GENETIC ANALYSIS OF THE ANTENNAPEDIA GENE COMPLEX (ANT-C) AND ADJACENT CHROMOSOMAL REGIONS OF *DROSOPHILA MELANOGASTER*. II. POLYTENE CHROMOSOME SEGMENTS 84A–84B1,2[1]

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ABSTRACT

The existence of a gene complex in the proximal right arm of chromosome 3 of *Drosophila melanogaster* involved in the development of the head and thorax was originally suggested by the phenotypes of several dominant homoeotic mutations and their revertants. A screen for mutations utilizing *Df(3R) Antp^{Ns+R17}* (proximally broken in salivary region 84B1,2) yielded, among 102 recovered mutations, 17 localized by deficiency mapping to the putative homoeotic cluster. These fell into four complementation groups, two of which were characterized by homoeotic phenotypes. To explore the limits of the Antennapedia gene complex (ANT-C) more proximally, a second screen has been undertaken utilizing *Df(3R)Scr*, a deficiency of 84A1–B1,2.——Of 2832 chromosomes screened, 21 bearing alterations localized to polytene interval 84A–84B1,2 have been recovered. Sixteen are recessive lethals, and five showing reduced viability display a visible phenotype in surviving individuals. Complementation and phenotypic analyses revealed four complementation groups proximal to those identified in the previous screen, including two new alleles of the recessive homoeotic mutation, proboscipedia (*pb*). Ten of the new mutations correspond to complementation groups defined previously in the *Df(3R)Antp^{Ns+R17}* screen, four to the *EbR11* group, two to the *Scr* group and four to the *Antp* group.——On the basis of the phenotypes of the 39 mutations localized to this region, plus their interactions with extant homoeotic mutations, we postulate that there are at least five functional sites comprising the ANT-C. Three have been demonstrated to be homoeotic in nature. The specific homoeotic transformations thus far observed suggest that these loci are critical for normal development of adult labial, maxillary and thoracic structures.

A SERIES of genes resident in the vicinity of salivary doublet 84B1,2 in proximal *3R* appears to control developmental decisions in the head and thorax much as the more distally localized bithorax complex (BX-C) mediates decisions in posterior segments (E. B. LEWIS 1978). These genes were initially implicated in this role on the basis of adult phenotypes of several homoeotic mutations

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associated with 84B1,2. A recent investigation (R. LEWIS *et al.* 1980) has recovered new mutations by screening EMS- and X-ray-treated third chromosomes over *Df(3R)Antp^{Ns+R17}* [Df(3R)84B1,2–84D11,12]. In that study, 17 of the recovered mutations were localized to 84B1,2 and fell into four complementation groups, at least two of which are homoeotic in nature.

The fact that the proboscipedia (*pb*) locus, mutations of which are characterized by a homoeotic transformation of labial palp to tarsus or antenna (KAUFMAN 1978), is localized to nearby 84A indicates that additional genes affecting head segmentation may be resident proximal to the limit of *Df(3R)Antp^{Ns+R17}*.

Thus, the analysis of the putative homoeotic gene complex, designated the Antennapedia complex (ANT-C), has been extended by the recovery and characterization of mutations in region 84A. These new mutations were identified by their failure to complement *Df(3R)Scr*, which was obtained from D. A. R. SINCLAIR and is deficient for 84A1 to 84B1 (KAUFMAN 1978). Specifically, we hoped to define functional sites not exposed in the previous screen, recover new alleles of proboscipedia and obtain additional alleles of genes located in the region of overlap of the two deficiencies. It was anticipated that an analysis of mutations within *Df(3R)Antp^{Ns+R17}* would provide a check on the extent of "saturation" achieved in the first screen and might clarify the nature of the two loci obtained in the initial analysis that are not associated with any obvious adult homoeotic phenotype.

MATERIALS AND METHODS

All flies were raised at 25° on standard cornmeal-agar-molasses-Brewer's yeast medium. Mutations and chromosomes of interest are described in Table 1 and in R. LEWIS *et al.* 1980. More complete descriptions may be found in LINDSLEY and GRELL (1968).

Generation and recovery of mutations: Males homozygous for the chromosome 3 markers red (*3–53.6*) and ebony (*3–70.7*) were treated with 0.0125 M ethyl methanesulfonate according to the method of LEWIS and BACHER (1968). These males were crossed to virgin females heterozygous for *Df(3R)Antp^{Ns+R17}* and the chromosome 3 balancer *In(3LR)TM3, ri p^{p} sep bx^{34e} Sb e^{s} Ser*, which is marked with the dominant bristle marker Stubble (*Sb*). A total of 2,832 F_1 virgin females heterozygous for a treated chromosome 3 and *TM3,Sb* were successfully mated to *Df(3R)Scr/TM3,Sb* males. The *Df(3R)Scr* chromosome is deficient for the polytene interval 84A1–84B1,2. The absence of the F_2 progeny class bearing wild-type (*Sb+*) bristles is indicative of the induction of a lethal mutation in the region deleted by *Df(3R)Scr*. Such lethal chromosomes were recovered in the sibling class and maintained in balanced stocks with the *TM3,Sb* chromosome. Visible and semi-lethal (defined by the critical class demonstrating less than 20% expected viability) mutations were recovered and maintained as well. Each mutation was retested over the screening deficiency.

An alpha-numeric code was used to identify recovered mutations. "E" refers to the mutagen, EMS; "f" designates the *red e* genetic background of the chromosome 3 on which the mutations were generated; the third letter identifies the discoverer; and the number indicates the order of discovery.

Complementation analysis: Heterozygous males from each of the balanced mutant stocks were crossed to *Df(3R)Antp^{Ns+R17}/TM3,Sb* females. The results of these crosses separated the new mutations into a proximal group, members of which complement this deficiency, and a distal group whose members fail to do so.

The new mutations were analyzed by complementation tests to mutations previously localized to this region (LEWIS *et al.* 1980). For any test, *mutation-i/TM3,Sb* males were crossed to

TABLE 1

Characteristics of deficiencies and extant homoeotics of the ANT-C

Deficiency or homoeotic mutation	Symbol	Description
Df(3R)Scr		*Df(3R)* 84A1,2–84B1, males show reduced sex-comb phenotype
Df(3R)Antp^{Ns+R17}		*Df(3R)* 84B1,2–84D11,12, males show reduced sex-comb phenotype
Antennapedia	*Antp*	Antenna → mesothoracic leg
Antennapedia-Extra Sex Combs	*Antp^{Scx}*	Meso- and metathoracic leg → prothoracic leg
Humeral	*Hu*	Extra bristles on humerus, sternopleura bristles on propleura when heterozygous with *Df(3R)* 84B1
Multiple Sex Combs	*Msc*	Males show reduced sex-comb phenotype, Meso- and metathoracic → prothoracic leg
proboscipedia	*pb*	Labial palps → antenna Labial palps → leg

mutation-i/TM3,Sb females, and the progeny examined for flies with wild-type (*Sb+*) bristles. The number of progeny heterozygous for the *TM3,Sb* chromosome was used to estimate the expected number of *mutation-i/mutation-j* individuals in order to assess the relative viability of latter class. Each cross was performed twice with a minimum of 100 progeny counted.

Phenotypic descriptions: For closer examination of some adult phenotypes, flies were boiled in 10% KOH for 10 min. Appropriate body parts were dissected free, mounted in Gurr's hydramount and viewed under the light microscope. To quantify the reduced sex-comb phenotype, a minimum of 30 male prothoracic legs were mounted and observed for each genotype.

For scanning electron microscopy (SEM), selected flies were anesthetized with CO_2, mounted on stubs with silver conductive paint and examined in an ETEC autoscan SEM.

A dominant marker Chubby (3–47.8) (VALENCIA 1968), which confers a characteristic "fatness" on pupae and adults, was used to distinguish certain mutant combinations that survive as far as pupation. Crosses of "mutant"/*Ch^v* × "mutant"/*Ch^v* or "deficiency"/*Ch^v* were performed. Lethal progeny (*i.e.*, "mutant"/"mutant" or "mutant"/"deficiency") were readily distinguished by their distinctly non-Chubby morphology. Individuals surviving long enough to yield pharate adults were sometimes dissected free from the pupa case, boiled in KOH, mounted in Gurr's hydramount and observed under the light microscope. The close linkage of *Ch^v* to the 84B–D interval (L. CRAYMER, personal communication) virtually insures its regular segregation from the lethals in the region in heterozygous females.

RESULTS

Among the 2832 EMS-treated third chromosomes screened over *Df(3R)Scr*, 21 mutations were recovered. Five were viable over the deletion, but appeared in lower than expected proportions. Mutations showing < 80% viability were classified as subvital, those with < 20% viability were considered semilethal.

Four of the mutations with reduced viability were associated with visible mutant phenotypes and will be described in more detail below. The remaining 16 mutations were lethal.

Complementation analysis: By testing the newly recovered mutations for complementation with *Df(3R)Antp*$^{Ns+R17}$, a proximal-distal grouping of the mutant sites was obtained. Mutations localized to the distal region deleted by both *Df(3R)Scr* and *Df(3R)Antp*$^{Ns+R17}$ were tested for complementation with the dominant homoeotic mutations, revertants of *Antp*^{Ns} and the EMS- and X-ray-induced recessive lethals previously localized to this region by LEWIS *et al.* (1980). The mutations localized to the proximal region exposed by *Df(3R)Scr* were tested in heterozygous combination with *pb* and with one another.

The results of these tests generated the complementation map seen in Figure 1. Although the linear order of some of the distal sites may be deduced from their interaction with rearrangements (KAUFMAN, LEWIS and WAKIMOTO 1980), the

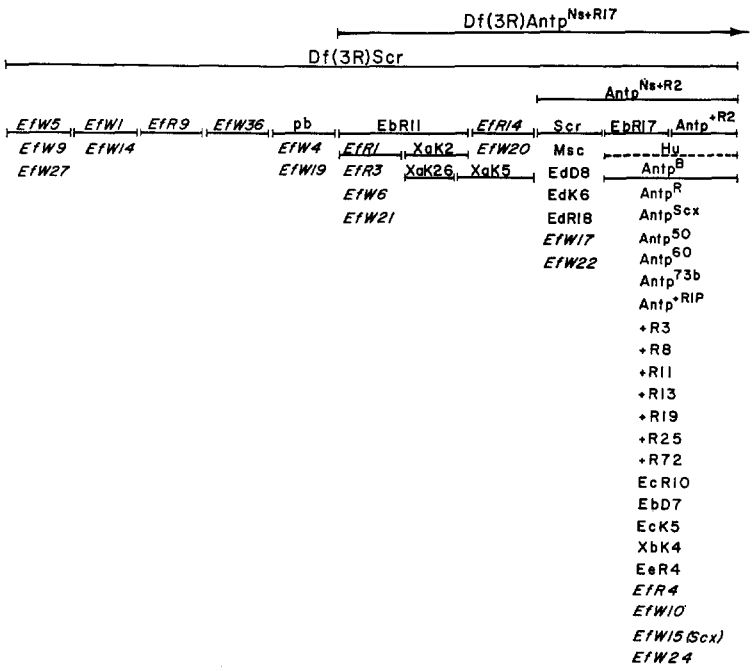

FIGURE 1.—Complementation map of the mutations in region 84A–B of the right arm of polytene chromosome 3, including the ANT–C. This map represents the results of complementation tests among mutations derived from 3 separate experiments plus several extant homoeotic mutations. *Antp*$^{+R}$ and *Antp*$^{Ns+R}$ denote revertants of *Antp*^{73b} and *Antp*^{Ns}, respectively. *Xa*, *Xb*, *Eb*, *Ec* and *Ed* are X-ray and EMS-induced mutations recovered by LEWIS *et al.* (1980). The *Ef* mutations are the EMS-induced lesions recovered in this study. The dashed line under Humeral (*Hu*) indicates that it is viable, but more extreme in phenotype, with the lethal members of the *Antp* group. With the exception of proboscipedia (*pb*), all other groups in the map are lethal. (For the other abbreviation used in the map, see text.)

proximal sites and their positions with respect to the *pb* locus are arbitrary at this point.

Nine *Ef* mutations complement *Df(3R)Antp*$^{N8+R17}$ and are believed to reside in section 84A. These lesions form five complementation groups. Two sites are defined by single members, *EfW36* and *EfR9*. *EfW1* and *EfW14* are allelic and form a third complementation group. Thus far, we can say nothing more as to the nature of these lesions other than that they are lethal over *Df(3R)Scr*.

The EfW5 *complementation group:* The *EfW5* complementation group consists of *EfW5*, *EfW9* and *EfW27*. The results of complementation tests among these three lesions and *Df(3R)Scr* are presented in Table 2. Both *EfW5* and *EfW27* are characterized by temperature effects on viability. When heterozygous for *Df(3R)Scr* or *EfW9*, the percent viability of the mutant *EfW5* or *EfW27* class is inversely correlated with temperature from 18° to 28°. In addition, the *EfW5* lesion is associated with temperature-sensitive mutant eye and bristle phenotypes. *Df(3R)Scr/EfW5* and *EfW5/EfW9* individuals appear normal when raised at 18°. However, at higher temperatures, the bristles appear reduced and twisted, and the surface of the eye becomes roughened due to a disruption of the normal pattern of eye facets. At 25°, the kinked bristle phenotype may be limited to the first four abdominal segments, but at 28° all bristles are affected. *EfW9* is lethal when heterozygous with *Df(3R)Scr* at 18°, 25° and 28°.

The pb *complementation group:* Two new proboscipedia alleles, *pb*^{W4} and *pb*^{W19}, were recovered. The phenotypes of individuals heterozygous for these alleles and *Df(3R)Scr* are shown in Figure 2. Both alleles fail to complement all five of the extant *pb* mutants. Like *pb*^{4}, (KAUFMAN 1978) the transformations of *pb*^{W4} and *pb*^{W19} are from labial palps to antennae as evidenced by the terminal aristae. However, the alleles are somewhat different in terms of the nature and extent of the transformed tissue. While the *pb*^{4} allele appears to be restricted in its action to the first three pseudotracheal rows of the proboscis (KAUFMAN 1978), the transformations associated with the new alleles extend to more medial tissue (Figure 2a, c). The labella of *Df(3R)Scr/pb*^{W4} individuals also show signs of proboscis-to-leg transformation; the aristal structure is thickened at its base and bracted bristles, apical bristles and a terminal claw are frequently observed. These leg-like structures appear less often in the mouthparts of *Df(3R)Scr/pb*^{W19} and are never observed in *pb*^{W4}/*pb*^{W19} heterozygotes. In individuals of the latter genotype, the entirety of the labella appears to be transformed into antennal structures. Additionally, all *pb*^{W4} and *pb*^{W19} transformations show an increase in the number of bristles on the prementum.

The chromosomes bearing the new *pb* alleles are lethal when homozygous. Since previous work suggests that *pb* is not a vital locus (KAUFMAN 1978), and since both *pb*^{W4} and *pb*^{W19} lesions are viable over *Df(3R)Scr*, the homozygous lethality is in all likelihood due to the presence of unrelated lethal mutations. Experiments attempting to separate the new *pb* alleles from the putative recessive lethal mutations by recombination are in progress.

The EbR11 *complementation group:* The *EbR11* complementation group was originally defined by the mutations *EbR11*, *XaK2*, *XaK5* and *XaK26*. Three

TABLE 2

Results of complementation tests among members of the EfW5 *group*

			Df(3R)Scr			*EfW9*			*EfW27*	
		18°	25°	28°	18°	25°	28°	18°	25°	28°
EfW5	No. individuals of critical class/total	57/205	14/69	3/254	9/123	1/116	1/493	50/181	94/229	156/538
	% viability	83.4%	60.8%	3.5%	21.9%	2.6%	0.6%	82.9%	87.4%	87.1%
	Eye phenotype	+	roughened	extreme roughened	+	roughened	extreme roughened	+	+	weak roughened
	Bristle phenotype	+	kinked on abdomen thorax	All bristles kinked	+	kinked on abdomen	All bristles kinked	+	+	few kinked on abdomen
EfW27	No. individuals of critical class/total	14/210	0/232	0/190	3/157	0/515	0/514			
	% viability	20%	0%	0%	5.7%	0%	0%			
	Eye phenotype	+			+					
	Bristle phenotype	+			+					
EfW9	No. individuals of critical class/total	0/204	0/217	0/205						
	% viability	0%	0%	0%						
	Eye phenotype									
	Bristle phenotype									

+ = wild type.

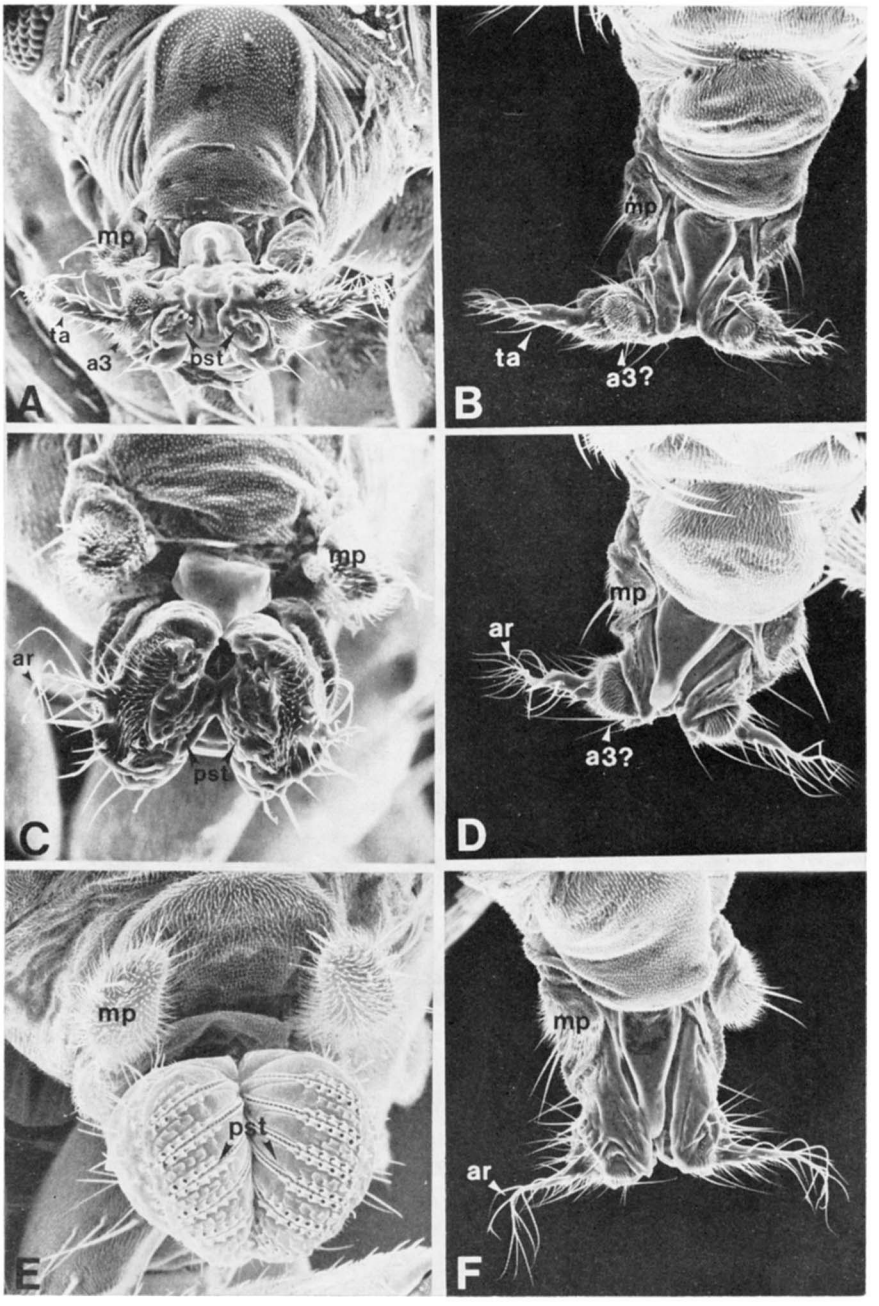

FIGURE 2.—Scanning electron micrographs of the labellar lobes of the *pb* mutations recovered in this study. (A) $pb^{W4}/Df(3R)Scr$ showing a remnant of pseudotracheal tissue (pst). (B) $pb^{W4}/Df(3R)Scr$ showing tarsal tissue (ta), as well as what may be third antennal segment (a3?) tissue replacing the labellar lobes. (C) $pb^{W19}/Df(3R)Scr$ showing the pseudotracheal remnant, as well as arista (ar) (D) $pb^{W19}/Df(3R)Scr$ showing a well developed arista, as well as more proximal antennal tissue. The arista is swollen basally and bears an apical bristle (apb) indicating the presence of some tarsal tissue. (E) Ore-R labellar lobes and maxillary palps (mp). (F) pb^{W4}/pb^{W19} showing a well developed arista and the absence of definitive tarsal structures.

of these mutations, designated *Xa*, were X-ray-induced lesions on a chromosome carrying the marker Deformed (*Dfd*). *EbR11* was EMS induced on a *Dfd*$^+$ chromosome (LEWIS *et al.* 1980).

Four new mutations recovered in the screen over *Df(3R)Scr* define a new subsite of *EbR11*, separable from the associated *Xa* site(s). These new lesions, *EfR1*, *EfR3*, *EfW6* and *EfW21*, all fail to complement *Df(3R)Antp*$^{Ns+R17}$, *EbR11* and each other, but complement the lethalities of *XaK2*, *XaK5* and *XaK26*. Two other newly recovered lesions, *EfR14* and *EfW20*, complement *EbR11*, but are lethal over each other, *Df(3R)Antp*$^{Ns+R17}$ and *XaK5*. Although *EfR14* and *EfW20* fully complement the recessive lethalities of *XaK2* and *XaK26*, they enhance the *Dfd* phenotype in the genetic background of these mutations. The results of *inter se* crosses among these lesions are presented in Table 3. Their complementation relationships are represented in Figure 1.

Interpretation of the complementation behavior of the *EbR11* group has been complicated by the pre-existing *Dfd* mutation on the *Xa* chromosome. *XaK2*, *XaK5* and *XaK26* were recovered by LEWIS *et al.* (1980) in a screen for mutations over *Df(3R)Antp*$^{Ns+R17}$. In this screen, the *Dfd p*^{p} chromosome used for mutagenesis was originally homozygous viable (SINCLAIR, 1977). However, this chromosome later proved too inefficient for continued use in the screen because the untreated chromosome demonstrated rather low viability (16.7% viability) when heterozygous with *Df(3R)Antp*$^{Ns+R17}$. However, seven X-ray treated *Dfd p*^{p} chromosomes were recovered that showed either significantly lower viability relative to the parent chromosome or were completely lethal over the screening deficiency. These were retained and tested for complementation with other proximal *3R* mutations. Three mutations (*XaK2*, *XaK5* and *XaK26*) mapped within the ANT-C, all falling within the *EbR11* complementation group. Additional complementation crosses (Table 3) demonstrate that the original *Dfd p*^{p} chromosome is lethal over *XaK2* and shows reduced viability (14.8%) over *EbR11*. These data, together with the confirmed localization of the *EbR11* and *XaK5* lethal sites to 84B1,2 by the recovery of the new *Ef* mutations, suggest that the

TABLE 3

Additional complementation relationships of XaK2, XaK5 *and* XaK26

	Percent viability		
	XaK2	*XaK5*	*XaK26*
Df(3R)Antp^{Ns+R17}	0%	0%	4%
Dfd p^p	2%	100%	100%
EfR14	100%*	0%	100%*
EfW20	100%*	0%	100%*
EbR11	0%	7.5%	26%
Control crosses			
EbR11/Dfd p^p = 14.8%; *Df(3R)Antp*$^{Ns+R17}$*/Dfd p*^{p} = 16.7%			

* *Dfd* phenotype enhanced.

Dfd mutation may interact with the ANT-C. The significance of this possibility will be examined in the DISCUSSION.

The Scr *complementation group:* We have defined in previous studies the *Scr* or Sex combs reduced locus (KAUFMAN, LEWIS and WAKIMOTO 1980; LEWIS *et al.* 1980). That this locus is haplo-insufficient is demonstrated by the *Scr* phenotype of males bearing *Df(3R)Scr*/+, *Df(3R)Antp*$^{Ns+R17}$/+ or a heterozygous deficiency of 84B1,2 generated by elements of *Y;3* translocations (KAUFMAN, LEWIS and WAKIMOTO 1980). This phenotype is also expressed in males heterozygous for the dominant homoeotic mutation Multiple sex combs (*Msc*). In the present work and that of LEWIS *et al.* (1980), five new mutations have been identified that fail to complement *Df(3R)Scr*, *Df(3R)Antp*$^{Ns+R17}$ and the recessive lethality of *Msc*. All of these mutations show the reduced sex comb phenotype over a normal chromosome *3*. Hence, the recessive lethality of *Msc* can be localized to the *Scr* locus, as can the dominant reduction of the number of sex-comb teeth seen in *Msc*/+ males.

The results of complementation tests among the *Scr* lesions are summarized in Table 4. Two of the mutations, *EdR18* and *EfW22*, are semi-lethal over *Df(3R)Scr*, while the other alleles are lethal when hemizygous. In various heterozygous combinations, the mutations exhibit a range of effects on both viability (0 to 100%) and on the reduced sex-comb phenotype.

Interactions of the new alleles, *EfW22* and *EfW17*, with other *Scr* lesions demonstrate clear reductions in the number of sex-comb teeth to a complete absence of the sex comb. In the more extreme cases, the bristle pattern and morphology of the foreleg of males and females are identical to those of the mesothoracic leg. Hence, these observations are consistent with the earlier interpretation that the reduced sex-comb phenotype represents a ventral prothoracic to mesothoracic transformation (KAUFMAN, LEWIS and WAKIMOTO 1980; LEWIS *et al.* 1980).

Like the previously described *EdR18* (LEWIS *et al.* 1980), *EfW22* shows an abnormal proboscis when heterozygous for a deficiency of 84B1,2. The six pseudotracheal rows present in the normal proboscis are reduced to three or less

TABLE 4

Results of complementation tests among members of the Scr *group*

	EfW22	*EdR18*	*EdD8*	*EdK6*	*EfW17*	*Msc*	*Df(3R)Scr*
TM3	100%/6.8	100%/7.29	100%/5.38	100%/5.68	100%/5.5	100%/5.96	100%/6.16
Df(3R)Scr	8.4%/0*	2%/1.45*	0%/–	0%/–	0%/–	0%/–	0%/–
EfW22	78.9%/3.36	100%/5.0	100%/0*	63%/0*	1.9%/0*	0.7%/0*	
EdR18			0.5%/–	0%/–	0%/–	25%/–	
EdD8				0%/–	0%/–	0%/–	
EdK6					0%/–	0%/–	
EfW17					0%/–	0%/–	
Msc						0%/–	

a/b*: a = % viability. b = Mean no. sex-comb teeth. * = Abnormal proboscis.

in *EfW22/Df(3R)Scr* individuals. The lateral aspect of the labellum shows an increase in the number of bristles and a change in the bristle pattern to resemble a maxillary palp. The abnormal proboscis phenotype is also observed in heterozygotes of *EfW22* with *EdD8*, *EdK6*, *EfW17* or *Msc*, but not in *EfW22/EdR18* individuals.

The results of the quantitative assessment of the effects of the *Scr* lesions on the reduced sex-comb phenotype and viability suggest that the *Scr* lesions represent a series of hypomorphic mutations that can be ordered from least to most stringent as: $Scr^{+} > EfW22 \geq EdR18 > EdD8 \geq EdK6 \geq EfW17 \geq Msc > Df(Scr^{+})$.

The Antp *complementation group:* Four new recessive lethal mutations, *EfW15*, *EfW24*, *EfW10* and *EfR4*, were recovered and localized by complementation to the *Antp* locus. The previous screen introduced six recessive lethal mutations to this site (*EcR10*, *EcK5*, *EdD7*, *EbR17*, *EeR4* and *XbK4*). *EeR4* has since been lost. *EfW15* is unique among the four new lesions in that it shows an $Antp^{Scx}$-like dominant transformation of meso- and metathoracic to prothoracic legs. However, it appears to effect a more extreme homoeotic transformation of mesopleura to propleura than does the original $Antp^{Scx}$, as is evidenced by the disruption or removal of the sternopleural bristles in *EfW15/+* individuals.

Determinations of the effective lethal phases of selected mutations localized to the *Antp* site have disclosed a developmental anomaly of the humeral region in heterozygotes surviving to pharate adults. Third-instar larvae bearing these lesions fail to evert their anterior spiracles at pupation, resulting in aberrant anterior closure of the pupal case and, thereby, blunt-ended pupae (Figure 3). This phenotype is observed in *EcR10/deficiency* individuals and in heterozygotes of *EcR10* with *EbR17*, *EfR4*, *EbD7*, *EcK5*, *EfW24*, $Antp^{Scx}$ and $Antp^{73b}$ as well. Individuals surviving to pharate adults were obtained in all heterozygous combinations involving *EcR10* and were dissected free from the pupal case. A variety

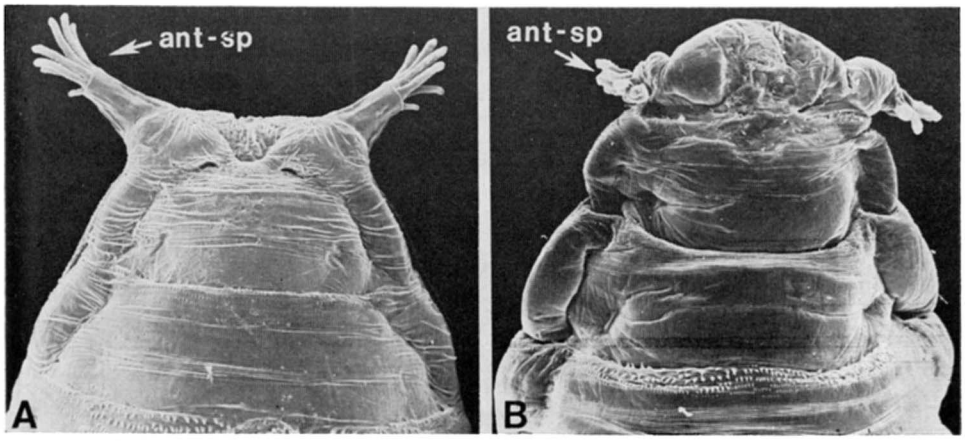

FIGURE 3.—Scanning electron micrographs of the anterior end of pupae (A) Ore-R. (B) *EcR10/Df(3R)Scr*; note the failure of normal eversion of the anterior spiracles (ant-sp) in the mutant individual, as well as the rounded, protruding nature of the head region between the two spiracles.

of defects associated with the humeral area was observed, including necrosis, amorphous bumps and stalks, and absence or duplication of tissue (Figure 4). This variable phenotype is similar to that noted in Polycomb/$Antp^{Ns+R25}$ and Polycomb/ $Antp^{Ns+R72}$ heterozygotes (DENELL 1973). *EcR10/EfW24* individuals survived longer and in greater numbers than other genotypes, often emerging from their pupa cases without assistance. These individuals showed many of the same humeral disc derivative defects described above.

Several other genotypes (*EbR17/deficiency*; *EbR17*/$Antp^{73b}$; *EfR4/deficiency*; *EfR4*/$Antp^{Scx}$; *EbD7*/$Antp^{73b}$; *EcK5*/$Antp^{73b}$) survived as far as early pupation and were characteristically blunt-ended. All other combinations involving a deficiency or any of the point mutations or dominant homoeotic mutations associated with the *Antp* site did not survive beyond the embryonic or larval stage.

DISCUSSION

Homoeotic mutations provide important model systems for investigating mechanisms of development. However, it must be emphasized that the end result of these mutations, *i.e.*, the homoeotic phenotype, does not necessarily imply that these lesions are directly involved in the determination of developmental pathways. As discussed by OUWENEEL (1976), DENELL (1978) and SHEARN, HERSPERGER and HERSPERGER (1978), it is possible that mutant genes controlling

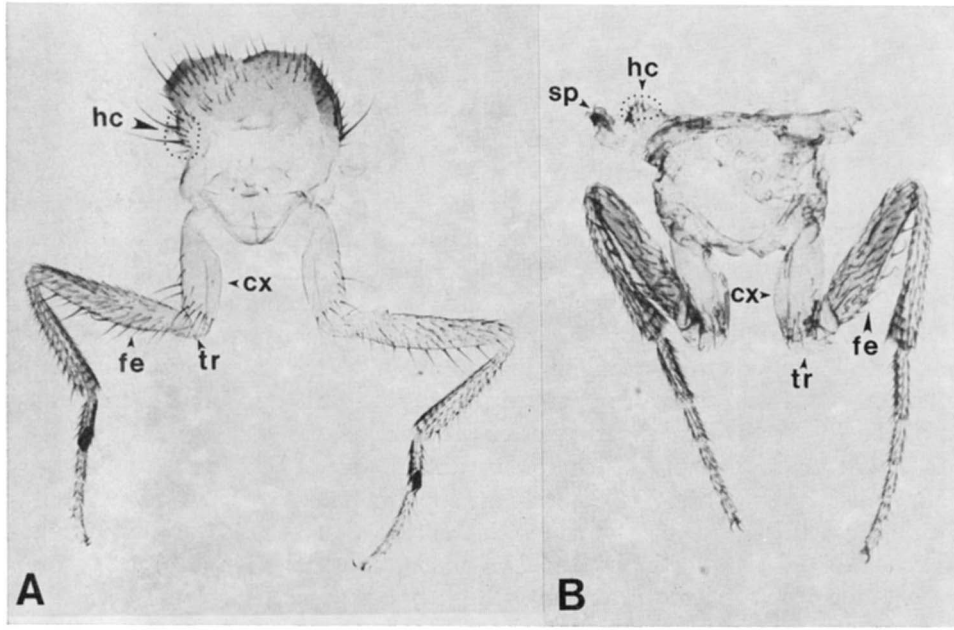

FIGURE 4.—Light micrographs of whole-body frontal sections of the prothorax. (A) Ore-R ♂. (B) *EcR10/EfW24* ♀. Note the stalk-like outgrowths in the mutant individual emanating from the pleural (lateral) area of the thorax. The stalk has as its terminus the mesothoracic spiracular opening (sp). The left stalk has a humeral callus (hc) proximally, while the right stalk does not. Other abbreviations: cx = coxa; tr = trochanter; fe = femur.

less specialized functions, such as cell proliferation, could result in homoeosis. Such genes then would not be true "selector genes" in GARCIA-BELLIDO's terminology (1977). Therefore, in order to ascertain the role of a homoeotic gene in development, it is vital to examine the genetic nature of the lesion and to establish the function of its wild-type allele. Until recently, only the bithorax complex (BX-C) had been subjected to a developmental genetic analysis sufficient to demonstrate its role in normal establishment of epigenetic fates (LEWIS 1978).

We have previously postulated that the Antennapedia complex consists of selector genes that control thoracic and head segmentation (KAUFMAN, LEWIS and WAKIMOTO 1980). Our approach in investigating this possibility has been to analyze this complex genetically by saturating the 84A–84B region with mutations. The characterization of the new lesions and their relationships to the existing dominant homoeotic mutations of proximal *3R* has led to a better understanding of the developmental function and genetic organization of this region of the genome.

Among the new complementation sites recovered in this screen and assigned to polytene section 84A is the *EfW5* group. The characteristics associated with this complementation group include temperature effects on viability, twisted bristles and roughened eyes. It is possible that the *EfW5* members are allelic to the dominant bristle mutation, Kinked (*Ki*). *Ki* was mapped initially by GRELL (1958) to proximal *3R*, to the left of pink. More recently, GREEN (1975) has shown that *Ki* maps to the left of *Antp*. Additionally, unconfirmed evidence of SINCLAIR (1977) is consistent with the localization of *Ki* to the region between the centromere and the *Dfd* locus. The relationship between *Ki* and the *EfW5* group is currently being investigated by recombination. Thus far, however, the evidence indicates that the *EfW5* mutations are not homoeotic in nature and that this site is not included in the ANT-C.

In light of the remaining mutations to be discussed, a careful reconsideration of the ANT-C is in order. The new mutations have confirmed the existence and the relative positions of the previously described *pb*, *Scr* and *Antp* complementation groups (KAUFMAN, LEWIS and WAKIMOTO 1980) and have added several new complementation sites to this region. The two newly recovered *pb* alleles demonstrate the potential of labial discs to undergo complete transformation to antennal structures, and an apparently new potential (proboscis to maxillary palp) is seen in individuals bearing lesions at the *Scr* site.

The nature of the *EbR11* complementation group (originally defined by *EbR11*, *XaK2*, *XaK5* and *XaK26* and now supplemented by six additional mutations) suggested that *Dfd* interacts with the mutations implicated in the hypothetical ANT-C. Interestingly, the *Dfd* phenotype was mapped by SINCLAIR (1977) to a region within proximal *3R* tightly linked to Kinked (*Ki*). The *Dfd* mutation causes a ventral and lateral decrease in ommatidial tissue and tufted vibrissae. The *XaK2*, *XaK5* and *XaK26* lesions could represent variant *Dfd* alleles, perhaps more severe than the original *Dfd* mutation. The complementation results among these lesions, the parent *Dfd* p^p chromosome and *EbR11* indicate some functional interaction of *Dfd* with the *EbR11* complementation

site. Whether or not this relationship is one of allelism is at present uncertain, but such a possibility is not inconsistent with SINCLAIR's (1977) tentative localization of *Dfd*.

Strong evidence confirms that prothoracic leg development is dependent upon the wild-type activity of the *Scr* locus. Reduction from the normal two doses of *Scr* caused by hypomorphic lesions or deficiencies of 84B1,2 results in the reduced sex-comb phenotype. In extreme cases, the ventral prothorax undergoes complete transformation to mesothorax. The five new lesions have defined the single *Scr* site as responsible for both the reduced sex-comb phenotype and the recessive lethality of *Msc*.

The interpretation of the mutation *Msc* remains difficult in that it appears to combine two seemingly contradictory phenotypes—an amorphic (null) reduced sex-comb phenotype (prothoracic to mesothoracic homoeosis) and the neomorphic extra sex-comb phenotype (meso- and metathoracic to prothoracic homoeosis). The new mutations localize the recessive lethality of *Msc* and the dominant reduced sex-comb phenotype to the single *Scr* site. One interpretation is that *Msc* is a double mutant with a second nonlethal lesion at the *Antp* site, resulting in the $Antp^{Scx}$-like homoeosis. The existence of *Antp* lesions that result in a dominant homoeotic phenotype without concomitant lethality is demonstrated by the homozygous viability of $Antp^{Ns}$. Alternatively, *Msc* may be a lesion in a control function common to both Scr^+ and $Antp^+$.

The *Antp* complementation group consists of lesions that share a common recessive lethal effect, yet show diverse adult phenotypes. One member, the mutation Humeral (*Hu*), does not share the recessive lethality common to most other *Antp* lesions. However, flies of the genotype *Hu/Df(3R)Scr* or *Hu/Df(3R)-*$Antp^{Ns+R17}$ display more extreme transformations than are observed in *Hu*/+ individuals, suggesting an interaction, possibly allelism, localized to the chromosomal region of overlap between the two deficiencies (KAUFMAN, LEWIS and WAKIMOTO 1980. Enhanced transformation of flies heterozygous for *Hu* and point mutations at the *Antp* site further localize the interaction of *Hu* to the 84B1,2 region, specifically to the *Antp* complementation group. KAUFMAN, LEWIS and WAKIMOTO (1980) have previously interpreted the *Hu* phenotype as a disruption of normal development of a portion of the humeral disc, resulting in an pleural prothoracic to pleural mesothoracic homoeosis.

Additional evidence that the *Antp* site is involved in normal development of the humeral disc derivatives is obtained from the phenotype of the EMS-induced lesion, *EcR10*. When *EcR10* is heterozygous with either *EfW24* or a deficiency of 84B1,2, a variety of humeral defects is observed. The exact nature of the humeral outgrowths that appear is uncertain. The phenotype may reflect a homoeotic transformation, although no distinguishing bristle types or patterns exist on the abnormal tissue to suggest the direction of such a transformation. It should be noted that the defects associated with *EcR10* lesions are very similar to those described by DENELL (1973) for mutant interactions involving other lethal lesions of the *Antp* complementation group. In DENELL's work, the two chromosomes associated with the mutant phenotypes were recovered as revert-

ants of the dominant $Antp^{Ns}$ phenotype and are lethal in combination with the other *Antp* lethals and with the 84B1,2 deficiency chromosomes. Like the EMS-induced point mutations *EcR10* and *EfW24*, these revertants are not associated with any dominant adult phenotypes and probably represent an inactivation of the $Antp^{+}$ function.

The $Antp^{Scx}$ and *Antp* homoeotic phenotypes indicate that the *Antp* site is involved in controlling ventral thorax. We view $Antp^{Scx}$ as resulting from activation of prothoracic genes in the meso- and metathorax. Similarly, the antenna to mesothoracic leg transformation seen in *Antp*/+ heterozygotes results from activity of ventral thoracic genes in the antenna segment, where they are normally inactive or repressed. Based on these interpretations, we propose that the primary action of the *Antp* site is to promote thoracic development and that the dominant homoeotic phenotypes attributed to this site are caused by the abnormal activation of pro- or mesothoracic genes in segments where they are normally inactive.

If the normal function of the $Antp^{+}$ gene is to control thoracic development, then the interpretations of the *Antp* and the $Antp^{Scx}$ homoeotic phenotypes are consistent with genetic evidence that these lesions are neomorphs (DENELL 1973, DUNCAN and KAUFMAN 1975). Additionally, our hypothesis is consistent with the developmental studies on $Antp^{Ns}$ revertants which conclude that the $Antp^{+}$ allele is not necesesary for establishing or maintaining the normal antennal state of determination (DENELL *et al.* 1980).

We do not have sufficient evidence thus far to suggest the functions of the remaining lethal sites. Although strong evidence exists that the homoeotic genes of proximal *3R* are involved in the developmental decisions leading to normal labial, ventral prothoracic, ventral mesothoracic and humeral elements, we do not know if additional, more anterior segments are controlled by these same or neighboring genes. If wild-type activity of the ANT-C is essential for normal maxillary or mandibular segmentation in the embryo, then some lethal sites may represent specific gnathocephalic genes (TURNER and MAHOWALD 1979). Preliminary developmental studies give credence to this possibility (WAKIMOTO and KAUFMAN 1980).

Lethal-phase determinations of the members of the *EbR11* and *EfW36* complementation groups show that these are prehatching lethals when heterozygous with a deficiency chromosome. The lethal individuals show anomalies in anterior development, most notably in head involution and mouth-hook formation. We are currently investigating the additional possibility that the true selector genes responsible for normal determination of the eye-antennal disc derivatives reside in this region. None of the remaining lethals proximal to $Df(3R)Antp^{Ns+R17}$, however, show any clear larval anomalies that can be construed as segmental tranformations (WAKIMOTO and KAUFMAN 1980).

What has become clear at this point is that the interpretation of the homoeotic transformations associated with individual members of the ANT-C requires an understanding of the complex as a whole. To single out, for instance, the *Antp* mutation without consideration of the genetic nature of the lesion and its rela-

tionship to other members of the ANT-C could lead to misinterpretation of its developmental role in determination and/or differentiation.

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LITERATURE CITED

DENELL, R. E., 1973 Homoeosis in Drosophila. I. Complementation studies with revertants of Nasobemia. Genetics **75:** 279–297. ——, 1978 Homoeosis in Drosophila. II. A genetic analysis of Polycomb. Genetics **90:** 277–289.

DENELL, R. E., K. R. HUMMELS, B. T. WAKIMOTO and T. C. KAUFMAN, 1980 Developmental studies of lethality associated with the Antennapedia gene complex in *Drosophila melanogaster*. Develop. Biol. (in press).

DUNCAN, I. W. and T. C. KAUFMAN, 1975 Cytogenetic analysis of chromosome 3 in *Drosophila melanogaster*: mapping of the proximal portion of the right arm. Genetics **80:** 733–752.

GARCIA-BELLIDO, A., 1977 Homoeotic and atavic mutations in insects. Amer. Zool. **17:** 613–629.

GREEN, M. M., 1975 Conversion as a possible mechanism of high coincidence values in the centromere region of *Drosophila*. Molec. Gen. Genet. **139:** 57–66.

GRELL, R. M., 1958 Report of new mutants. Drosophila Infor. Serv. **32:** 80.

KAUFMAN, T. C., 1978 Cytogenetic analysis of chromosome 3 in *Drosophila melanogaster*: isolation and characterization of four new alleles of the proboscipedia (*pb*) locus. Genetics **90:** 579–596.

KAUFMAN, T. C., R. LEWIS and B. WAKIMOTO, 1980 Cytogenetic analysis of chromosome 3 in *Drosophila melanogaster*: The homoeotic gene complex in polytene chromosome interval 84A–B. Genetics **94:** 115–133.

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

LEWIS, E. B. and F. BACHER, 1968 Method of feeding ethyl methanesulfonate (EMS) to *Drosophila* males. Drosophila Inform. Serv. **43:** 193.

LEWIS, R., T. C. KAUFMAN, R. E. DENELL and P. TALLERICO, 1980 Genetic analysis of the Antennapedia gene complex (ANT-C) of *Drosophila melanogaster*. I. Polytene chromosome segments 84B–D. Genetics **95:** 367–381.

LINDSLEY, D. L. and E. M. GRELL, 1968 *Genetic Variations of* Drosophila melanogaster. Carnegie Inst. Wash. Publ. No. 627.

OUWENEEL, W. J., 1976 Developmental genetics of homoeosis. Adv. Genet. **18:** 179–248.

SHEARN, A., G. HERSPERGER and E. HERSPERGER, 1978 Genetic analysis of two allelic temperature-sensitive mutants of *Drosophila melanogaster* both of which are zygotic and maternal-effect lethals. Genetics **89:** 341–353.

SINCLAIR, D. A. R., 1977 Genetic and developmental studies of proximal segments of chromosome 3 of *Drosophila melangaster*. Ph.D. dissertation, University of British Columbia.

TURNER, F. R. and A. P. MAHOWALD, 1979 Scanning electron microscopy of *Drosophila melanogaster* embryogenesis. III. Formation of the head and caudal segments. Devel. Biol. **68:** 96–109.

VALENCIA, R., 1968 New mutant report. Drosophila Inform. Serv. **43:** 60.

WAKIMOTO, B. T. and T. C. KAUFMAN, 1980 Analysis of larval segmentation in lethal genotypes associated with the Antennapedia gene complex in *Drosophila melanogaster*. Develop. Biol. (in press).

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