HOMEOESIS IN DROSOPHILA. I. COMPLEMENTATION STUDIES
WITH REVERTANTS OF NASOBEMIA

R. E. DENELL*

Department of Genetics, University of Edinburgh, Edinburgh, Scotland

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ABSTRACT

A number of homoeotic mutants have been localized to the proximal right arm of chromosome 3 of Drosophila melanogaster. These include seven alleles of Antennapedia (Antp), which is associated with a transformation of antennae into legs; Nasobemia (Ns), which causes the same phenotype as Antp but was considered by GURARIO (1966) not to be an allele; and three genes causing a transformation of second and third legs into first legs: Extra sex comb (Sex), Polycomb (Pc), and Multiple sex comb (Msc). The alleles of Antp and Sex share a common recessive lethal effect, and Pc maps 0.2-0.3 units to the left of Sex.—In the present investigation, rearrangements associated with the reversion of Ns suggest that its cytological location is in or just distal to salivary chromosome doublet 84B1-2. Although Ns is viable when homozygous, four of its revertants share a common recessive lethal effect. These revertants fail to complement the recessive lethality of Antp and Sex. Furthermore, they show a complex pattern of functional interaction with Pc and with Humeral (Hu), a dominant mutation associated with a rearrangement with one breakpoint just distal to 84B1-2. Finally, analysis of a revertant of Msc indicates that Msc is also located very close to 84B1-2. It is concluded that Ns and Sex are alleles of Antp. Pc shows many functional similarities to the Antp locus, but is probably not allelic. Evidence is presented that these dominant homoeotic genes are neomorphic in nature.

THE elucidation of the mechanisms underlying the genetic control of eukaryotic development remains one of the most important problems of modern biology. Such control is now generally envisioned in terms of the temporal and spatial regulation of gene activity. Development may be conceptually subdivided into determination, which HADORN (1965) defined as "a process which initiates a specific pathway of development by singling it out from among various possibilities for which a cellular system is competent", and its observable consequences of differentiation and morphogenesis. In practice, it is often difficult to distinguish between determination and other aspects of development, but in tissues showing a clonal inheritance of a determined state, such as the insect imaginal disc, such a distinction is enhanced. It has been recognized that the genetic basis of this clonal inheritance probably involves a molecular feedback system (ABERCROMBIE 1967; HADORN 1967). BRITTEN and DAVIDSON 1969; (DAVIDSON and BRITTEN 1971) have presented a model accommodating information on

* Present address: Division of Biology, Kansas State University, Manhattan, Kansas 66506.

reiterated DNA sequences to explain the regulation of gene transcription during development; their model incorporates a genetic explanation of the clonal transmission of a determined state.

Among the genetic tools that decades of research with *Drosophila melanogaster* have provided is a group of variants called homeotic mutations. Bateson (1894) defined homeosis as the replacement of an organ of one segment by the homologous organ of another segment. Current usage has relaxed the criterion of homology, and homeosis is generally regarded as the replacement during development of one structure by another normally developing elsewhere. Gehring and Nöthiger (1973) list the homeotic mutations presently known in this organism. Virtually all mutations affect adult structures elaborated at metamorphosis from imaginal discs. Imaginal discs are set aside early in embryogenesis, each determined to form a specific complement of adult structures. Present evidence suggests that in some cases homeosis represents a change in the developmental fate of the cells of an imaginal disc during the third larval instar, rather than an initial error in determination (see Postlethwait and Schneiderman 1971).

Two general interpretations of the mechanism of homeotic mutations have been proposed. Waddington (1940) postulated the existence of a class of genes whose function is the control of other genes, and suggested that homeotic mutations are such control genes. Alternatively, a number of investigators have followed Goldschmidt (1938) in concluding that homeosis is an indirect consequence of these mutations. Recently, Hadorn (1967) suggested that these genes change the proliferation dynamics in the affected disc, causing the molecular feedback mechanism maintaining a state of determination to break down and allowing a new state to be established. Postlethwait and Schneiderman (1971) have argued convincingly against changes in proliferation dynamics as the sole mechanism of homeosis. Thus it is possible that the ability to mutate to a homeotic allele may, in at least some cases, serve to define a set of genes which act specifically to establish or maintain a state of determination. This possibility provides ample rationale for the careful study of these mutations, and of their normal alleles.

A number of homeotic mutations are located in the proximal right arm of chromosome three of *D. melanogaster*. Because this region shows an abnormally low frequency of recombination in relation to cytological length, the spatial relationships of these mutations are not well known. The purpose of this report is to present evidence reflecting on the genetic nature of these homeotic mutations, and their interrelationships.

**MATERIALS AND METHODS**

There is a severe inhibition of recombination in the proximal portion of the third chromosome. The most proximal gene in the left arm whose salivary chromosome position is known is radius incompletus (*ri*), located at 47.0 on the recombinational map, and in region 77 (or possibly 78) of the salivary map (Arajärvi and Hannah-Alava 1969). Only one percent recombination separates *ri* from pink (*p*), located at 85A-B in the right arm (see Lindsley and Grell 1968). This region represents a considerable portion of the mitotic chromosome as well, as evi-
TABLE 1

*Characteristics of the known homoetic mutants (and of the mutant Humeral) with loci in the proximal right arm of chromosome three. Recombinational positions are not indicated because of the uncertainties described in the text, but pb, Pc, Ns, and Scx are known to map between scarlet and pink.*

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Symbol</th>
<th>Phenotype</th>
<th>Salivary chromosome characteristics</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>proboscipedia</td>
<td>pb</td>
<td>oral lobes transformed to tarsus or arista</td>
<td>normal</td>
<td>BRIDGES and DOBZHANSKY 1933</td>
</tr>
<tr>
<td>Polycomb$^1$</td>
<td>Pc$^1$</td>
<td>2nd &amp; 3rd legs transformed to 1st legs; antennae, wings, and thoracic bristles also affected</td>
<td>normal</td>
<td>P. LEWIS 1947</td>
</tr>
<tr>
<td>Polycomb$^2$</td>
<td>Pc$^2$</td>
<td>2nd &amp; 3rd legs transformed to 1st legs; antennae, wings, and thoracic bristles also affected</td>
<td>normal</td>
<td>LINDSLEY and GRELL 1968</td>
</tr>
<tr>
<td>Antennapedia of Yu</td>
<td>AntpY$^a$</td>
<td>antenna transformed to 2nd leg</td>
<td>T(2;3)22B;83E-F + T(2;3)38E;98A</td>
<td>E. B. LEWIS 1956</td>
</tr>
<tr>
<td>Antennapedia of Rappaport</td>
<td>Antp$^R$</td>
<td>antenna transformed to 2nd leg</td>
<td>In(3R)83F;86C</td>
<td>FALK 1964</td>
</tr>
<tr>
<td>Antennapedia of Bacon</td>
<td>Antp$^B$</td>
<td>antenna transformed to 2nd leg</td>
<td>In(3R)84A;85E</td>
<td>E. B. LEWIS 1956</td>
</tr>
<tr>
<td>Antennapedia of Le Calvez</td>
<td>Antp$^{LC}$</td>
<td>antenna transformed to 2nd leg</td>
<td>In(3R)84A5-6;92A5-6</td>
<td>LE CALVEZ 1948</td>
</tr>
<tr>
<td>Antennapedia$^{49}$</td>
<td>Antp$^{49}$</td>
<td>antenna transformed to 2nd leg</td>
<td>possible abnormality in 83EF-84AB</td>
<td>HANNAH-ALAVA, personal communication</td>
</tr>
<tr>
<td>Antennapedia$^{59}$</td>
<td>Antp$^{59}$</td>
<td>antenna transformed to 2nd leg</td>
<td>not examined</td>
<td>HANNAH-ALAVA, personal communication</td>
</tr>
<tr>
<td>Antennapedia$^{59}$</td>
<td>Antp$^{59}$</td>
<td>antenna transformed to 2nd leg</td>
<td>possible abnormality in 83D-84B</td>
<td>HANNAH-ALAVA, personal communication</td>
</tr>
<tr>
<td>Nasobemia</td>
<td>Ns</td>
<td>antenna transformed to 2nd leg</td>
<td>normal</td>
<td>GEBRING 1966</td>
</tr>
<tr>
<td>Extra sex comb</td>
<td>Scx</td>
<td>2nd and 3rd legs transformed to 1st legs</td>
<td>normal</td>
<td>HANNAH-ALAVA 1958</td>
</tr>
<tr>
<td>Multiple sex comb</td>
<td>Msc</td>
<td>sex comb teeth on 2nd and 3rd legs of males</td>
<td>In(3R)84B1-2;85C</td>
<td>TORKUNAGA 1966; this report</td>
</tr>
<tr>
<td>Humeral</td>
<td>Hu</td>
<td>extra bristles on humerus</td>
<td>In(3R)84B2-3;84F2-3; 86B1-Cl</td>
<td>LINDSLEY and GRELL 1968</td>
</tr>
</tbody>
</table>
denced by the findings that breakpoints associated with \(Dp(1;3)\)\(\text{int}^{77}\) (also in salivary region 77) and with \(T(3;4)\)A9 (in region 87) are in the centers of the left and right arms of mitotic third chromosomes, respectively (Hannah-Alava 1971; Brown 1940). This depression of recombination, as well as the variability of results from experiment to experiment, demonstrates the difficulties of recombinational mapping of the third chromosome centromeric region (Hannah-Alava 1969; Arajarvi and Hannah-Alava 1969).

Table 1 summarizes the current state of our knowledge about the locations of the homoeotic mutations in the proximal right arm of chromosome 3 (3R). A series of dominant mutations, denoted Antennapedia (Antp), causes antennae to be transformed to second legs. These mutations are lethal when homozygous and lethal in heterozygous combination with one another (Hannah-Alava, personal communication), and are usually associated with rearrangements with one breakpoint in salivary chromosome region 83F-84A. Another dominant mutation with the same phenotype, Nasobemia (Ns), is viable when homozygous and in heterozygous combination with Antp\(^{6}\). Ns is not associated with a rearrangement, and Gehring (1966) tentatively concluded that it is not allelic to Antp.

Dominant mutations at three loci in this region cause males to develop sex combs on the second and third pairs of legs. Two alleles of Polycomb, \(Pc^{c}\) (hereafter denoted \(Pc\)) and \(Pc^{a}\), are lethal when homozygous and in heterozygous combination. Extra sex comb (Scx) is also lethal when homozygous, but it is viable in heterozygous combination with either allele of Polycomb and separated from them by about 0.3 recombination units (Hannah-Alava 1969). \(Pc\) and \(Pc^{a}\) also cause aberrant wing morphology, aberrant humeral, notopleural, and sternopleural bristles, and a rare antennal-leg transformation (P. Lewis 1947). \(Pc\) and \(Pc^{a}\) enhance all Antp alleles thus far tested, except that \(Pc/\text{Antp}^{40}\) may be lethal (Hannah-Alava, personal communication).

Another leg-transformation mutation, Multiple sex comb (Msc), causes sex combs to develop on the second and the third pairs of legs in males and causes a reduction of the number of teeth in the sex combs of first legs. Although the Msc-bearing chromosome is lethal when homozygous, Msc/\(Pc\) Scx individuals are viable, and Msc is considered not to be allelic to either (Torkunaga 1966). Msc is associated with a rearrangement described by Torkunaga as \(\text{In}(3\text{R})84B;84F\). In the stretched chromosome shown in Figure 1A, the proximal breakpoint appears to be in or very near the 84B1–2 doublet. (The three pairs of bands figured by Bridges [1935] in this region, 84A1–2 and 3–4 and 84B1–2, usually appear as heavy single bands.) I would place the distal breakpoint at 85C.

Humeral (Hu) is a dominant mutation causing extra bristles to develop on the humerus and in a line towards the base of the first leg and passing posterior to it (see Lindsley and Grell 1968). It is viable when homozygous, and its expression is greater in homozygous than in heterozygous individuals, and in females than in males. \(Hu\) is associated with a rearrangement cited as \(\text{In}(3\text{R})84B2–3;84F2–3;86B4–C1\). I have confirmed that the proximal breakpoint of this rearrangement is distal to 84B1–2.

Denell (1972a) has described the generation of X-ray-induced revertants of Ns (denoted Ns\(^{+R}\) followed by an identifying number). Of the five revertants fully characterized, three were associated with rearrangements, and these have been reexamined in the present investigation. Ns\(^{+R72}\) (Figure 1C) is at least a three-break rearrangement, with one break within the 84B1–2 doublet (or just distal to it), and breakpoints at 85A and in the heterochromatin. Ns\(^{+R72}\) (Figure 1D) is a short deficiency. The exact localization of its proximal breakpoint has proven to be a difficult problem, but the best estimate from many observations of Ns\(^{+R72}/\text{In}(3\text{R})84B;84F\) (the latter causing a disruption of the usually tight synapsis in this region) implies that all or most of 84B1–2 is intact. The deficiency is therefore probably \(\text{Df}(3\text{R})84B3;84D\).

Figure 1.—Salivary chromosome configurations for the indicated genotypes, each heterozygous with Canton S. a: Msc: \(\text{In}(3\text{R})84B1–2;85C\). b: Msc\(^{+R}\): \(\text{In}(3\text{R})84B1–2;85C\). c: Ns\(^{+R}\); breakpoints in the heterochromatin and at 84B1–2 and 85A. d: Ns\(^{+R72}\); \(\text{Df}(3\text{R})84B3;84D\). e: Ns\(^{+R72}\); \(\text{In}(3\text{R})84B1–2;94C\). f: Ns\(^{+R72}\); breakpoints in the heterochromatin and at 84B1–2.
$Ns+R^{as}$ is another three-break rearrangement, and genetic results indicate that it is $In(3R)84B1-2;94C + Y(3)Y;94C$ (Figure 1E), with the proximal euchromatic break within the $84B1-2$ doublet. A sixth reversion, $Ns+R^{as}$, was lost before it could be fully characterized. Salivary chromosome analysis, however, showed that it was also associated with a rearrangement, with one breakpoint in the heterochromatin and one within the $84B1-2$ doublet (Figure 1F).

Of the reversion-bearing chromosomes characterized by Denell (1972a), $Ns+R^{11}$, $Ns+R^{as}$, $Ns+R^{as}$, and $Ns+R^{as}$ were lethal when homozygous, while $Ns+R^{70}$ was viable as a homozygote. These revertants of $Ns$ were tested for complementation inter se and with many of the homeotic mutants of proximal 3R. These tests consisted of reciprocal crosses with the tested chromosomes each heterozygous with a dominantly-marked, multiply-rearranged homolog, with the exception of $Ns+R^{as}$, which was tested only as a male.

With two exceptions, I utilized a qualitative assessment of the phenotypes of viable heterozygous combinations. Some $Pc/Ns+R$ combinations greatly enhance the antennal-leg transformation associated with $Pc$. Thus I made a series of crosses in which the degree of antennal-leg transformation was measured utilizing the scale of Postlethwait and Schneiderman (1971). Each appendage was given a numerical score according to the following classes: 1) a normal antenna; 2) leg bristles appearing on the third antennal segment; 3) an outgrowth of femur material from the third antennal segment; 4) femur tissue occurring on the medial side and tibial tissue on the lateral side of the third antennal segment, with a bridge of cuticle between; 5) as in class 4, but with the tibia joining the femur in the normal tibia-femur articulation; 6) a homeotic antennal leg approaching the size of a second leg; and 7) a normal second leg. Observations were made on intact flies with a dissecting microscope at a magnification of $\times 50$ or $\times 100$. In addition, the number of sex comb teeth on the second and third legs of $Pc/Ns+R$ males was recorded. Legs were examined using a dissecting microscope at a magnification of $\times 50$ or $\times 100$, and were often removed from the thorax for better viewing.

RESULTS

Revertants of $Ns$ were crossed inter se and with many of the homeotic mutants of proximal 3R for complementation testing. Table 2 presents the results with respect to complementation of lethal effects. For each heterozygous combination of mutations which fails to survive, the total number of viable adult progeny from each of the appropriate crosses is given.

TABLE 2

Results of complementation tests between certain mutations, with $v =$ viable combination and $l =$ lethal combination. For heterozygous combinations of mutations with noncomplementing recessive lethal effects, the total number of adult progeny of other genotypes scored from the complementation cross is presented.

<table>
<thead>
<tr>
<th>$Ns+R^{11}$</th>
<th>$Ns+R^{22}$</th>
<th>$Ns+R^{10}$</th>
<th>$Ns+R^{72}$</th>
<th>$Ns+R^{26}$</th>
<th>$Ns$</th>
<th>Antp$^{10}$</th>
<th>Scx</th>
<th>Pc</th>
<th>Msc</th>
<th>Hu</th>
<th>Msc$^{2}$R^{23}</th>
<th>pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Ns+R^{11}$</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>v</td>
<td>v</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(217)</td>
<td>(267)</td>
<td>(577)</td>
<td>(278)</td>
<td>(234)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>$Ns+R^{25}$</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>v</td>
<td>v</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>(361)</td>
<td>(100)</td>
<td>(434)</td>
<td>(405)</td>
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<td></td>
</tr>
<tr>
<td>$Ns+R^{70}$</td>
<td>v</td>
<td>v</td>
<td>v</td>
<td>v</td>
<td>v</td>
<td>v</td>
<td>1</td>
<td>1</td>
<td>v</td>
<td>v</td>
<td>v</td>
<td>v</td>
</tr>
<tr>
<td>$Ns+R^{72}$</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>v</td>
<td>v</td>
<td></td>
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</tr>
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<td></td>
<td>(217)</td>
<td>(221)</td>
<td>(206)</td>
<td>(158)</td>
<td>(287)</td>
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<td></td>
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<tr>
<td>$Ns+R^{96}$</td>
<td>v</td>
<td>v</td>
<td>v</td>
<td>v</td>
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<td>v</td>
<td>1</td>
<td>1</td>
<td>v</td>
<td>v</td>
<td>v</td>
<td>v</td>
</tr>
</tbody>
</table>
Of the five \( N_s^{+R} \)-bearing chromosomes tested, all but \( N_s^{+R70} \) are lethal when homozygous. Any heterozygous combination of the other revertants also fails to survive. These revertants must share a common recessive lethal mutation, presumably induced at the \( N_s \) locus simultaneously with the reversion event.

Individuals carrying \( \text{Antp} \) alleles in heterozygous combination with \( \text{Scx} \) are inviable (Hannah-Alava, personal communication), but this lethal interaction could either indicate that these mutations are allelic or that the simultaneous impact of these dominant genes is inconsistent with survival. The four revertants of \( N_s \) sharing a recessive lethal effect fail to complement the recessive lethality of \( \text{Antp}^R \) and \( \text{Scx} \) (Table 2). Since these revertants have no dominant phenotype, these results indicate that \( \text{Antp} \) alleles, \( \text{Scx} \), and \( N_s \) are allelic, or possibly pseudo-allelic.

All revertants tested are viable in heterozygous combination with \( N_s \). For \( N_s^{+R11} \), \( N_s^{+R25} \), \( N_s^{+R70} \), and \( N_s^{+R90} \) there is no apparent difference in the expression of the antennal-leg transformation phenotype between \( N_s/N_s^{+R} \) individuals and their \( N_s/\text{In}(3LR)/\text{TM6} \) sibs. \( N_s/N_s^{+R70} \) individuals, however, showed a marked decrease in this expression; the basis of this decrease is under investigation.

**Figure 2.—Degree of antennal-leg transformation in \( N_s^{+R}/\text{Pc} \) and \( \text{TM6}/\text{Pc} \) flies.** Each horizontal group of histograms is comprised of four of the genetic classes arising from crosses between \( \text{Pc}/\text{TM1} \) and \( N_s^{+R}/\text{TM6} \) individuals. Antennae were assigned to one of seven phenotypic classes indicating the degree of transformation, ranging from a normal antenna (class 1) to a completely developed leg (class 7).
The revertants of *Ns* are also viable in heterozygous combination with *Pc*. These revertants interact with *Pc* to give a complex complementation pattern for its various pleiotropic effects. This pattern will be described for each effect.

**Extra sex comb phenotype:** The number of sex comb teeth on second and third legs of *Pc/Ns*~+R~ and of *Pc/TM6* males was scored; these data are presented in Figure 3. The extra sex comb phenotype of *Pc* is quite variable, and strongly dependent on experimental conditions and background genotype (Hannah-Alava 1964). A critical experiment requires standardization of background genotype, rigorously controlled experimental conditions, and replicate experiments to allow a meaningful statistical interpretation. The data presented here were not collected in such a rigorous fashion, and must be regarded only as sug-

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**Figure 3.—** Extra sexcomb phenotype of *Ns*~+R~/*Pc* and *TM6/Pc* males. The histograms give the percentage of legs with the indicated number of extra sexcomb teeth. Each horizontal group of histograms is comprised of four of the genetic classes arising from crosses between *Pc/TM1* and *Ns*~+R~/*TM6* individuals. *TM6/Pc* sons are not recovered from *T(Y;3)* *Ns*~+R~/*TM6* fathers. The number of males scored and the mean number of sexcomb teeth per leg are indicated for each genotype.
gestive. An additional difficulty of interpretation lies in the utilization of \textit{Pc/TM6} males as a standard. \textsc{hannah}-\textsc{ala}va (1964) has shown that \textit{Ubx} (an allele of which is carried by the \textit{TM6} chromosome) enhances the effect of \textit{Pc} on leg transformation relative to \textit{Pc/+}.

The data in Figure 3 show clearly that the extra sex comb phenotype of \textit{Pc} is not enhanced by revertants of \textit{Ns} relative to \textit{Pc/TM6} brothers, since the former are invariably associated with a lower level of expression. It is possible that the expression of \textit{Pc/Ns+R_{11}} and \textit{Pc/Ns+R_{72}} males corresponds to that of \textit{Pc/+} rather than that of \textit{Pc/TM6} males. The data for \textit{Pc/Ns+R_{32}}, and especially for \textit{Pc/Ns+R_{99}}, strongly suggest that these revertants suppress the formation of extra sex combs.

\textit{Antennal transformation:} \textit{Pc} is associated with a second homoecotic effect—transformation of antennae to legs. \textsc{postl}ethw\textsc{ait} and \textsc{schnei}der\textsc{man} (1971) have studied a similar transformation associated with \textit{AntpR} and have described the seven classes of expression presented previously. From some of the complementation crosses, \textit{Pc/Ns+R} and \textit{Pc/TM6} males and females were scored according to this system of classification. These data are presented in Figure 2. The degree of penetrance and expression of this transformation is consistent for \textit{Pc/TM6} males and females from cross to cross, and confirms previous observations that the effect is greater in females than in males (\textsc{p. lew}is 1947). For this character, we have no quantitative comparison of the expression of \textit{Pc/TM6} relative to \textit{Pc/+} individuals, but casual examination suggests that the degree of antennal-leg transformation is also enhanced by \textit{TM6}.

When \textit{Pc/Ns+R} males and females are compared to \textit{Pc/TM6} males and females, respectively, it is clear that the revertants of \textit{Ns} differ in their interactions with \textit{Pc}. Both \textit{Ns+R_{11}} and \textit{Ns+R_{72}} enhance the antennal-leg-transformation phenotype associated with \textit{Pc}, and females bearing such heterozygous combinations are virtually fully penetrant for this trait if both antennae are scored. \textit{Ns+R_{32}}, on the other hand, appears to suppress this phenotype, and none of the 214 individuals examined specifically for this character nor any of the many more that have been casually examined showed any antennal transformation.

\textit{Wing morphology:} \textsc{p. lew}is (1947) noted that the effect of \textit{Pc} on wing morphology exhibits marked sexual dimorphism. This phenotype is also strongly affected by culture conditions, and in the present experiments the flies which eclose early in a culture vessel are much more drastically affected than those eclosing thereafter. The following description is of the most drastically affected individuals of each genotype. The wings of \textit{Pc/TM6} females are usually slightly divergent and tilted with respect to the horizontal plane, with the distal margin higher than the medial one. There is sometimes a short terminal gap in the fifth longitudinal vein and a slight crumpling of the wing along its medial margin. The wings of \textit{Pc/TM6} males are usually normal. Among females bearing \textit{Pc} and \textit{Ns+R_{11}}, \textit{Ns+R_{32}}, or \textit{Ns+R_{99}} the wings are much more severely affected. They are spread in a position perpendicular to the anterior-posterior axis of the fly, strongly bowed downwards, and very crumpled; often there are terminal gaps in the fifth longitudinal vein. Males bearing \textit{Pc} and any of the four revertants of
Rs tested show a less dramatic enhancement—their phenotype is similar to that of Pc/TM6 females.

Scx is known to similarly enhance the effect of Pc on wing morphology (Hannah-Alava 1958), and Hu does as well. In other crosses, however, this effect is also enhanced in Pc/TM1 individuals, and a careful study of a number of heterozygous combinations of Pc seems necessary before a meaningful interpretation of the specificity of these interactions is possible.

Thoracic morphology: Details of normal thoracic morphology are described by Zalokar (1947) and Ferris (1950). Figure 4A shows a lateral view of the thorax of a wild-type fly. The two main lateral plates of the dorsal prothorax, the humeral callus (commonly called the humerus) and the propleurum, are indicated, as is the main lateral plate of the mesothorax (the mesopleurum). The anterior thoracic spiracle is located in the sclerotized suture between the two anterior thoracic segments.

Pc/TM6 individuals show a variety of thoracic bristle effects. The humeral setae may be shorter and thicker than normal and either bowed or bent at a sharp angle. The ventral humeral setae is more often affected than the dorsal setae. The anterior notopleural setae may be similarly affected, though much less frequently

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**Figure 4.**—Thoracic morphology of a wild-type (Canton S) female (A) and of Rs+Rzs/Pc females (B–E), showing a series of prothoracic alterations. The wings have been removed. A, B, and C are seen from lateral view, and D and E from an oblique lateral view from a more posterior point. H, humeral callus; MS, mesopleurum; PP, propleurum; S, anterior thoracic spiracle.
than the humeral setae. In flies bearing heterozygous combinations of Pc and the revertants of Ns, the penetrance of these effects is considerably enhanced. In addition, the sternopleural setae are sometimes also affected. Scoring the thoracic setae as a whole, Pc becomes virtually fully penetrant in such females and nearly as penetrant in males.

An additional phenotype occurs in Pc/Ns+Rss flies—the development of outgrowths from the prothorax at or ventral to the site of the humerus. Figure 4B–E shows a series of typical effects. The least extreme expression of this change is a stronger definition of the humerus, and/or a raising of the rim of the anterior thoracic spiracle (Figure 4B); these traits occasionally occur in wild-type flies as well (Herskowitz 1949). The humerus may form a fleshy projection, which is characteristically located ventral to the normal site of the humerus (Figure 4C). The most typical expression is a roughly spherical growth with the opening of the spiracle at its most distal point (Figure 4D). The base of the sphere may be flush with the thorax, as in this case, or the sphere may project on a short stalk. Finally, some growths are roughly cylindrical, with the spiracle invariably located at the apex (Figure 4E). Structures of the latter two classes may have creases giving the appearance of weak segmentation. The hairs on these structures range from the normal complement of the humerus to a complete absence of bristles. Rarely, the projecting structures are heavily melanized.

Ns+Rss shows some evidence of a similar interaction with Pc in heterozygous combinations, but the prothoracic effects are less well developed. Some growths similar to those depicted in Figure 4C occur, but there are no more extreme types of expression. Neither Ns+R11 nor Ns+Rss interacts with Pc to produce changes in prothoracic morphology. From cultures specifically scored for effects similar to those in Figure 4D and E, the number of affected flies was 9 of 78 for Pc/Ns+Rss, and none of 260 and 197 for Pc/Ns+R11 and Pc/Ns+Rss, respectively.

In all flies observed from the initial crosses the expression was unilateral. After affected Pc/Ns+Rss individuals were inbred and subjected to casual selection, however, the penetrance was slightly increased, and occasional flies were bilaterally affected.

As noted earlier, the homozygous viable dominant mutant Humeral (Hu) is associated with an inversion with one breakpoint just distal to 84B1–2. Hu was subjected to complementation analysis with the revertants of Ns; flies of the genotype Hu/Ns+ survive (Table 2). The Humeral phenotype is slightly better expressed in Hu/Ns+ progeny than in their Hu/TM6 sibs, but does not reach the same degree of expression of Hu/Hu individuals.

In addition, both Hu/Ns+Rss and Hu/Ns+Rss individuals have prothoracic outgrowths. The range of phenotypes for Hu/Ns+Rss is similar to that for Pc/Ns+Rss. Hu/Ns+Rss individuals have outgrowths similar to that illustrated in Figure 4D, making their expression greater than that of Pc/Ns+Rss but less than that of Hu/Ns+Rss. In individuals specifically scored for outgrowths similar to those shown in Figure 4D and E, 8 of 56 Hu/Ns+Rss individuals showed this phenotype, while 1 of 54 Hu/Ns+Rss and none of 96 Hu/Ns+R11 fulfilled this criterion. There was no indication of any interaction between Ns+R11 or Ns+Rss and Hu to
produce any prothoracic alteration, and all Hu/Hu individuals examined were also unaffected. The Hu\(^{/+}\) flies originally scored were unilaterally affected. Inbreeding of affected individuals markedly enhanced this expression, and bilaterally affected individuals were often observed.

Since Pc and Hu show parallel interactions with Ns\(^{R85}\) with respect to prothoracic changes, Pc/Hu individuals were examined. These genes interact in a strictly additive fashion. Flies bearing this heterozygous combination show no prothoracic growths and no antennal-leg-transformation, although the effect of Pc on wings is slightly enhanced. The phenotype with respect to prothoracic hairs is typical of Hu heterozygotes.

Several workers have described morphological changes of the dorsal prothorax. Waddington (1942) and Villee (1946) found that outgrowths were induced by X-ray treatment. Also, Waddington (1943) found that in individuals simultaneously homozygous for dachsous and combgap, two second chromosome recessive mutations affecting legs and other organs, the thorax is sometimes extended as large "shoulders". Herskowitz (1949) described Hexaptera (Hx), a second chromosome dominant homeotic mutation which causes wings, halteres, and legs to form at the suture between the humerus and the propleurum.

It is attractive to interpret the structures observed here as the products of a homeotic change as well. The series of phenotypes observed suggests that the humerus is progressively replaced by the structures formed. The structures do sometimes grossly resemble appendages, with the stalked spheres similar to halteres and many of the structures appearing to be weakly segmented. Of course, no appendage normally incorporates a spiracle and its associated trachea in the manner that these outgrowths do. In the larva the dorsal prothoracic disc, which forms the humerus, surrounds the main dorsal tracheal trunk just posterior to the anterior spiracle (Zalokar 1947). Perhaps the abnormal growth that this disc undergoes to form these structures somehow entraps the trachea, causing it to remain associated with the structures formed. Such an entrapment could not be considered a mandatory consequence of such abnormal growth of the dorsal prothoracic disc, since Herskowitz (1949) does not describe any involvement of the spiracle in the homeotic growths associated with Hx.

Sublines of flies heterozygous for Ns\(^{R85}\), especially those also carrying Pc, include rare individuals with other developmental anomalies. Legs are sometimes shortened and aberrantly formed. Occasionally, the humerus is missing. Flies are sometimes missing the portion of the thorax normally elaborated by the dorsal mesothoracic disc. Such individuals have the humerus in juxtaposition to the haltere, and the anterior and posterior thoracic spiracles lie side by side. These flies always have melanized tissue, observable through the cuticle, lying anteriorly in the abdomen on the same side on which no external dorsal mesothoracic structures are present. In several cases these flies were dissected, and the internal structure proved to be a derivative of the missing disc. A wing was usually present, and a vesicle of thoracic cuticle with the thoracic bristles, if present, protruded to the interior of the vesicle. This type of development, typical of transplanted discs, is due to the failure of the disc to evert; eversion is appar-
ently dependent on an association of the disc with the pupal epidermis (Bhas- 
karan and Sivasubramanian 1969). The humeral and leg anomalies are also 
probably due to eversion abnormalities in \textit{Ns}^{+Rss} heterozygotes, although it is not 
clear how this developmental behavior might be related to the interactions of 
\textit{Ns}^{+Rss} with \textit{Pc} and \textit{Hu}.

Multiple sex comb, which has a phenotype similar to that of \textit{Pc} and \textit{Scx}, is 
associated with a rearrangement which has one of its breakpoints at 84B1–2. In 
an experiment currently in progress, a number of putative revertants of \textit{Msc} 
were induced by X-rays and are being analyzed. The salivary chromosome com-
plement of one of these putative revertants, \textit{Msc}^{+Rss}, has been determined. The 
\textit{Msc}^{+Rss} chromosome carries, in addition to the original inversion, a new inver-
sion with one breakpoint just proximal to the 84A3–4 doublet, and another at 
100A (Figure 1B). \textit{Msc}^{+Rss} is a partial revertant: \textit{Msc}^{+Rss}/+ males have no sex 
combs on second or third legs, but in the presence of a dominant second chromo-
sonal enhancer of \textit{Msc} (Denell, unpublished) such males have a slight \textit{Msc} 
phenotype. These data suggest that \textit{Msc} is associated with the 84B1–2 breakpoint 
of \textit{In(3R)Msc}, and that the newly-induced inversion is suppressing \textit{Msc} by a 
stable position effect (Lewis 1950).

Another homoeotic mutation, proboscipedia (\textit{pb}), is known by recombin-
tional analysis to be located in proximal 3R but has not been localized cytologi-
cally. \textit{pb}/\textit{Df(3R)Ns}^{+Rss} individuals survive and do not have the proboscipedia 
phenotype. Thus \textit{pb} is not located within the limits of this deficiency.

**DISCUSSION**

**General Interpretation**

The noncomplementing recessive lethal effect of \textit{Scx}, the alleles of \textit{Antp}, and 
some revertants of \textit{Ns} indicate that these mutations are allelic. The phenotypic 
difference between \textit{Scx} and the antennal-leg-transforming mutations causes one 
to suspect that more than one functional unit is involved, although Chovnick 
et al. (1969) have cautioned against the indiscriminate use of this logic. The inter-
pretation (discussed in detail below) of these dominant genes as neomorphic 
(Muller 1932) provides an alternative explanation for the functional diver-
sity. Critical evidence will require a recombinational analysis of the locus. In a 
preliminary note on this investigation, Denell (1972b) suggested that Naso-
bemia be renamed Antennapedia-Nasobemia (\textit{AntpNs}). It also seems appro-
priate to rename Extra sex comb as Antennapedia-Extra sex comb (\textit{AntpScx}), 
although I will continue to utilize the original terminology throughout this report.

The conclusion that \textit{Ns} is an allele of \textit{Antp} implies that \textit{Antp} is located at 
84B1–2. How should this location be reconciled with the results that \textit{Antp}-asso-
ciated rearrangements have an array of breakpoints well proximal to this doublet 
(Table 1)? The possibility that \textit{Antp} alleles are associated by chance with re-
arrangements sharing breakpoints in this limited region is sufficiently improbable 
to be discounted. It is possible that the rearrangements affect the functioning 
of a mutationally unaltered \textit{Antp} locus by position effect. Rearrangement break-
points eliminate the possibility of variegated position effect, however, and only stable (S-type) position effects need be considered (Lewis 1950). Lindsley and Grell (1968) describe many rearrangements with similar breakpoints and no phenotype. Also, Antp$^b$ is still expressed when separated from In(3R)Antp$^b$ (Hannah-Alava, personal communication). Thus the position effect model is not well supported. Alternatively, Hannah-Alava (personal communication) has suggested that alleles of Antp might have been isolated with accompanying rearrangements because the rearrangements act as enhancers. This hypothesis is supported by her observations that independently-derived rearrangements enhance both Antp and the extra sex comb mutations, and that Antp$^b$ shows a reduction in its expression when separated from its associated inversion.

We now have a considerable amount of information about Polycomb, and its interactions with Antp locus. Both Pc and the members of the Antp locus are associated with the transformations of second and third legs to first legs and of antennae to legs. Furthermore, chromosomes bearing mutational events at the Antp locus associated with the reversion of Ns show marked, though dissimilar, interactions with Pc, including the development of a new phenotype. These interactions, which are summarized in Table 3, show no simple enhancement or suppression of the Pc phenotype, but instead give a complex pattern in which different pleiotropic effects of Pc seem to be independently affected.

TABLE 3

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Extra sexcomb phenotype</th>
<th>Antennal-leg transformation</th>
<th>Prothoracic outgrowths</th>
<th>Wing effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>TM6/Pc</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Ns+R$^R_1$/Pc</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Ns+R$^R_2$/Pc</td>
<td>+</td>
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<td>++</td>
</tr>
<tr>
<td>Ns+R$^R_3$/Pc</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Ns+R$^R_4$/Pc</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>++</td>
</tr>
</tbody>
</table>

Is Pc allelic or pseudoallelic to the Antp locus? Pc and Pc$^a$ act as recessive lethal mutations, but individuals bearing Pc and the revertants of Ns (including Df(3R)Ns+R$^R_2$) in heterozygous combination survive to adulthood. If the recessive lethality of Pc is due to the absence of its wild-type allele in homozygotes, this result suggests that Pc is not allelic to the Antp locus, and further that it is located proximal to the left breakpoint of this deficiency. Alternatively, lethality may be due to the presence of two doses of this dominant gene, an interpretation which does not clarify its allelic relationship to the Antp locus. The possibility of nonallelism is supported by the 0.2–0.3 percent recombination between Pc and Scx (Hannah-Alava 1964), since the most widely separated members of
complex loci presently known show 0.14 percent recombination (GREEN and GREEN 1956; WELSHONS 1965). If salivary chromosome region 84B has escaped the centromeric inhibition of recombination, Pc probably resides in one of the heavy bands of 84A (LEFEVRE 1971). If recombination is suppressed in this region, the physical separation of Pc and Scx could be even greater. Thus we can tentatively conclude that Pc is not allelic to the Antp locus, but that they have very similar genetic functions that act as the basis of these interactions.

It is interesting to note that the similarity in phenotypic traits shared by Pc and the Antp locus are also associated with a third locus: spineless (3-58.5). The phenotypic effects associated with this locus include reduced bristles, an antennal-leg-transformation, malformed legs, sex combs on second legs, and drooping wings (LINDSLEY and GREL 1968). Some alleles are recombinationally separable (HEXTER, LOZNER and BUNN 1967), and the results imply that more than one functional unit is involved.

Based on the frequency of reversion of Ns and the recessive lethality of most revertants, DENELL (1972a) concluded that reversions occurred by X-ray induced inactivation of the gene. The heterogeneous behavior of revertant-bearing chromosomes in heterozygous combinations with Pc, however, indicates that the situation is more complex. Before the induction of reversions, Ns was maintained for many generations as a freely-recombining homozygous stock. Since the revertants were induced, the genetic integrity of these chromosomes has been maintained by heterozygosity with the TM6 chromosome. Thus, the mutagenized chromosomes were genetically fairly homogeneous, and although modifiers of Pc might had been induced by X-rays it seems most probable that the differences in interactions of these reversion-bearing chromosomes with Pc are due to the reversion events themselves. This conclusion is strengthened by the result that Ns+8R25 gives prothoracic outgrowths in heterozygous combination with either Pc or Hu, but that Pc/Hu or Hu/Hu individuals are normal for this trait. The implication is that Pc and Hu are modifying a primary event associated with Ns+8R25. The interpretation of the origin of revertants of Ns by an inactivation of the gene may still be the proper one. The differential genetic behavior of the revertants may be due to the location of Ns among functionally related genes. Thus the genetic differences between revertants could be due to induced alterations of structure and/or function of contiguous genes. In addition, inactivation of Ns by events in nearby genes could explain the high rate of reversion of this gene (DENELL 1972a).

We presently have little data on the relationships of Hu with the other homeotic mutations in this region. It seems most reasonable to continue to interpret Hu as nonallelic to these genes, and to conclude that its association with prothoracic outgrowths in heterozygous combinations with Ns+8R25 and Ns+8R2 indicates some functional interaction. It is tempting to speculate that Hu is associated with the 84B2-3 breakpoint of In(3R)Hu, but no evidence exists to support this interpretation.

Evidence has been presented that Msc is located near the 84B1-2 doublet. The Msc-bearing chromosome is lethal when homozygous, but survives in het-
erozygous combinations with Pc and Scx, themselves associated with recessive lethality. These results caused Tokunaga (1966) to conclude that Msc is not allelic to either of these genes. Msc has never been separated from In(3R)Msc, and no evidence exists to distinguish whether this recessive lethality is associated with the dominant mutation itself or with the inversion. It is possible that the Msc mutation is itself homozygous viable. Thus while we can continue to consider Msc as tentatively nonallelic to Pc and Scx, we must recognize that the survival of Msc/Pc Scx individuals does not critically demonstrate this relationship.

The Genetic Nature of These Genes

It is interesting to note that Antp and Ns share the same phenotype, whereas Antp and some revertants of Ns share the same lethal. One explanation is that Antp and Ns are neomorphs (Muller 1932), and that a single wild-type gene mutated to Antp with a concomitant loss of its normal function (producing recessive lethality) and to Ns with no loss of function. Reversions of Ns occurring by its inactivation would also cause the loss of original function, yielding a recessive lethal. An alternative explanation is that Ns is a duplication, with one gene performing the original function and another the neomorphic function, with lethal revertants formed by an inactivation of both loci. There is no basis on which to choose between these models at present.

Ns is clearly not a hypomorph or an amorph, since it can be reverted by deletion. The observation that Ns/Ns+R individuals (including Df(3R)Ns+R7+) are phenotypically indistinguishable from Ns/+ implies that Ns is not a hypomorph and is consistent with the interpretation that it is a neomorph. This observation is supported by a preliminary examination of flies heterozygous for Ns and an interstitial deficiency of salivary chromosome region 83E-84D, constructed by the method described by Lindsley et al. (1972). The corresponding interstitial duplication, however, suppresses the Ns phenotype, and if this effect proves to be characteristic of Ns+/+/+, the gene might better be interpreted as an antimorph.

As part of a collaborative investigation into the effects of segmental aneuploidy of the autosomes (Lindsley et al. 1972), I also generated and examined flies of both sexes hypoploid or hyperploid for salivary chromosome region 83E-84D. Phenotypically, these flies showed no evidence of a homoeotic transformation. In addition to the Antp locus this region probably includes the loci of Pc and Msc, and possibly Hu. The observation that a heterozygous deficiency for this region is not associated with a phenotype makes it unlikely that any of the included dominant mutations are hypomorphs or amorphs. Further, the absence of a dominant transformation phenotype associated with an interstitial duplication of the region suggests that these mutations are not hypermorphs. Thus we can tentatively conclude that these mutations are neomorphic dominants as well.

These speculations can most interestingly be applied to Antp², which is among the developmentally best-described of homoeotic mutations. From their studies of this mutation, Postlethwait and Schneiderman (1969, 1971) concluded...
that cells of the early third instar larva undergo a transformation of developmental fate. Although previously determined to form antennal structures, these cells are redetermined to follow a developmental pathway leading to the formation of a leg, and transmit these new state of determination clonally until overt differentiation begins. This change occurs in a small population of cells (about ten) related by proximity rather than by pedigree. From the results given above, we can postulate that \textit{Antp} was induced by a mutational event which eliminated its wild-type function and endowed the gene (or its product) with a new, homoeotic function. It is still not clear, however, whether \textit{Antp} acts by a direct or indirect mechanism.

\textbf{E. B. Lewis (1963)} has performed gene dosage studies on the members of the bithorax series. The recessive members and one of the dominants, Ultrabithorax, act as hypomorphs. Lewis interprets another dominant, Contrabithorax, as a neomorph.

\section*{Conclusions}

The present interpretation of the available data is that \textit{Ns} and \textit{Scx} are allelic to \textit{Antp}, with the \textit{Antp} locus located in or just distal to salivary chromosome doublet 84B1-2; that \textit{Pc} bears considerable functional similarity to the \textit{Antp} gene but is not allelic to it; that \textit{Msc} is also located close to 84B1-2 and its allelic relationship to the other genes is unclear; and that \textit{Hu} also shows functional similarities to the \textit{Antp} gene. Moreover, these dominant mutations are probably neomorphic in nature. It is now critical to determine the spatial relationship of \textit{Pc} and \textit{Antp}. It will be necessary either to gather more recombinational information on the region to allow a meaningful interpretation of the 0.2-0.3 units separating them, or to map \textit{Pc} on to the salivary chromosome.

Although these mutations appear to be neomorphic, it seems most reasonable to assume that their wild-type alleles also function in the control of developmental process. In spite of the allelism of \textit{Antp}, \textit{Ns}, and \textit{Scx}, the impression remains that there is an unusual clustering of genes of similar function in this region. Two explanations (not mutually exclusive) have been proposed as the basis of clusters of functionally related genes: that the genes are derived from a single ancestor by gene duplication and subsequent diversification of function; and that the genes are clustered to facilitate their coordinate control (E. B. Lewis 1967). Future investigations of this region can be orientated to answer two complementing questions: what is the mechanism of action of these homoeotic mutations and of their wild-type alleles, and what role does the genic organization of this region play in their control?

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LITERATURE CITED


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