 1988).

In *C1/Ubx*$^-$, the equations would be:

$$b=100/(1 + K_{br}*r^4)$$
$$a=50/(1 + K_{ab}*b + K_{ar}*r^4) + h=50/(1 + K_{hr} * r^4)$$
$$u=h=50/(1 + K_{hr} * r^4)$$
$$t=100/(1 + K_{ta}*a + K_{tb}*b + K_{trp}*r_p)$$

As observed experimentally by Casanova *et al.*, (1988), the contribution of the normal chromosome to *abd-A* gene levels allows the more normal development of the abdominal segments. The lower *Antp* level in A1 than that observed in *C1/DfP9* explains the more normal A1 segment found in this case, whereas the relatively low level in *Ubx* explains the *Ubx*$^-$ phenotype. In this case, we must invoke the partial or total lack in the hybrid product of functional activity necessary for the correct development of the A1 segment.

Finally, we have applied our method of comparison to the larval ventral denticle belts in the case of this deletion. We have compared the segments from one *C1/C1* larva vs four wild type larvae. An example of the empirical distances obtained is shown in Fig. 7B together with the empirical distances from wild type to wild type. Many significant differences are found in the comparison between A2-A8 segments of *C1/C1* vs wild type, showing a homeotic transformation of all of these segments towards more anterior segments. Thus, A1- A3 resembles T3 wild type more than other wild type segments, A4 transforms to A1, A5 to A2 or A3 (not shown), A6 to A3, A7 (not shown) and A8 to A6. This distribution of empirical distances is well explained by the estimated distances obtained from the estimated expression levels shown in Fig. 7C, where it can be seen that the values for the *Abd-B* gene are the same as in wild type. The hybrid levels extend from T3 to A8 and the corresponding values of *Antp* expression obtained are strongly increased along the abdomen. All of this is in good

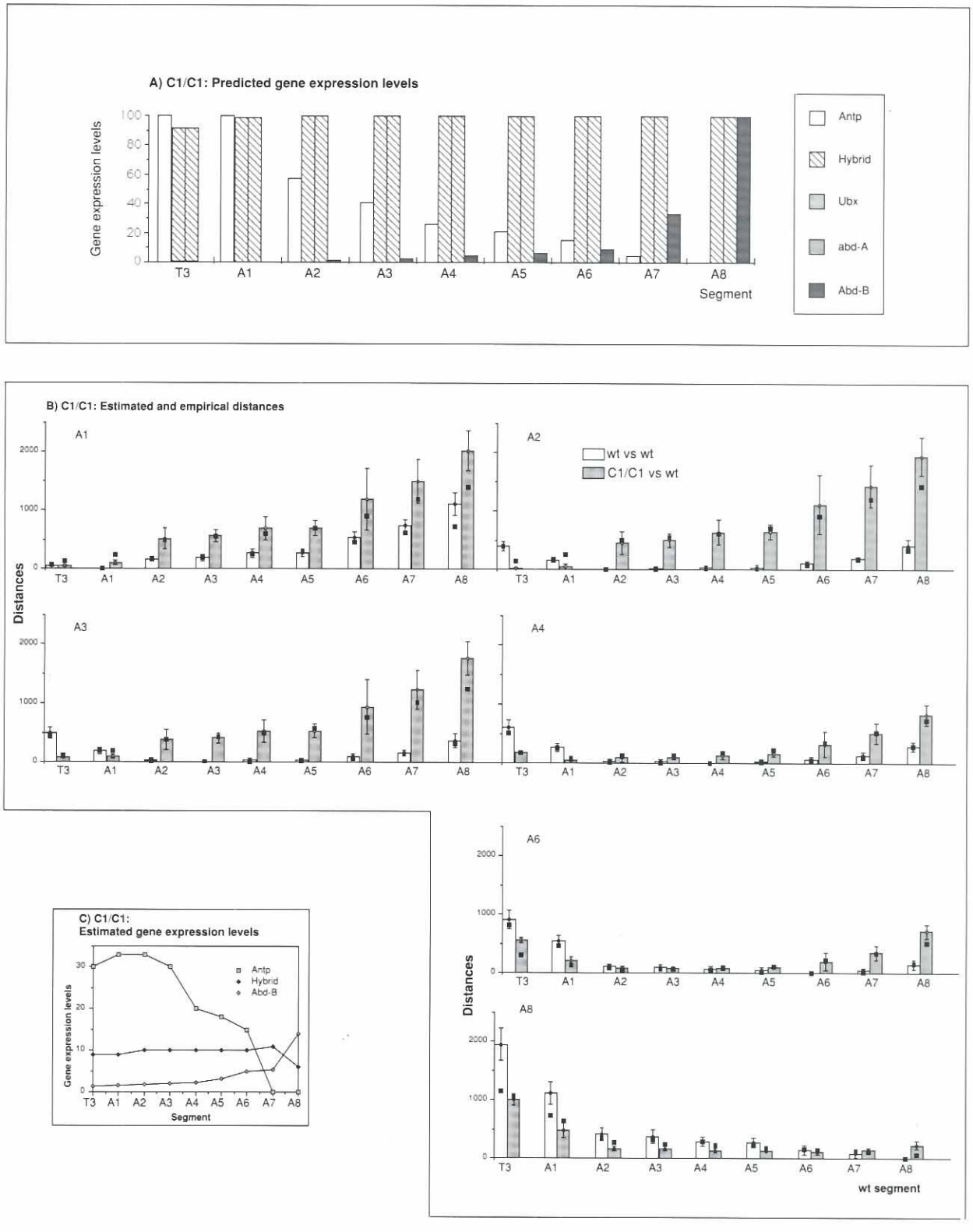

Fig. 7. (A) Predicted gene expression levels for C1/C1 larvae, *obtained from the model equations with the appropriate changes (see text) and using the levels for the general repressor system and posterior repressor element shown in Fig. 1.* **(B)** *Estimated and empirical distances from each segment of the wild type and* C1/C1 *larvae to wild type segments. Empirical distances are plotted as bars together with their standard deviation intervals (the units are* 10^{-4}*in*^{2}*/point). Filled squares are the estimated distances in arbitrary units obtained from the estimated gene expression levels shown in* **(C)**. *The same values of the hybrid gene have been used twice in the equations, once for* Ubx *and one for* abd-A *gene expression levels.*

agreement with the profiles predicted by the network gene model and shows that the proposed model is able to explain the C1 phenotypic alterations.

Discussion

The major conclusion of this paper is that a surprisingly good agreement is achieved between experimental and predicted values using a relatively simple approach to the problem of the establishment of pattern directed by the set of selector homeotic genes, namely *Antp* and the bithorax complex (García-Bellido, 1975; Lewis, 1978; Gehring, 1987), from a minimal quantitative model starting from the level of gene regulation to the phenotypic expression of the pattern controlled by these selector genes. The feasibility of this approach was established in a preceding paper (Carratalá *et al.*, 1989) using the known data of *in situ* expression of these genes in the embryo and the phenotypic ventral denticle belt patterns of wild type and homozygous Ubx^{-}/Ubx^{-}, $abd\text{-}A^{-}/abd\text{-}A^{-}$ and $Abd\text{-}B^{-}/Abd\text{-}B^{-}$ larvae. The validity of any model must be established by comparisons between its predictions and the experimental data obtained in situations not taken into account in the initial set of data from which it was deduced. Here we have examined the situation in the case of the larval heterozygotes for the 3 genes of the bithorax complex. The agreement between empirical and calculated distances in this case is also fairly good, as was the case in our initial analysis of the homozygotes.

In addition to these results in terms of the model, our method of phenotypic analysis of the larval ventral denticular patterns has allowed us to detect a previously undescribed (to our knowledge) dominant effect or haploinsufficiency produced by the presence of only a single dose of each of the three genes of the bithorax complex. Moreover, this result is clearly predicted by our model. This subtle dominant effect of the presence of a single mutant gene is especially clear in the case of the $abd\text{-}A^{-}$ heterozygotes where the statistical significance of the data is more firmly established (Fig. 4C).

This model system also allows us to interpret the phenotypic and genotypic results of a very unusual combination, i.e., the hybrid gene product in the C1 deficiency in which the 5' end of *abd-A* is fused to the 3' end of *Ubx*. In fact the model can also be used to predict the consequences of what will happen if particular types of regulation are lost. For instance what would be the level of gene expression and ventral denticle belt pattern if *Ubx* loses the regulation by *abd-A* gene product, or other similar cases. This work would also be of help in localizing particular regions in the sequence that may be candidates for the regulatory sites of the genes in the network. In fact, if particular regulatory sites in the genes were mapped, this approach might be used to interpret the phenotypes of the mutations in which these sites were altered (Carratalá *et al.*,1988). The complexity of the controlling regions of many of these genes is well established at least for the bithorax complex genes (Hogness *et al.*, 1985), *Antp* (Schnewly *et al.*, 1986), *engrailed* (Drees *et al.*, 1987) and *hairy* (Howard *et al.*, 1988).

Finally, it must be pointed out that the model has to be further refined. The model should incorporate the known property that many of the genes are capable of autoactivation (Beachy *et al.*, 1988) or of showing the phenomenon of transvection, i.e. that the degree of chromosome pairing affects the level of gene expression (Lewis 1985; Benson and Pirrotta 1987; Biggin and Tjian 1988; Biggin *et al.*, 1988). In addition such refinement should take two directions. First, it has to be extended from one dimension to two or even three dimensions and should include the differentiation between anterior and posterior compartments and the presence of additional cuticular landmarks in the larvae. The analysis could also be extended to later stages in development, to interpret features of the adult cuticle.

Other features of the regulatory gene network are currently being incorporated. For example, additional genes - both members of the network and regulatory genes. With the exception of the secondary controlling genes, modeled here as a relatively simple gradient decreasing towards the posterior end of the larva, the rest of the system is self-stabilized and would behave in a cell autonomous fashion, as the mutants in the selector genes are known to be (for a review see Duncan, 1987). We realize that the genes controlling the expression of the homeotic genes must achieve the predicted level of expression by a more complex mechanism than that implied by the simple gradient described here, for instance a self-regulating network of genes that produces several stable states of gene products corresponding to the levels obtained in the model. Furthermore, our model so far addresses the question of the *maintenance* of the regulation of pattern and gene expression along the larva *but not how it is initially established* in the embryo. Much data (Duncan, 1986; Scott and O'Farrell, 1986; White and Lehman, 1986; Martínez-Arias and White 1988) indicate that the segmentation genes are involved in the switching on of the selector genes. Our current work indicates that further refinement and/or extension of the model system will be able to cope with at least some of these aspects, taking our model to a stage at which it may be capable of predicting and/or explaining a significant amount of the experimental information that is rapidly accumulating in the exciting problem of pattern formation in the insect body.

Materials and Methods

The stocks used were Oregon-R wild type strain (Fig. 3A), Ubx^{130}/TM1 (Fig. 3B), $abd\text{-}A^{MX1}$/TM1 (Fig. 3C), $Abd\text{-}B^{M5}$/TM1 (Fig. 3D) and *C1/C1* (Fig. 3E) mutants (Sánchez-Herrero *et al.*, 1985)

Larval cuticles were prepared and mounted from recently hatched embryos as described (van der Meer, 1977).

The method of comparison

To analyze the pattern of the denticle bands, larvae of the genotypes indicated above were mounted and microphotographs of the ventral cuticle were taken (x160) and digitized with 0.001 inches resolution. The digitization consisted of storing the relative x, y coordinates of the different denticles.

The pattern of points obtained after digitizing had a width of

less than 6 inches and an anterioposterior length of less than 1.5 inches. Therefore, a 6 x 1.5 in window was selected to write the digitized coordinates. The spatial patterns of the points were oriented and centered by fitting a straight line by a least squares approximation through the center of the denticle belts, rotating and moving the line and the pattern until they were parallel to the x axis. The central x coordinate was made equal to 300 making the y coordinate of the more posterior row of denticles zero in the pattern. To minimize the effects of the different sizes of embryos the following procedure was used. The segment in the larva with the greatest width, (usually A3 or A4), was made equal to a fixed width (600 units) and the rest of the segments in the larva were modified to maintain the same relative difference to the widest segment.

To compare two segmental patterns and to obtain a value for the degree of similarity between them, every two segments were overlaid (Fig. 2) and the sum of Euclidean distances between each point of the first segment to the nearest neighbor of the second, and vice versa, was calculated. The sum of the square of the distances was divided by the number of denticles in the comparison, giving an initial empirical distance D_{ijo} (see Fig. 2A). Finally, the difference in denticle patterns in identical segments in different larvae is used as an estimation of the error (or 'noise') introduced in the comparisons. Therefore the corrected estimated distance is given by the following expression

$$D_{ij} = D_{ijo} - (D_{iio} + D_{jjo}) / 2$$

where D_{ij} is the corrected distance from the ith segment to the jth segment between two larvae.

A value for each of the expression levels of the four genes *Abd-B* (b), *abd-A* (a), *Ubx* (u) and *Antp* (t) in each segment is initially arbitrarily chosen (Fig. 2B). A second measure of distance, the *estimated distance,* is determined between every two segments by taking the sum of square differences between the four estimated levels of the genes in these two segments (Fig. 2C). By an iterative process these initial estimated levels are modified to obtain the best fit by least squares between estimated and empirical distances (Fig. 2D). In this way, the estimated distances and gene expression levels are obtained.

The estimated levels of gene expression are thus obtained from the differences in spatial pattern in the denticle bands in the different segments, given the basic assumption of a system of four genes. The estimated levels (as an indication of 'actual' gene activity) can then be directly compared with the predicted levels obtained from the model equation system.

In this way the distances between the segments of four different wild type larvae (mm strain), three *Ubx-/+* larvae, three *abdA$^-$/+* larvae, three *Abd-B$^-$/+* larvae and a single *C1/C1* larva were obtained.

The calculations for the comparisons were performed on a VAX 11/780 using Fortran or on a Macintosh Plus or an Apple IIe using compiled Basic.

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