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THE UNIVERSITY OF ALBERTA

MUTATIONAL ANALYSIS OF SEGMENTATION IN THE EMBRYONIC DEVELOPMENT OF *DROSOPHILA MELANOGASTER*

BY

PATRICIA LEIGH ONOFRECHUK

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled MUTATIONAL ANALYSIS OF SEGMENTATION IN THE EMBRYONIC DEVELOPMENT OF DROSOPHILA MELANOGASTER submitted by Patricia L. Onofrechuk in partial fulfilment of the requirements for the degree of M.Sc. in Genetics.

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Supervisor

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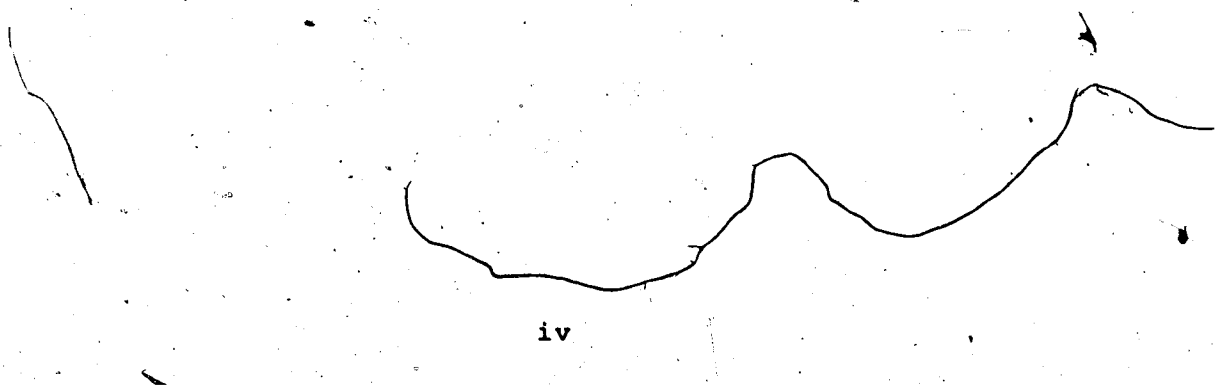
September 12, 1986

To my parents, Peter and Rita, for their neverending encouragement and support.



ABSTRACT

The research described in this thesis was performed to test several current models of Drosophila development. Each model is capable of simulating the phenotypes of specific segmentation mutants in this organism. Zygotic mutants affecting embryogenesis have been found on every chromosome of Drosophila and comprise three major classes. These are the gap class, pair rule, and segment polarity class of mutants. By extrapolating the models, predictions of the phenotypes expected for combinations of pair rule and segment polarity mutants were made. The original purpose of the experiment was to garner evidence to test the models. If a model correctly predicted many of the phenotypes, it was felt that it may be simulating the actual mechanisms involved in pattern formation. The result of the experiment however, was to support specific features of each model and therefore none of the three could be discarded in its entirety. The results raised some interesting questions concerning dosage effects and interactions between segmentation genes that were not expected. Several double mutant phenotypes were less extreme than the single mutant phenotypes suggesting interactions between the two loci. As well, in some cases the control larvae exhibited defects associated with one of the recessive, lethal mutants even though the larvae were heterozygous at that locus.

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1. INTRODUCTION

Most organisms, by necessity, must reproduce to ensure the continued existence of their species. This process, integral to survival is therefore under rigorous genetic control. The initial product, in higher eukaryotes the zygote, must in turn develop into a fully differentiated adult capable of reproduction. The progressive development of the zygote from a unicellular entity into a multicellular organism, continues to fascinate and perplex observers.

The adult organism contains many different cell types and tissues. Although all of these cells are originally derived from the unicellular zygote, after differentiation they express different sets of genes peculiar to the organ or specialized tissue to which they belong. Laser ablation fate mapping experiments (Lohs-Schardin et. a., 1979) reveal that all cells that contribute to the formation of larval epidermal anlagen are found in specific spatial relationships to each other at blastoderm e.g. in Drosophila when specific cells in the blastoderm are irradiated with an ultraviolet laser microbeam, specific structures in the larva are absent, (Lohs-Schardin et. al. 1979). Irradiation of this location always results in the same defect. When this is done, in turn, to all of the blastoderm cells, a fate map can be generated. This correlates all of the larval epidermal structures with the location of the

blastoderm cells that produce them. When this same type of experiment is performed on pre-blastoderm embryos, no defects in the differentiated larval pattern are observed. This implies that the nuclei become aware of their developmental fates at a particular stage of development. When this occurs they are 'determined'. Since this state is cell heritable they cannot usually change their fate. Larval defects are observed when the cells are irradiated at blastoderm because other cells and their descendents cannot alter their determined state to replace the missing cells. Determination occurs sometime before cellularization of the blastoderm is complete (Lohs-Schardin et. al., 1979).

The origin of determined states is under genetic control, therefore this study approaches the problem of pattern formation at this level. The organism of choice is Drosophila melanogaster due to the plethora of information, genetic and developmental, that is available. The larva of the fruit fly also has a highly organized segmentation pattern on its ventral surface which simplifies analysis of mutant phenotypes. (See Materials and Methods for a description of the pattern.)

A Drosophila melanogaster egg is less than .5 mm long. Ventral and dorsal surfaces are distinguished by the convex shape of the former, and the slightly flattened appearance of the latter. Immediately surrounding the egg is a transparent, hydrophobic,

vitelline membrane. External to this, the opaque chorion is found with its two anterior appendages.

A few minutes after sperm entry, the male and female pronuclei fuse at a position approximately 2/3 of egg length (EL) as measured from the posterior pole. The cleavage nuclei divide synchronously, the first seven divisions occurring in the egg's interior. Each nucleus accumulates cytoplasm; these 'energids' are then distributed evenly throughout the egg. After the seventh or eighth division, the energids migrate to the periplasm, beneath the plasma membrane, to form the syncytial blastoderm. Approximately 100 remain in the centre to become yolk nuclei, while 18 at the posterior pole form the pole cells.

The syncytial blastoderm nuclei divide synchronously four more times to produce approximately 6000 nuclei (Foe and Alberts, 1983; Fullilove and Jacobson, 1978; Poulson, 1950). Cellularization of the blastoderm occurs through the inward invagination of membrane between adjacent nuclei from the plasma membrane. Although the final product of this process is referred to as cellular blastoderm, cytoplasmic connections between the cells and the yolk can still be observed until the initial stages of gastrulation (Rickoll and Counce,1980). The formation of the blastoderm is generally complete by 3.5 hours after egg laying (AEL) at 25 ^{o}C, at which time gastrulation begins. This entails a major rearrangement

of the single cell layered blastoderm to produce ectoderm, endoderm and mesoderm. Ventral furrow (VF) formation is the first movement observed after which the cephalic furrow (CF) appears. By 4 hours AEL, the germ band, composed mainly of ectoderm, elongates. At 7.5 hours segmentation of the germ band becomes apparent as a series of transverse grooves on the ventral surface, past the posterior pole of the egg and up along the dorsal surface, due to germ band extension. Head involution begins, resulting in the disappearance of the CF. The germ band shortens so that it again occupies the ventral surface of the embryo. The edges of the germ band extend laterally until they meet on the dorsal midline, thus, enclosing the embryo. After dorsal closure, the entire surface is covered by a single layer of epidermal cells which subsequently secrete the cuticle of the first instar larva; this hatches by 22 hours AEL, at 25 ^{o}C.

The first somatic cells in this organism are formed at blastoderm. Simcox and Sang (1983) removed cells from embryos at this stage and transplanted them into other embryos. Both donor and host embryos that survived until they emerged as adults were studied to determine if the transplanted cells developed according to their original fate (autonomous behavior) and were therefore determined, or if they were affected by their new location and differentiated adult structures appropriate to it. When cells from cellular blastoderm

stage donors were used, they developed autonomously. When this same experiment was performed with nuclei from stages prior to cellularization, they participated in the formation of host tissues, exhibiting nonautonomous, undetermined behavior. Laser ablation experiments have been used to support these results. When embryos in syncytial blastoderm are irradiated with an ultraviolet laser microbeam, defects in the hatched larva are very rare. When irradiated at cellular blastoderm, as many as 90% of the first instar larvae exhibited defects, (Lohs-Schardin et. al., 1979). Studies in which Drosophila eggs are ligated at different positions and different times of development reveal that when performed at blastoderm, a large number of segments are absent from the cuticular pattern. At progressively later times of development, smaller gaps in the pattern are observed. When eggs are ligated at blastoderm, all of the cuticular pattern elements are formed to the extent that both halves together contain the wildtype number of segments (Schubiger et. al., 1977). All of these experiments indicate that segment determination occurs during blastoderm formation. They do not however, suggest the mechanism of determination nor explain what the determined state is.

More information on this subject is provided by clonal analysis. If embryos heterozygous for bristle and hair markers are irradiated with specific doses of X-

rays, mitotic recombination in one or more cells can be induced. This produces cells that are homozygous for the markers, in a background of heterozygous cells. This technique is ideally suited for the study of imaginal disc development. These discs are the progenitor cells of some adult tissues. Throughout larval development they remain overtly undifferentiated until metamorphosis when they replace the larval tissue. Using clonal analysis, Garcia-Bellido et. al. (1973) found that a sequence of clonal restrictions appear at specific points in larval development resulting in the progressive restriction of the developmental competence of imaginal disc cells. e.g. When clones are induced in wing discs at an early stage of development, they never cross a 'line' separating the anterior portion of the wing from the posterior portion. This line, or compartment boundary subdivides the disc into anterior and posterior compartments. Clones induced later reveal another, dorsal/ventral, compartmental restriction. The observation that the restrictions occur in a specific temporal sequence implies that determination is a gradual process relying on complex interactions and gene activities.

Several mutants are known in D. melanogaster that affect compartment specification. Viable alleles of engrailed transform the posterior wing compartment into a mirror-image of the anterior compartment. When

engrailed clones are made in *en*/+ individuals, clones originating in the posterior compartment can now cross the boundary and populate the anterior compartment (Lawrence and Morata, 1976). Clones with mutant alleles at other gene loci affecting posterior wing morphology do not exhibit this type of behavior. These results ~~suggest~~ that determination of the difference between posterior and anterior wing depends on the activity of the *engrailed* gene. According to this idea, *engrailed* is 'on' in the posterior compartment of the wing disc, and 'off' in the anterior one and, in turn, controls other genes that are differentially activated in these two compartments.

This 'selector gene hypothesis', proposed by Garcia-Bellido (1975), suggests further that progressive restrictions of developmental potential caused by subsequent compartmentalization events are dependent on other selector genes and that the basis of determination is a combinatorial binary code of selector gene activity. When the wing is subdivided into anterior and posterior compartments, the code for anterior would be '0' and posterior '1', indicating *en*$^{+}$ activity in the posterior compartment. After the dorsal/ventral restriction, the combinatorial code specifying the anterior-dorsal compartment would be '00', anterior-ventral '01', posterior-dorsal '10', and posterior-ventral '11', where the second digit represents a different, hypothetical

selector gene.

A mechanism which has been suggested to control the selective expression of developmentally important genes, including selector genes, is 'positional information' (Wolpert, 1969) which tells cells where they are in the developing embryo. Evidence supporting its existence is garnered from embryo ligation experiments (Herth and Sander,1973). Schubiger et. al. (1977) ligated Drosophila eggs and then pierced a hole in the membrane that separated the ligated parts. When this was performed on early embryos, the normal segmentation pattern was restored. This implies that communication between the two portions of the egg is necessary for proper specificity of segments. Information in the form of a diffusible substance might provide the positional information used to specify the sequence of segments.

The idea of positional information has also been found necessary to explain the regeneration of imaginal discs. Discs can be removed, fragmented and after transplantation into an appropriate host, regeneration patterns assessed. If a disc is cut into two pieces, one piece always regenerates a complete disc, the remaining piece duplicates itself. French, Bryant and Bryant (1976) explained this behavior through a 'clock' model of positional information. Two coordinates are placed on the disc: one specifies circumferential values of 1 to 12/0 (compartment boundary) the other specifies the

proximal-distal axis of the disc. Briefly, the model states that when a disc is bisected, intercalation of intermediate values progresses by the shortest route. Since one portion of the disc is larger, it will contain more clock values and therefore will regenerate the intermediary values. The portion of the disc that duplicates does so because the shortest route of confronted values is through the values that are already present. This indicates that the positional information needed to produce a complete disc is present and that disc cells communicate this information to each other .

The mechanisms by which positional information acts and the form it takes are unknown, but several theories have been developed. Turing (1952) first demonstrated mathematically that a uniform array of cells could produce biologically significant patterns of morphogens. Wolpert (1969) suggested that positional information may be specified by one or more gradients of diffusible morphogens. If morphogen 'a' is found in high concentration at one embryonic pole (e.g. anterior) and diffuses to the posterior pole, it will set up a gradient of high to low concentration. Every cell in the developing embryo could be specified by the concentration of the morphogen at its position. This implies that specialized areas or sources, produce the morphogen, and that other regions or sinks, dispose of it. These unique properties are assigned to boundary regions, and they

should therefore behave differently from other portions of the embryo. Boundaries are also present between segments, thus the existence of these inherent properties can be tested.

Locke (1959) found that when small pieces of abdominal integument from the blood feeding bug Rhodnius prolixus were removed, rotated 180° and then replaced, the patterns that resulted could best be explained if every segment contained reiterations of homologous information. Wright and Lawrence (1981) performed a series of experiments that not only duplicated these results, but expanded them to show that boundaries do not possess unique, specialized properties but are simply another pattern element on the insect integument. They found that extirpation of a boundary in Oncopeltus fasciatus could have several results depending on the proportion of the segment that was removed with it. If only the segment boundary was removed, when the animal was observed after moulting, it had regenerated. When a segment-sized piece was transplanted from the middle of one segment to the middle of the next, including a segment boundary, a large, stable mosaic segment was the result. The boundary was not regenerated.

These results suggest that when less than half of a segment is removed, cells are confronted during the healing process that contain very similar values in the gradient. Therefore, the absent material is intercalated

and a normal segment is produced. When an entire segment is removed, the values juxtaposed are nearly identical and therefore the cells do not regenerate the missing material, nor the segment boundary. One interesting result observed can only be explained by invoking one of the rules from the clock model. When more than half, but less than an entire segment (excluding the segment boundary) is removed, an ectopic boundary in reversed polarity results. If intercalation by the shortest route is followed, then the cells would intercalate through the segment boundary in orientation opposite to that of the other segments.

Recently, many mutants in D. melanogaster have been isolated which affect segment identity, size and number (Wieschaus et. al. 1984, Nusslein-Volhard et.al. 1984, Jurgens et. al.,1984). Several types of mutations were recovered including homoeotic, maternal effect and segmentation mutants. Ultrabithorax is an homoeotic mutant that transforms the identity of the metathoracic segment (T3) into mesothorax (T2). Maternal effect mutants such as dorsal result in genotypically wildtype embryos differentiating only dorsally derived structures (Nusslein-Volhard et. al., 1980). Three classes of segmentation mutants were isolated, segment polarity, pair rule and gap mutants, (Wieschaus et. al. 1984, Nusslein-Volhard et. a. 1984, Jurgens et. al. 1984). Segment polarity mutants such as patch contain the

wildtype number of segments but only the anterior portion of each denticle belt is normal. The posterior portion is a duplication of the anterior rows in reverse polarity. In this case, this includes the segment boundary, thus, twice the normal number of boundaries are present. All of the mutants in this class contain mutant specific duplications and polarity reversals. Pair rule mutants such as evenskipped are missing segment-sized pieces of their pattern. The endpoints of the deleted material do not necessarily coincide with the segment boundaries although this may occur in some mutants. The pattern presented by the gap class of mutants reveals that several contiguous segment-sized pieces are absent. Again, the endpoints of the deleted material are not obliged to coincide with the segment boundaries, although this may occur.

Several theories have been proposed to attempt to explain mutants which can affect segmentation. Each is capable of simulating single mutant phenotypes of members of the pair rule and segment polarity classes. By extrapolating these simulations, the aim of this project was to use them to predict phenotypes of pair-wise or double mutant combinations of mutants in these two classes. Five pair rule and three segment polarity mutants of D. melanogaster were used in this study. They were specified by mutant alleles at eight different loci on chromosome two. Double mutants were generated through

crossing over. By using this procedure no ambiguity should arise concerning the genotypes of the lethal embryos, as can occur if mutants on two separate, segregating chromosomes are used. Comparing the predictions of each model to the observed phenotypes of the constructed double mutants should determine which model best simulates not only the mutant phenotypes, but perhaps also the mechanisms by which segmentation is achieved.

2. MATERIALS AND METHODS

CULTURE OF FLIES

All flies were raised in temperature regulated incubators at 25 +/- 1 °C unless otherwise indicated, and fed a yeast-sucrose-agar medium supplemented with chloramphenicol to suppress bacterial growth (Table 1). They were bred in 33 ml vials plugged with cotton balls.

DROSOPHILA STRAINS

All mutants were obtained from Dr. Eric Wieschaus, Department of Biology, Princeton University except gooseberry which was ordered from Bowling Green Stock Centre, and Df(2)en28 which was produced in this laboratory, (Eberlein and Russell, 1983). All markers and special chromosomes used are described in Tables 2 and 3 respectively. A diagram indicating the cytological and genetic map positions of important markers and segmentation mutants used in this study is shown in Figure 1.

SEGMENTATION MUTANTS

Eight segmentation mutants found on the second chromosome of D. melanogaster were used in this study. Their location was important as unambiguous double mutant chromosomes could be produced through recombination. Five of the mutants were pair rule mutants: oddskipped^{IIID36}, sloppy paired^{IIM105}, paired^{IIB42}, evenskipped^{ID19ts}, en^{1M99} and Df(2)en28. The remaining three belonged to the segment polarity class of mutants: wingless^{IL114ts},

Table 1: CONSTITUENTS OF FLY MEDIUM

1% agar
10% sucrose
10% Brewer's yeast
10% chloramphenicol, 1 g/l solution
1% propionic acid
phosphate buffers to pH 7.4

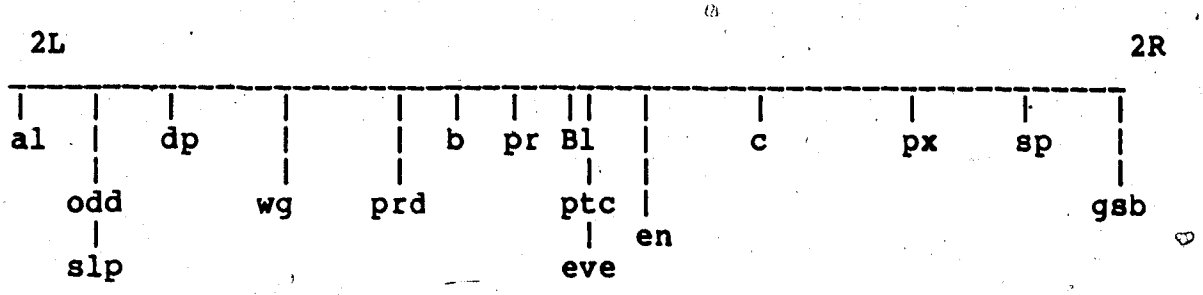
Table 2: MARKERS USED IN THE STUDY

Marker	Phenotype
[illegible] staless	feather-like extensions on antennae absent
[illegible]py	wings have square shape
b black	black coloured body
pr purple	ruby eye colour
Bl Bristle	bristles are shorter, blunter and thicker
c curved	wings are curved downward and held out from body
px plexus	wings have extra veins, more pronounced at tips
sp speck	black specks at base of wings
cn cinnabar	bright red eye colour
bw brown	brown eye colour
Cy Curly	wings curled upwards

Table 3: SPECIAL CHROMOSOMES AND MARKERS FOUND ON THEM

Chromosomes	Markers
all	al dp b pr c px sp
all-Bl	al dp b pr Bl c px sp lvi 2
CyO	dp Cy pr cn 2 lvi 2p 2
SM6a	al Cy dp cn sp

Figure 1:GENETIC MAP POSITIONS AND CYTOLOGICAL LOCATIONS OF SEGMENTATION MUTANTS AND MARKERS ON THE SECOND CHROMOSOME



Marker of Mutant	Genetic Map Position	Cytological Location
al	.1	21C1-2
odd	8	24E-25A
slp	8	24C-D
dp	13	24E-25A
wg	30	??
prd	45	33B6-E3
b	48.5	34E6-35C5
pr	54.5	37B-40B4
Bl	54.8	38A6-E9
eve	59.0	46C3-11
ptc	59.0	44B5-F
en	62.0	??
c	75.5	??
px	100.5	58E-59
sp	107.0	60B13-C5
gsb	107.6	60E9-F

'??' cytological location not known

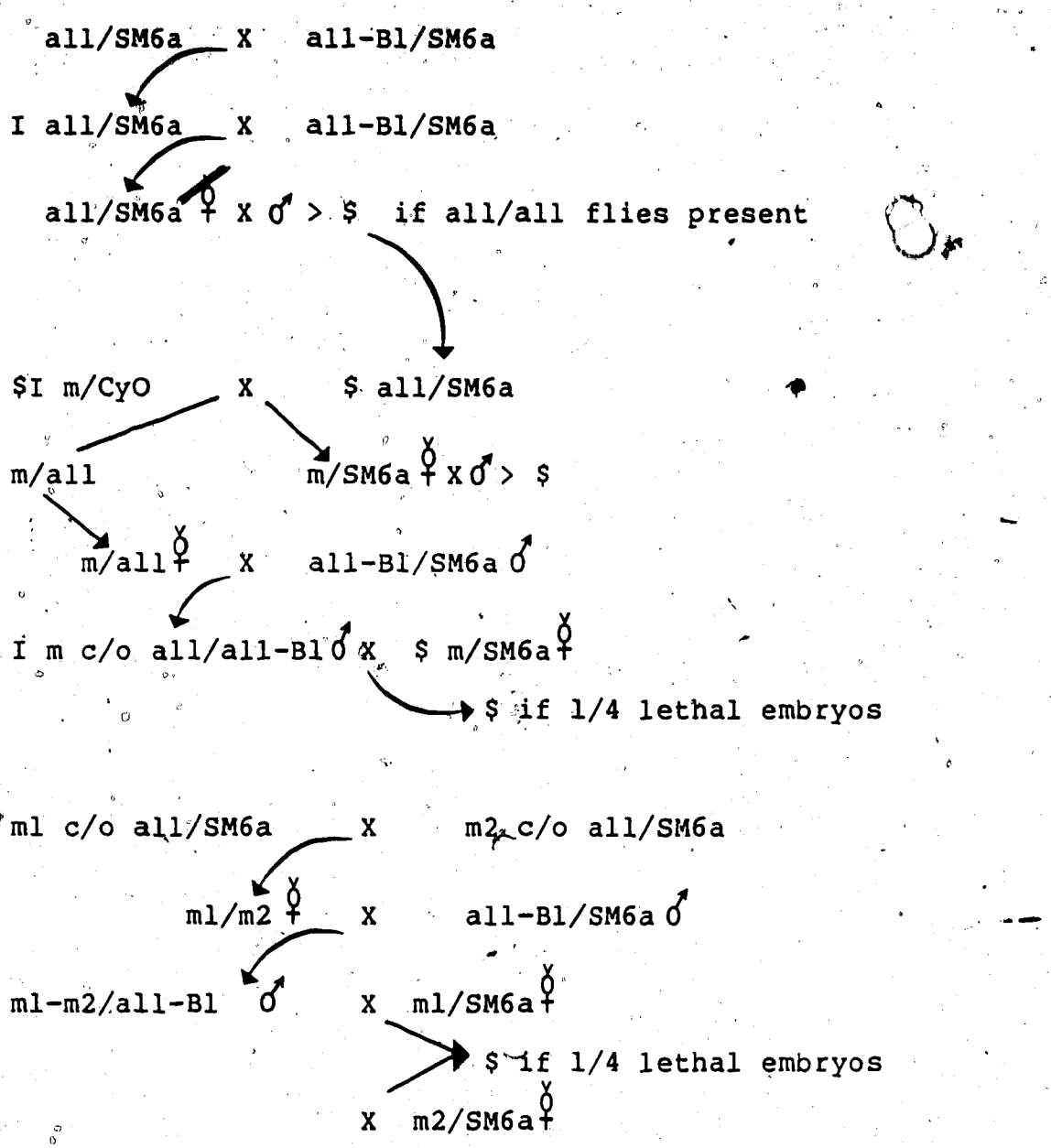
patch IN108, and gooseberry. All of these mutants were kept as balanced stocks. Recombination was prevented through use of a multiply inverted chromosome, in this case SM6a, which carries several markers, one of which is dominant (see Table 3). Since all of the segmentation mutants used in this study are recessive lethals, the homozygous segmentation mutant embryos die before hatching. The homozygous balancer embryos are lethal in the first instar larval stage, therefore all animals surviving to adulthood are heterozygous. In this way, all of the single and double mutant stocks were maintained without further recombination.

CONSTRUCTION OF NEW STOCKS

Elimination of Possible Modifiers from Segmentation Mutant Chromosomes

The double mutant stocks used in this study were constructed taking advantage of the fact that meiotic crossing over occurs only in Drosophila females, not males. The crossing scheme used is shown in Figure 2. The first step was to try to remove all but closely linked enhancer or modifying mutations which may have been present on the original mutagenized chromosomes (Wieschaus, personal communication). Individuals from the original mutant bearing stocks were therefore crossed to individuals from a stock containing a homozygous-viable, multiply marked, second chromosome carrying the markers al dp b pr c px and sp, hereafter referred to as 'all'.

Figure 2: CROSSING SCHEME USED TO GENERATE DOUBLE MUTANTS



Symbols:

'$' balanced stock

'I' individual fly used in cross

'm' segmentation mutant

'm c/o all' segmentation mutant chromosome with markers from the 'all' chromosome

Unmated 'virgin' females of this genotype were then crossed to all-Bl/SM6a males, 'all-Bl' referring to an 'all' chromosome which carries an additional, dominant bristle mutation. Recombinant male progeny were selected using published map positions (Figure 1). These males were tested to determine whether they retained the segmentation mutant by backcrossing to the original stock. For example, when this procedure was followed for the odd mutant, males that carried the markers b pr c px and sp were selected. These males (to ensure no further recombination) were crossed to virgin females of the original odd stock which carried the markers cn and bw. If the selected recombinant did not carry the odd mutation, a wildtype phenotype class of flies would be present among the backcross progeny. If this class of flies was absent, the odd mutation was presumed to have been present on both second chromosomes resulting in lethality. Males and females carrying the recombinant chromosome balanced over SM6a were then selected to obtain the required, balanced, single mutant stock.

Derivation of Double Mutant Stocks

After all of the single mutant stocks had been obtained as described above, they were intercrossed to produce a series of females heterozygous for each pair of segmentation mutants. For example, the odd b pr c px sp/SM6a stock was crossed to the al dp ptc px sp/SM6a stock. odd +/+ ptc virgin females were selected that were

homozygous for px and sp. These were crossed to males that were al-Bl/SM6a. Using the markers on the al-Bl chromosome, recombinant progeny resulting from a crossover in the odd-ptc interval could be selected to contain both segmentation mutants on one second chromosome. In this example, to select for an odd-ptc recombinant chromosome, male flies that were homozygous for b+ pr+ and c+ were selected. Each putative double segmentation mutant male was placed separately in a vial and backcrossed successively to females carrying the first segmentation mutant, then the other, to confirm the presence of both lethals on the same chromosome. In the example, when the putative double mutant males were crossed to the odd b pr c px sp/SM6a virgin females, if the odd mutation was included on the male chromosome, all of the progeny would carry either the dominant Bl or Cy mutations. If progeny were observed that were b px and sp, they were obviously not homozygous for the odd mutation and therefore it was assumed to not be present in the sperm of male. These flies were then discarded. If this class of flies was absent, the cross to the al dp ptc px sp SM6a females was checked. If px and sp progeny were present, the ptc mutation was not homozygous and the flies were discarded. If absent, a stock containing the putatively double mutant chromosome balanced over SM6a was established using virgin flies from one of the crosses.

When the putatively double mutant stock was produced, males were crossed again to single mutant females. This time, the eggs were collected (see below) and 24 hours after the adults had been removed, unhatched eggs were dissected and mounted on microscope slides (see below). Only if the phenotype of these lethal embryos was a segmentation defect, i.e. odd when mated to the odd stock virgin females, ptc when mated to ptc stock virgin females, was the double mutant stock retained. This test was included as a control to ensure that the lethal phenotypes used to select putative double mutant chromosomes was indeed due to the segmentation mutants and not to other linked lethals.

At the same time, the double mutant lethal embryos were dissected and mounted. These formed the material used for the studies reported in the Results section where their phenotypes are compared with the corresponding pairs of single mutant controls.

METHODS OF EGG COLLECTION, DISSECTION AND MOUNTING OF LARVAE

To assess each segmentation phenotype, flies from a segmentation mutant/Balancer stock culture were used as parents. About 1/4 of the eggs from the parents would be homozygous for the recessive lethal segmentation mutant(s). These eggs would not hatch. The remaining eggs would be viable mutant/Balancer heterozygotes or Balancer homozygotes.

The egg laying apparatus consisted of a baby food jar, whose lid contained standard fly food. Premated adults were introduced to the apparatus and allowed to lay eggs. After enough eggs had been laid the adults were removed. The length of time needed to lay a sufficient number of eggs was different for each mutant but was normally about 24 hours. Usually the lids were checked visually and when they contained more than thirty eggs, the adults were removed.

Twenty four hours later, after all viable embryos would have hatched, the remaining unhatched eggs were transferred with a dissecting needle onto a piece of double sided sticky tape on a microscope slide. The opaque chorion was then removed by rolling the egg on the tape. The larva, enclosed within the thin, transparent vitelline membrane was then placed on the slide next to a drop of mounting medium (9 Parts 85% lactic acid: 1 Part 95% ethanol, Lewis, 1978). If possible, each larva was dissected out of the vitelline membrane by rolling it very gently with an insect pin on the dry slide. The membrane would tear open, and a small drop of mounting medium was brought over to the larva to engulf it. Due to its hydrophobic nature, the vitelline membrane would float off, liberating the larva which was then transferred to a fresh drop of mounting medium on another slide. (For certain genotypes it proved impossible to remove the vitelline membrane without destroying the

larva.) These were simply mounted after dechorionation, as described below.

After 15-25 larvae had been accumulated in this fashion, a #1 coverglass was placed on top of the embryos, and the slide placed on a slide warmer set at approximately 40 °C. The mounting medium rendered the internal organs of the larva transparent, allowing an unhindered view of the ventral cuticular pattern. For this reason the location of the larvae on the slide were noted by drawing a circle around them on the underside of the slide prior to incubation on the slide warmer. After allowing several days for the excess mounting medium to evaporate, the slides were made semi-permanent by sealing the edges of the coverslip with nail polish. They were then examined and photographed using phase contrast optics on a Wild M20 microscope equipped with a 35mm camera with automatic exposure control. All photographs were taken with Kodak Panatomic-X black and white film and processed with Kodak D19 developer.

ANALYSIS OF DOUBLE MUTANT PHENOTYPES

The double mutant phenotypes are described below by analyzing the denticle belts present on the ventral surface of unhatched first instar larvae. By identifying the portions of the wildtype pattern that remain, the size and types of deletions that are occurring could be ascertained. Not only was the denticle belt pattern analyzed, but also the polarity of the denticles within

them. Normally, the first and fourth rows of denticles point anteriorly; the remainder posteriorly. If this pattern was altered in the double mutant, it was noted and if possible, conclusions drawn as to how the combination of mutations were interacting to affect this characteristic. For each double mutant, two control embryos' phenotypes were also examined. This was very important as it verifies the presence of the specific mutation on the double mutant chromosome.

Analysis of double mutant phenotypes was complicated by the variability exhibited within a sample of single mutant embryos. For example, slp homozygotes displayed a spectrum of phenotypes. A pair-wise 'leaning' of denticle belts toward each other, or a pair-wise, partial fusing of denticle belts can occur. Both describe the sloppypaired phenotype yet are extremes, demonstrating pair-wise deletions of varying size. The difficulty in interpreting the double mutant phenotype was compounded when both mutants exhibited such variability. Photographs of the larvae have been chosen to reflect, in most cases, the average or generalized phenotype due to the subjectivity inherent to this type of study.

3. RESULTS

ANALYSIS OF MUTANT PHENOTYPES

The wildtype larval pattern as represented by the Oregon-R strain is described first, followed by the phenotype of each single, segmentation mutant.

Oregon-R

The principal phenotypic trait of the larva analyzed in this study was the ventral cuticular pattern of the fully differentiated but unhatched first instar larva, (Figure 3). The ventral surface of the larva contains a highly organized pattern and has been described by Lohs-Schardin et. al. (1979). It contains three thoracic and eight abdominal segments, many of which are distinguishable from each other. The dorsal surface of the larva is covered by very fine hairs which are more difficult to see than the ventral denticles, especially in mutant larvae which show poor cuticle pigmentation. For this reason I decided not to score the dorsal surface when analyzing segmentation mutant phenotypes.

On the ventral surface, the first thoracic segment, T1 is the most anterior visible segment. This is due to head involution which occurs during embryogenesis (Poulson,1950). T1 contains two patches of denticles, one immediately posterior to the other. The anterior patch has large, darkly pigmented denticles found in a belt across the width of the larva, while the posterior belt has finer denticles in a long and narrow medial patch.

Figure 3: Wildtype Larva-Oregon R

This figure illustrates a normal or wildtype larva. See text for detailed discussion.

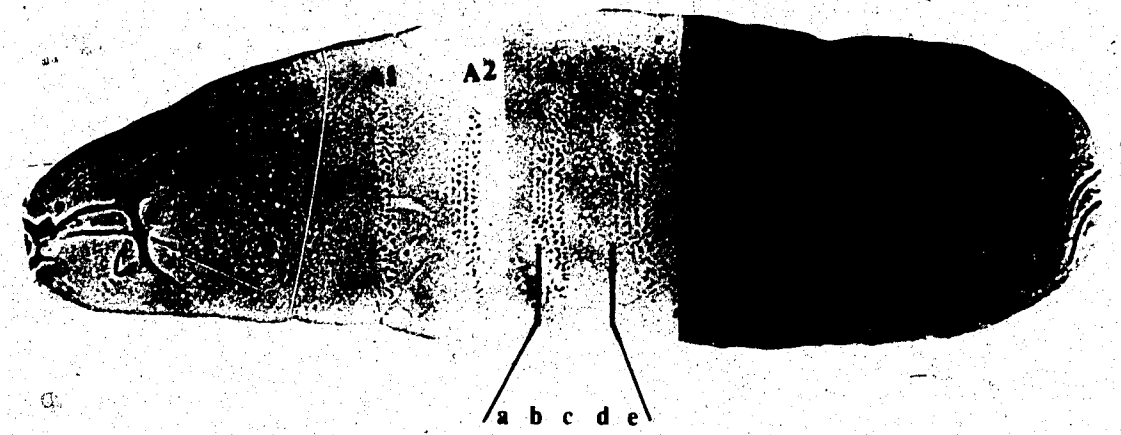
a= anterior portion of denticle belt, medium sized denticles.

b= middle portion of denticle belt, large, darkly pigmented denticles.

c= most posterior portion of denticle belt and beginning of naked cuticle. Denticles are very and darkly pigmented.

de= naked cuticle, no markers are present except in thoracic segments where Keilin's organs are found.

Note: In all subsequent figures, as in Figure 3, the anterior portion of the larva is oriented to the left of the page. In figures with double mutant and control embryo, the double mutant larva is in the top position with the controls underneathe.


A2
a b c d e

The second and third thoracic segments (T2 and T3) both contain three to four rows of fine, lightly pigmented denticles, that extend the width of the ventral surface of the larva. All thoracic denticles point posteriorly.

Each of the three thoracic segments have two Keilin's organs (KO) associated with them. These sense organs are found on the anterior/posterior compartment boundary (Struhl,1984) and consist of three protruding hairs. Lateral of the two KO's, are small, campaniform sensilla . These markers are very useful for determining the size of segmental deletions since their positions are precisely defined. However, their usefulness is limited since they are not found in the abdominal segments.

A1, the first abdominal segment, contains a wide denticle belt which crosses the entire ventral surface of the larva. It consists of three to four rows of larger, darkly pigmented denticles. The most important feature of this belt that distinguishes it from the other abdominal belts is that all of its denticles point posteriorly. Abdominal segments A2-A8 contain belts consisting of 6 rows of denticles. The first and fourth rows in belts A2 to A8 are directed anteriorly and the remaining rows posteriorly. One feature shared by all of the abdominal denticle belts is the presence of the smallest denticles at the posterior edge of the belt. Intermediately positioned denticles are the largest. This feature is very useful for determining whether the anterior or

posterior portion of the denticle belt is deleted by a mutation. It is particularly helpful when analyzing segment polarity mutants where all denticle belts are affected by the mutation.

Abdominal belts A2-A7 are all trapezoidal in shape, narrow at the anterior and wider at the posterior. As one progresses posteriorly from A2, the shape of the denticle belts become narrower, with the result that A8 appears almost rectangular. This feature is sometimes useful when identifying which portion of the pattern is present in pair rule mutants. However, because most abdominal denticle belts are very similar to those immediately adjacent, unambiguous identification of mid-abdominal segments is not always possible. When this occurs, the thoracic denticle belts, A1 and A8 are used to establish the phase of the pattern deletion involved. If we assume that the portion of the pattern removed by each mutation is reiterated in every or every other segmental repeat, the deletion pattern can be inferred from the effect on the most distinctive segments.

MUTANT PHENOTYPES

1) oddskipped:

Larvae homozygous for the oddskipped IIID36 allele exhibit a pair rule repeat pattern, (Nusslein-Volhard et. al.,1984). As Figure 4 (top) demonstrates, this mutant is named for the deletion of every second denticle belt. The denticle belts associated with T1, T3, A2,A4,A6,and A8

are complete and normal in shape and denticle polarity. Those associated with the odd numbered segments are either absent, or greatly reduced. Often, an insufficient number of denticles are present to unambiguously establish whether or not a normal polarity pattern is present in these denticle belts. Pieces of these denticle belts are usually present, however, the belt associated with A1 is preferentially deleted from these larvae. This denticle belt was completely missing in nine of thirteen larvae studied. Three of the remaining four larvae had less than five denticles representing this denticle belt. The Keilin's organs associated with T1 and T3 are present in the mutant embryos while those associated with T2 are either rarely present or perhaps difficult to discern from the overall pattern.

2) sloppy paired:

As demonstrated by Figure 4 (middle), larvae homozygous for the sloppypaired IIM105 allele contain a wildtype number of denticle belts. The repeat pattern is of the pair rule type however, since the naked cuticle between every other denticle belt is shorter than normal resulting in a pair-wise 'leaning' of denticle belts towards each other. T1 is reduced in size, since only a few denticles from the posterior belt are observed. All of the other denticle belts appear to be normal in size although this is difficult to judge since the shape of each belt is distorted. The denticle belts brought

together in pair-wise associations are as follows; T2-T3, A1-A2, A3-A4, A5-A6, and A7-A8. Rarely, this association results in fusion of two denticle belts, producing a large, composite belt. The Keilin's organs associated with segment T2 are never observed. Although the organs from T1 and T3 are present and can be distinguished, they usually contain only two hairs.

3) paired:

Figure 4 (bottom) illustrates a larva homozygous for the paired IIB42 allele and its pair rule repeat pattern, previously described by Nusslein-Volhard and Wieschaus, (1980). One large thoracic belt and four large abdominal belts are present. The first and fourth rows of the three posterior belts point anteriorly, the remainder posteriorly. The denticle belt associated with T1 is present but is smaller and its shape is distorted. Earlier studies have shown that this pattern is generated by the deletion of the posterior half of each odd numbered segment and the anterior half of the posteriorly adjacent even numbered segment (Nusslein-Volhard and Wieschaus, 1980). This would result in the formation of composite denticle belts, the anterior half of one belt being juxtaposed to the posterior half of the next denticle belt. With the mutant allele used here, embryos are never found which show separation of these belts. Although the same number of rows of denticles are present in each belt as in wildtype, the belts appear to be

Figure 4: oddskipped, sloppypaired and paired larvae

top- oddskipped larva. The denticle belts associated with T1,T3,A2,A4,A6,and A8 are present and normal. Small pieces of the oddskipped numbered denticle belts can be seen.
Magnification = 294X

middle- sloppypaired larva. A pair-wise 'leaning' of denticle belts T2-T3, A1-A2,A3-A4,A5-A6,A7-A8 is observed.
Magnification = 278X

bottom- paired larva. Large composite denticle belts are present.
Magnification = 284X

larger. This is likely due to the tendency of the remaining pattern to expand and cover more of the larva. Keilin's organs were observed in only one larva, in this case one was present, posterior to a denticle belt identified as T3.

4) evenskipped:

A larva raised at 25°C and homozygous for the evenskipped^{ID19ts} allele is presented in Figure 5 (middle). The figure shows a pair rule repeat pattern with one thoracic and four abdominal belts, with characteristics similar to wildtype segments T2, A1, A3, A5 and A7. The denticle belts of T1 and T3 are absent but the entire segment is not deleted as the Keilin's organs associated with them are often observed. The denticle polarity in the belts is unaffected by the mutation. This figure also shows the almost wildtype pattern observed when the embryos develop at 18°C (top) and the extreme phenotype produced when development occurs at 29°C (bottom).

5) engrailed:

Mutants with two different alleles at this locus were used in this study. The first, en^{IM99} was induced with ethylmethane sulfonate (Nusslein-Volhard et. al.,1984) the second, Df(2)en28 was induced with X-rays (Eberlein and Russell, 1983).

a) en^{IM99}

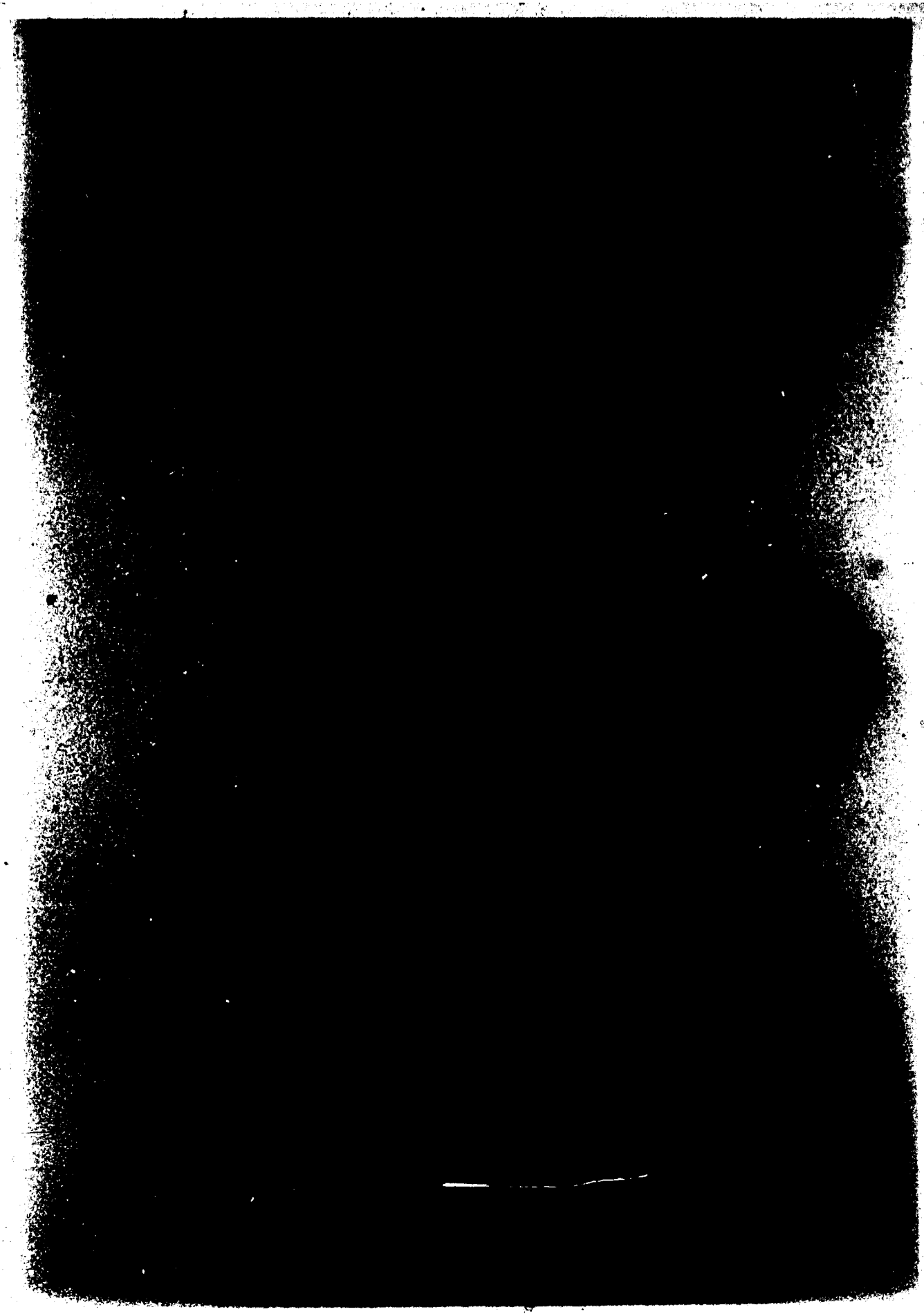
As shown in Figure 6 (top), larvae homozygous for

Figure 5: evenskipped larvae at various temperatures.

top- 18oC. All denticle belts are present.
Magnification = 274X

middle- 25 °C. Pair-rule phenotype with the even numbered abdominal denticle belts absent.
Magnification = 292X

bottom- 29 °C. Extreme evenskipped phenotype. Apparent loss of polarity and segmentation.
Magnification = 329X



this allele exhibit a pair rule repeat pattern complicated by additional defects. The basic phenotype of six large denticle belts is generated by two defects. The first is a pair rule defect resulting in a pair-wise deletion of most of the naked cuticle of segments T1,T3,A2,A4,A6 and presumably A8 although this is difficult to ascertain. In the absence of intervening naked cuticle the denticle belts of T1T2, T3A1,A2A3, A4A5,A6A7 are juxtaposed. This results in the formation of the large, composite denticle belts observed in these larvae. Two types of polarity reversals are observed in these larvae, the first occurs in the denticle belts, the second can only be recognized when large amounts of naked cuticle are deleted, bringing denticle belts close together.

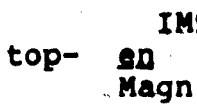
Secondary fusions of the fused denticle belts also often occur and are readily identifiable since usually only the lateral edges of the concerned belts are involved. The large region of naked cuticle between the composite belts is never completely removed. The second defect manifests itself in the anterior rows of each denticle belt (Nusslein-Volhard et.al., 1984). Although anteriorly directed denticles are present at the anterior edge of each denticle belt, they are disorganized. Instead of an entire row of these denticles, only a few are present. Their positions in the belts are variable, often appearing only at the ventral midline or at the

lateral edges. Small, ventrally pointing denticles are observed at the lateral edges of the denticle belts. Keilin's organs are not observed in these larvae.

b) Df(2)en 28:

An embryo homozygous for this deficiency which deletes the engrailed region (Eberlein and Russell, 1983) is found in Figure 6 (bottom). The pattern is somewhat similar to that observed in en^{IM99} homozygotes and might be interpreted as more extreme. The T1T2 belt contains few denticles and all are lightly pigmented. The remaining belts also contain fewer denticles and exhibit unusual polarity. The anterior edge of each composite belt contains small anteriorly directed denticles immediately followed by medium sized denticles also directed anteriorly. Posterior to this are denticles of the same size pointing posteriorly. These are immediately followed by small denticles also directed posteriorly. This polarity pattern is repeated in all of the composite denticle belts and is indicative of two different polarity reversals per repeat unit. Secondary fusions occur to such an extent that many larvae have a 'rug' of denticles, rectangular in shape as it is longer that it is wide. The rug is bounded on the lateral edges by small denticles pointing ventrally. A8 is represented by a circular patch of denticles, all pointing outwards or away from the circle's centre. Keilin's organs are never found in these larvae.

Figure 6: *en* alleles.



top- *en* allele.
Magnification = 323X

bottom- *Df(2)en28* allele.
Magnification = 295X

6) wingless:

A larva homozygous for wingless IL114ts, is shown in Figure 7 (top). One thoracic denticle belt appears at the anterior of the larva but its identity is unclear since very few denticles are present and because the head and thorax regions are severely affected by the mutation. In the abdomen, the larva displays a wildtype repeat pattern in that a normal number of abdominal pattern repeats are present. The first abdominal denticle belt, A1, can be identified by its characteristic shape. Normally, all of the denticles in this belt point posteriorly, but in this mutant the vast majority of the denticles are directed anteriorly. A small number of posteriorly directed denticles can be observed in some larvae, but most have only anteriorly pointing denticles in this belt. The remaining abdominal denticle belts share a common pattern. At the anterior edge of each denticle belt are small, posteriorly pointing denticles. These are followed immediately by large, posteriorly pointing denticles which continue until the centre of the belt. At this point are large, anteriorly directed denticles immediately followed by small, anteriorly pointing denticles. The next denticle belt then begins with small, posteriorly directed denticles. This pattern continues the length of the larva, with little or no naked cuticle separating the denticle belts. The number of belts can be ascertained by counting the number of

polarity reversals. Difficulties arise however as the well organized denticle polarity described above breaks down progressively toward the posterior region of the larva. Another feature of this mutation is the complete absence of Keilin's organs. As well, all of the denticles at the lateral edges of the belts are directed ventrally. One phenotypic detail is particularly intriguing. A small percentage of larvae contain one or more denticle belts in which the medial denticles point dorsally, instead of anterior or posterior.

7) patch:

A larva homozygous for the patch IN108 allele, a segment polarity mutation is shown in Figure 7 (middle) and demonstrates a pattern with wildtype number of repeats. Although no thoracic denticle belts are present, the Keilin's organs associated with these segments are all visible, often containing four hairs instead of the normal three. Keilin's sensory organs have been precisely localized and found to straddle the anterior/posterior compartment boundary (Struhl,1984). Therefore two of the hairs may be derived from the anterior compartment cells, the third from the posterior compartment cells. Keilin's organs containing four hairs could be the result of a duplication including the anterior compartment but not the posterior. All of the abdominal belts are present, and separated by naked cuticle. Denticle belt A1 is represented by a small

patch of denticles always centered on the ventral midline. These denticles do not exhibit an obvious polarity pattern. All of the other abdominal denticle belts have several features in common. First, the anterior row of each belt contains small denticles which are directed anteriorly. The second row contains larger denticles pointing anteriorly and posteriorly. The remaining rows are directed posteriorly. Secondly, all of the belts contain either five or six rows of denticles. Thirdly, because the belts are longer along the ventral midline than at the outer, lateral [redacted] they are all 'eye'-shaped.

8) gooseberry

A larva homozygous for this [redacted]ation is shown in Figure 7 (bottom). A wildtype number of repeats are present, each containing a polarity reversal. The denticle belt associated with T1 is present, however the small patch of denticles that is normally found on the ventral midline posterior to the larger T1 denticle belt is absent. Often, midline defects are observed in other segments as well, notably T2 and T3. The denticle belts associated with these latter two segments are present and contain posteriorly directed denticles in the anterior portions of the belts and anteriorly directed denticles in the posterior portions of the denticle belts. The Keilin's organs associated with these segments are absent. The abdominal segments follow a similar pattern

Figure 7: Segment Polarity Mutants.

top- wingless larva. Square bracket indicates one repeat unit. Magnification = 341X

middle- patch larva. Square bracket indicates one repeat unit. Magnification = 284X

bottom- gooseberry larva. Square bracket indicates one repeat unit.
Magnification = 279X

of posteriorly directed denticles in the anterior portion of the pattern and anteriorly directed denticles in the posterior portion. Various sizes of denticles participate in the pattern. They closely resemble those found in the posterior portion of wildtype denticle belts.

DESCRIPTION OF DOUBLE MUTANT PHENOTYPES

1.*wingless patch* (*wg ptc*/*wg ptc*)

This double mutant, as shown in Figure 8 (top), exhibits a very complex phenotype. The anteriormost portion of the larva contains denticles whose identities are thoracic, although their precise origin is ambiguous. Posterior of this area the ventral surface of the larva is covered by a large expanse of denticles. Close examination of this region reveals that the polarity of these denticles is highly organized. Counting the number of anterior-posterior polarity reversals along the length of the larva reveals a wildtype number of repeats. Two lateral 'organizing' regions, toward which denticles point, can be identified in most denticle belts. More than two in one repeat unit has never been observed, although less than two is fairly common. One possible explanation is that two of these special regions are in fact present in each denticle belt, but cannot always be observed since a fairly high density of denticles is necessary in order to distinguish such specific polarity patterns. All of the surrounding denticles are directed toward the centres of these regions. Denticles at intermediate positions, or between two of these regions point toward the one which is the closest to it. This feature has been identified in other double mutants containing the *wg* allele.

wingless control (*wg ptc*/*wg* +)

Figure 8 (middle) illustrates a larva in which a wildtype number of repeats are present. The polarity of the denticles in each pattern repeat is typical of the wingless phenotype. There are several minor differences that should be noted. First, some posteriorly pointing denticles can be observed at the anterior of A1. All of the denticles associated with A1 in the single mutant wg larvae are directed anteriorly. Secondly, A3,A5 and A7 exhibit disruptions in their patterns along the ventral midline. These defects in the odd numbered denticle belts are not observed in all larvae of this genotype. However, the ventral denticle pattern with its characteristic wingless polarity is evidence that this mutation is indeed present on the double mutant chromosome.

patch control (wg ptc/ + ptc)

Figure 8 (bottom) illustrates a larva with a typical patch phenotype.

2. wingless gooseberry (wg gsb/wg gsb)

The double mutant shown in Figure 9 (top) exhibits a phenotype very similar to the wingless phenotype. It appears to contain a wildtype number of repeats, with a polarity reversal in each. The denticles are large as in wg homozygotes. The only apparent difference between the double mutant phenotype and the wingless phenotype is that the thoracic denticle belt is not as easily identified in the double mutant larvae. It appears to be

Figure 8: *wingless* *patch* Double Mutant and Control Larvae.

top- *wingless* *patch* double mutant larva.
Magnification = 480X

middle- *wingless* control larva.
Magnification = 328X

bottom- *patch* control larva.
Magnification = 281X

reduced in size.

wingless control (wg gsb/wg +)

The larva in Figure 9 (middle) exhibits a wingless phenotype.

gooseberry control (wg gsb/ + gsb)

The larva in Figure 9 (bottom) exhibits a gooseberry phenotype.

3. patch gooseberry (ptc gsb/ptc gsb)

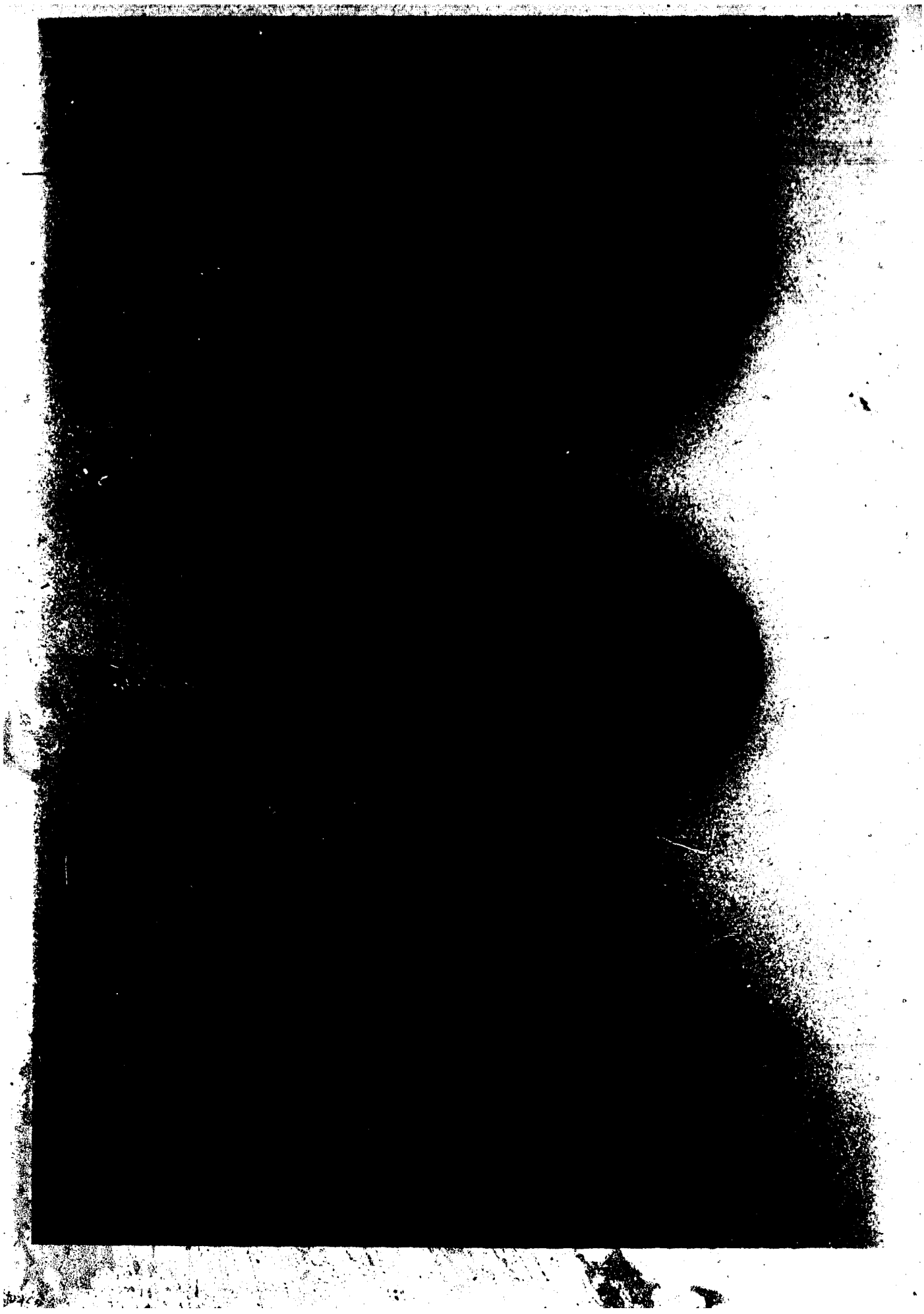
A larva with this genotype is illustrated in Figure 10 (top). Analysis of the repeat pattern is difficult due to the complex phenotype exhibited by these larvae. By counting the alternating pattern of small and large denticles, and noting the polarity reversals present in the pattern, five to six polarity repeats can be observed. This indicates a wildtype repeat pattern. Most of the denticles on the lateral edges of the ventral surface are directed ventrally while those denticles present on the ventral midline point anteriorly or posteriorly. No Keilin's organs were observed but this could be due to the large number of 'holes' present in the pattern. The holes are regions where no differentiated cuticle is present. The edge of the wound is darkly pigmented and would obscure any Keilin's Organs that could be present. The occurence of the holes in the cuticle is directly correlated to the presence of the gsb allele. They are sometimes present in larvae of different genotypes but occur very rarely. When gsb is

Figure 9: wingless gooseberry Double Mutant and Control Larvae.

top- wingless gooseberry double mutant larva.
Magnification = 438X

middle- wingless control larva.
Magnification = 43X

bottom- gooseberry control larva.
Magnification = 240X



present every larva has at least one of these cuticular anomalies, most will contain several.

patch control (ptc gsb/ptc +)

A larva of this genotype is shown in Figure 10 (middle). It closely resembles the patch phenotype in that a wildtype number of repeats are present which display the polarity pattern associated with the patch phenotype. The Keilin's Organs exhibit duplications so that four hairs are present rather than the normal three. This is convincing evidence that the patch mutation is present on the double mutant chromosome.

gooseberry control (ptc gsb/ + gsb)

The larva shown in Figure 10 (bottom) displays a phenotype that is very similar to gsb homozygotes.

4. oddskipped wingless (odd wg/odd wg)

This double mutant is shown in Figure 11 (top). It exhibits a pair rule pattern, like oddskipped.

Two morphologically different types of denticle belts are observed, A2 A4 A6 A8 and A3 A5 A7. These differ in the number of denticles per belt, the odd numbered segments containing fewer than the even numbered ones. This is expected as the odd mutant does not completely remove the odd numbered denticle belts in the single mutant leaving small portions of each. Examination of the denticles leads to the conclusion that polarity reversals are occuring in each segmental repeat. At the anterior and posterior edges of each belt the large

Figure 10: patch gooseberry Double Mutant and Control Larvae.

top- patch gooseberry double mutant larva.
Magnification = 352X

middle- patch control larva.
Magnification = 256X

bottom- gooseberry control larva.
Magnification = 300X

denticles point in a posterior and anterior direction respectively. Thus the denticle polarity in each belt resembles wingless.

oddskipped control (odd wg/odd +)

Figure 11 (middle) illustates the pair rule pattern seen in these larvae. The polarity of the denticles in each band is normal, the first and fourth rows pointing anteriorly, the remaining rows posteriorly. The odd numbered denticle belts contain many fewer denticles than the even numbered belts. These observations support the conclusion that the larva is homozygous for the odd mutant.

wingless control (odd wg/ + wg)

Figure 11 (bottom) shows a typical larva and the aberrant denticle pattern present in these larvae. The pattern is similar to wingless with no naked cuticle and regular polarity reversals. Surprisingly, the odd numbered denticle belts are morphologically different from the even numbered belts. As the arrows in the figure indicate, the odd numbered repeats contain fewer denticles at the midline region. This suggests that odd is exerting a slight dominant pair rule effect.

5. wingless evenskipped (wg eve/wg eve)

A larva of this genotype is shown in Figure 12 (top). Analysis of the segmentation pattern is complicated for several reasons. First, the number of pattern repeats present is usually ascertained by counting such features

Figure 11: oddskipped wingless Double Mutant and Control Larvae.

top- oddskipped wingless double mutant larva. Arrow points to the interior of the odd numbered repeat units where less denticles are present than in the even numbered belts.
Magnification =379X

middle- oddskipped control larva.
Magnification = 256X

bottom- wingless control larva. Arrow points to odd numbered repeat units where fewer denticles are present than in even numbered repeats.
Magnification = 382X

as the number of polarity reversals in the denticle belts on the ventral surface. In these larvae, the posterior half is sparsely populated by denticles and the polarity of the denticles is not obvious and appears random. Secondly, denticles representing even as well as odd numbered segments are apparently present. The even numbered polarity repeats often appear to contain fewer denticles than the odd numbered ones but this is not always clear. Due to these problems, the repeat pattern can only tentatively be designated as pair rule. The polarity of the denticles themselves are as follows; the anterior half of each polarity repeat points posteriorly, the posterior half anteriorly. The anterior row of the first abdominal belt is directed posteriorly, in contrast to the single mutant wg homozygous phenotype. Table 4 demonstrates the number of polarity repeats that can be visualized in several of these larvae.

wingless control (wg eve/wg +)

This control larva, as illustrated in Figure 12 (middle) has a wildtype number of polarity repeat units. It is identical to single mutant wg homozygotes with two wild-type copies of the eve gene with one exception, the anterior row of denticle belt A1 points posteriorly rather than anteriorly. This could be due to interactions between these loci.

evenskipped control (wg eve/ + eve)

Further evidence of interactions between these

mutants is seen in this control. Figure 12 (bottom) demonstrates an example of one of these larvae. Only two of twenty-one larvae exhibited a normal evenskipped pattern. All others had portions, and often the entirety of even numbered segments present. This is in striking contrast to the evenskipped embryos with two wildtype copies of the wg locus. These embryos have highly invariable phenotypes and rarely contain any denticles that can be associated with even numbered segments. Compare Table 5 with Table 6. Although the phenotype is highly variable, the eve mutant is present on the double mutant chromosome as these embryos are missing portions of the even numbered denticle belts, and are lethal.

6. wingless paired (wg prd/wg prd)

Figure 13 (top) illustrates the intriguing phenotype of this double mutant. The segmentation pattern is obviously pair rule as four, double sized, abdominal denticle belts are present. Each belt exhibits polarity reversals as in wg homozygotes. The anterior half of each denticle belt points posteriorly, the posterior half anteriorly. This phenotype is convincing evidence that both mutants contribute additively to the double mutant phenotype.

wingless co[illegible]rd/wg +)

Figure [illegible] middle) demonstrates that a larva of this genotype is very similar to larvae with two wildtype copies of the prd locus. However, there is one minor

Figure 12: *evenskipped*, *wingless* Double Mutant and Control Larvae.

top- *wingless evenskipped* double mutant.
Magnification = 335X

middle- *wingless* control larva.
Magnification = 335X

bottom- *evenskipped* control larva.
Magnification = 271X

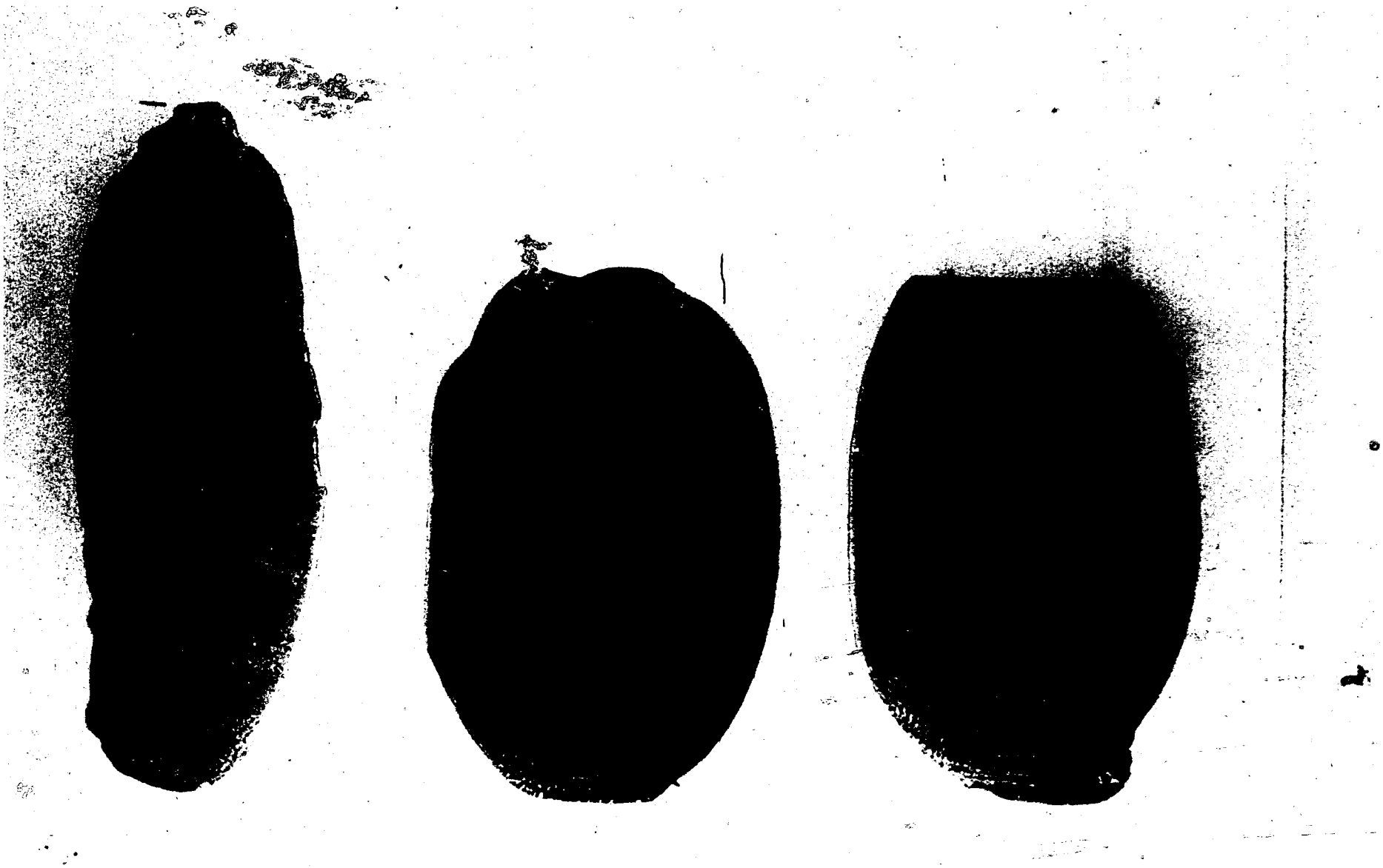


Table 4: NUMBER OF POLARITY REPEATS PRESENT IN *wg eve* HOMOZYGOTES

Number of larvae	Number of Polarity Repeats Present
3	4
8	5
7	6

Table 5: ENTIRE OR PARTIAL DENTICLE BELTS PRESENT IN *eve* + / *eve wg* CONTROL LARVAE

Number of larvae	Denticle Belts Present											
	T1	T2	T3	A1	A2	A3	A4	A5	A6	A7	A8	**
2		X		X		X		X		X		
6		X		X		X		X		X	X	
1		X		X		X	X	X		X		
2		X		X		X	X	X		X	X	
2	X	X		X		X		X		X	X	
3	X	X		X	X	X	X	X		X	X	
1	X	X	X	X		X	X	X		X	X	
4												X

'**'- denticle belts cannot be identified

Table 6: DENTICLE BELTS PRESENT IN *evenskipped* HOMOZYGOTES AT 25 °C

Number of larvae	Denticle belts present										
	T1	T2	T3	A1	A2	A3	A4	A5	A6	A7	A8
13		X		X		X		X		X	
1		X		X		X		X		X	X

difference. In the first abdominal belt of control larvae, there is usually a row of denticles at the anterior edge of the belt that points posteriorly. This differs from wg homozygotes with two wildtype copies of the prd gene where the denticles of this belt are all directed anteriorly.

paired control (wg prd/ + prd)

The larva in Figure 13 (bottom) demonstrates a typical paired phenotype.

7. sloppypaired wingless (slp wg/slp wg)

Figure 14 (top) shows a typical example of a larva with this genotype. In this case it is difficult to determine the number of repeat units in the double mutant due to the absence of completely naked cuticle between denticle belts. Since the wg mutant belongs to the segment polarity class, the repeat pattern could be analyzed by counting the number of discrete polarity reversals along the length of the larva. This was done by observing the direction in which groups of denticles point. Table 7 presents the result of this analysis for the slp wg homozygotes. It illustates that the number of polarity repeat units for the abdominal region appears wildtype rather than pair rule. This conclusion is tentative due to the absence of completely naked cuticle between denticle belts. The thoracic region is uninterpretable for several reasons. Not only is this area poorly differentiated in these larvae, any denticles

Figure 13: *wingless paired* Double Mutant and Control Larvae.

top- *wingless paired* double mutant.
Magnification = 307X

middle- *wingless* control larva.
Magnification = 327X

bottom- *paired* control larva.
Magnification = 267X

present cannot be assigned a segmental identity nor their polarity analyzed due to their disorganization. Thoracic-like denticles are completely absent in a large proportion of the larvae. As well, the most posterior area of denticles does not have well-defined polarity, therefore the region anterior to this and posterior to the thoracic area was used in the analysis of the double mutant phenotype.

At the lateral edges of the belts are relatively small denticles, as you move toward the ventral midline their size increases until you reach the midline, itself populated by small denticles. All denticles point toward the ventral midline with two exceptions. First, small denticles along the ventral midline are usually directed posteriorly. Secondly, singularities toward which all surrounding denticles point similar to those observed in the wingless patch homozygotes are present. These regions are located on either side of the ventral midline, in the larger sized denticles. The regions cannot be identified in all denticle belts, one possible explanation for this could be the paucity of denticles in some belts. A higher density of denticles might be necessary to impart an observable effect.

sloppypaired control (slp wg/slp +)

Figure 14 (middle) illustrates the phenotype of these larvae. The repeat unit is two segments large and is a result of the deletion of a portion of the naked cuticle

associated with T2 A1 A3 A5 and A7. This causes the denticle belts on either side of the deleted cuticle to be closer together than normal. In single mutant slp homozygotes, this pair-wise association rarely results in fusions of the denticle belts. In this control however, apparently random fusions of denticle belts do occur, Table 8. A probable explanation of this is that a larger amount of naked cuticle is being deleted when wg is heterozygous than in slp homozygotes alone. Table 9 details the occurrence of fusions in slp homozygotes. Other than the appearance of fusions, the phenotype is identical to that of single mutant slp homozygotes, Figure 4 (middle).

wingless control (slp wg/ + wg)

A larva of this genotype is seen in Figure 14 (bottom). The control larvae are identical to the single mutant wg larvae (Figure 7, top) with the exception that smaller denticles than those normally present, are found at the anterior and posterior edges of the denticle belts. The polarity of these denticles is variable, however, in general if the region between the denticle belts is divided in half perpendicular to the ventral midline, those in the anterior half point anteriorly, those in the posterior half, posteriorly. The ventro-lateral singularities toward which surrounding denticles point, are present as observed in the double mutant, but not in all denticle belts. The presence of polarity

Figure 14: sloppypaired wingless Double Mutant and Control Larvae.

top- sloppypaired wingless double mutant larva.
Magnification = 345X

middle- sloppypaired control larva.
Magnification = 262X

bottom- wingless control larva.
Magnification = 323X

Table 7: NUMBER OF POLARITY UNITS PRESENT IN slp wg HOMOZYGOTES

Number of Larvae	Number of Polarity Units
10	7
6	6
1	5
1	7+
1	6+
1	5+

'+' denticles are present but pattern repeat [redacted] not be defined due to disorganization in posteri[redacted] portion of embryo.

Table 8: DENTICLE BELT FUSIONS PRESENT IN slp wg/slp + LARVAE

Number of Larvae	Denticle Belt Fusions Observed {A1-A2}	{A3-A4}	{A5-A6}	{A7-A8}
1		**		
2	X			
1			X	
1		X	X	
1	X	X	X	
1	X	X	X	X
1	X	[X -----	X]	X

'[X--X]' secondary fusion of these denticle belts
'**' no fusions present

Table 9: DENTICLE BELT FUSIONS PRESENT IN slp HOMOZYGOTES

Number of Larvae	Denticle Belt Fusions Observed {A1-A2}	{A3-A4}	{A5-A6}	{A7-A8}
10		**		
2			X	
1	X		X	X

'*' no fusions present

reversals is conclusive evidence that the wg mutant is present on the double mutant chromosome.

8.oddskipped patch (odd ptc/odd ptc)

A larva of this genotype is shown in Figure 15 (top). It has only four, enlarged denticle belts as in pair rule mutants but each belt shows polarity reversals as found in patch.

The shape of the denticle belts is abnormal. Instead of the wildtype trapezoidal pattern observed in Figure 3, the belts are rectangular. The polarity of the denticles are as follows; the first or anterior row of each belt points in an anterior direction, the last or posterior row points in a posterior direction and all other denticles are directed perpendicular to the anterio-posterior axis. They can point towards or away from the ventral midline. As in ptc homozygous embryos the thoracic denticle belts are usually absent. The Keilin's organs associated with them are very difficult to find, and in some cases may be absent. When they can be observed however, instead of possessing the normal number of hairs (3) they now have four or five hairs per organ. As with the shape of the denticle belts, this is undoubtedly an effect of the ptc mutant as these characteristics are all observed in ptc homozygotes (Figure 7, middle).

Many double mutant larvae contain large denticle belts alternating with smaller pieces of denticle belts.

This can be attributed to the odd pair rule mutant, (Figure 4, top). In ptc larvae, A1 is usually represented by a small region of denticles. The odd mutant removes A1 very efficiently compared to other odd numbered segments. This would explain why A1 is never observed in odd ptc homozygotes, while the presence of other odd numbered denticle belts is common.

oddskipped contol (odd ptc/odd +)

A pair rule phenotype is observed in these larvae as shown in Figure 15 (middle). Six repeat patterns can be seen, including the denticle belts of T1 T3 A2 A4 A6 A8. These denticle belts exhibit normal denticle polarity. As well, small pieces of the odd numbered denticle belts are observed. Since these larvae are lethal and possess a pair rule phenotype identical to oddskipped alone, it can be concluded that the odd mutant is present on the double mutant chromosome.

patch control (odd ptc/ + ptc)

The larva shown in Figure 15 (bottom) displays a phenotype that is very similar to that observed in ptc homozygotes.

The lethality of these larvae and the characteristic shape of the denticle belts is convincing evidence that the ptc mutant is present on the double mutant chromosome.

Figure 15: oddskipped patch Double Mutant and Control Larvae.

top- oddskipped patch double mutant larva.
Magnification = 282X

middle- oddskipped control larva.
Magnification = 277X

bottom- patch control larva.
Magnification = 288X

9. sloppypaired patch (slp ptc/slp ptc)

The phenotype of these double mutants combines the features of the two single mutant phenotypes. As seen in Figure 16 (top), the denticle belts are closely associated in a pair-wise fashion. T2-T3, A1-A2, A3-A4, A5-A6, and A7-A8 demonstrate the sloppypaired 'leaning' of denticle belts towards each other. The polarity of the denticles exhibit the typical patch phenotype. The belts are square instead of trapezoidal, with the anterior row directed anteriorly, the posterior row posteriorly and intervening denticles pointing ventrally, although a small percentage point dorsally. Therefore, both mutants contribute to the double mutant phenotype.

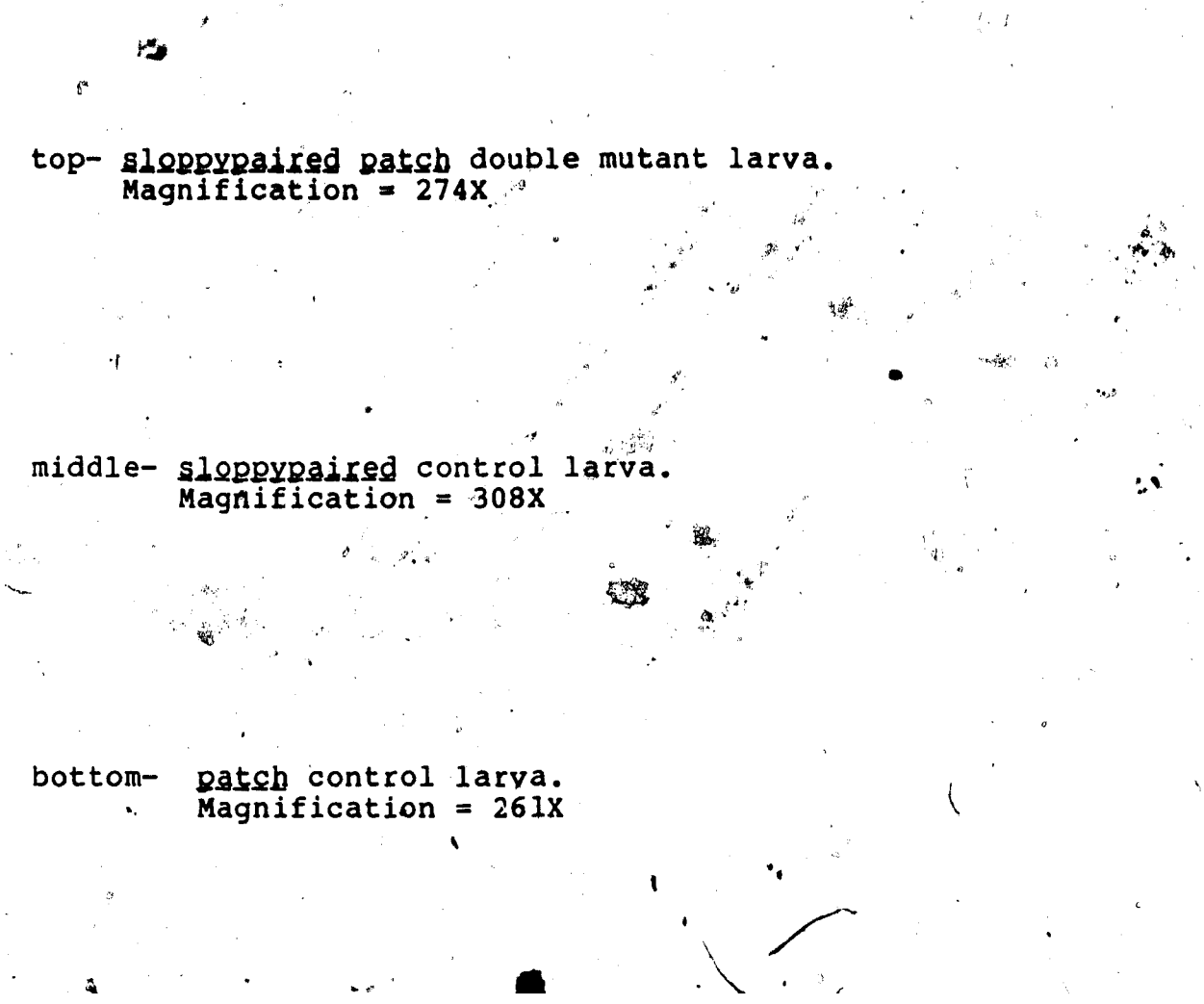
sloppypaired control (slp ptc/slp +)

Figure 16 (middle) illustrates a larva of this genotype exhibiting a typical sloppypaired phenotype. The normal number of denticle belts are present and 'lean' towards each other in a pair-wise fashion, characteristic of the sloppypaired phenotype.

patch control (slp ptc/ + ptc)

These larvae, as shown in Figure 16 (bottom) have a typical patch phenotype. The denticle belts are square instead of trapezoidal, and the polarity is characteristic of polarity reversals. The anterior row of each denticle belt points anteriorly, the posterior row posteriorly, the intervening denticles ventrally, although some are directed dorsally.

Figure 16: sloppypaired patch Double Mutant and Control Larvae.



top- sloppypaired patch double mutant larva.
Magnification = 274X

middle- sloppypaired control larva.
Magnification = 308X

bottom- patch control larva.
Magnification = 261X

10. sloppy paired (slp prd/slp prd)

This double mutant, pictured in Figure 17 (top) has a pair rule segmentation pattern. It contains four large abdominal denticle belts, one large thoracic belt and anterior to this, pieces of the T1 belt. Between the abdominal belts are large areas of naked cuticle. However in a small percentage of larvae, a number of denticles can be found in this region. This is interpreted as weak secondary fusions of the large belts. Polarity in the large belts is normal except at the lateral edges. Here, the denticles point dorsally, but as you move ventrally in the belt the denticles are directed ventrally. Only small patches of denticles participate in these polarity changes. These disruptions are present in most of the large denticle belts whether they are participating in secondary fusion events or not.

Judging by the size of the denticle belts, the increased number of rows present in each and the genotype of these larvae, it is reasonable to conclude that these denticle belts are a result of fusions. The anterior half of A1 fuses with the posterior half of A2, this continues down [illegible] producing the large denticle belts observed.

This is identical to the phenotype observed in single mutant prd homozygotes. The only observable difference lies in the tendency for secondary fusions of the large denticle belts in the double mutant larvae. Since they

are not observed in the single mutant, this suggests that the double mutant phenotype is more extreme than the paired phenotype.

sloppypaired control (slp prd/slp +)

Figure 17 (middle) illustrates a larva with a pair rule segmentation pattern. As can be seen, T2T3, A1A2, A3A4, A5A6, and A7A8 associate together. The phenotype is highly variable ranging from a pair-wise 'leaning' toward each other to complete fusion into double sized composite segments.

It can be concluded by comparison with the single mutant phenotype, that more naked cuticle is being deleted, the smallest deletion resulting in the less extreme phenotype and the largest deletion in segmental fusions. This indicates that not only is the slp mutant present on the double mutant chromosome, but that the prd allele is exerting a slight dominant enhancing effect on the sloppypaired phenotype.

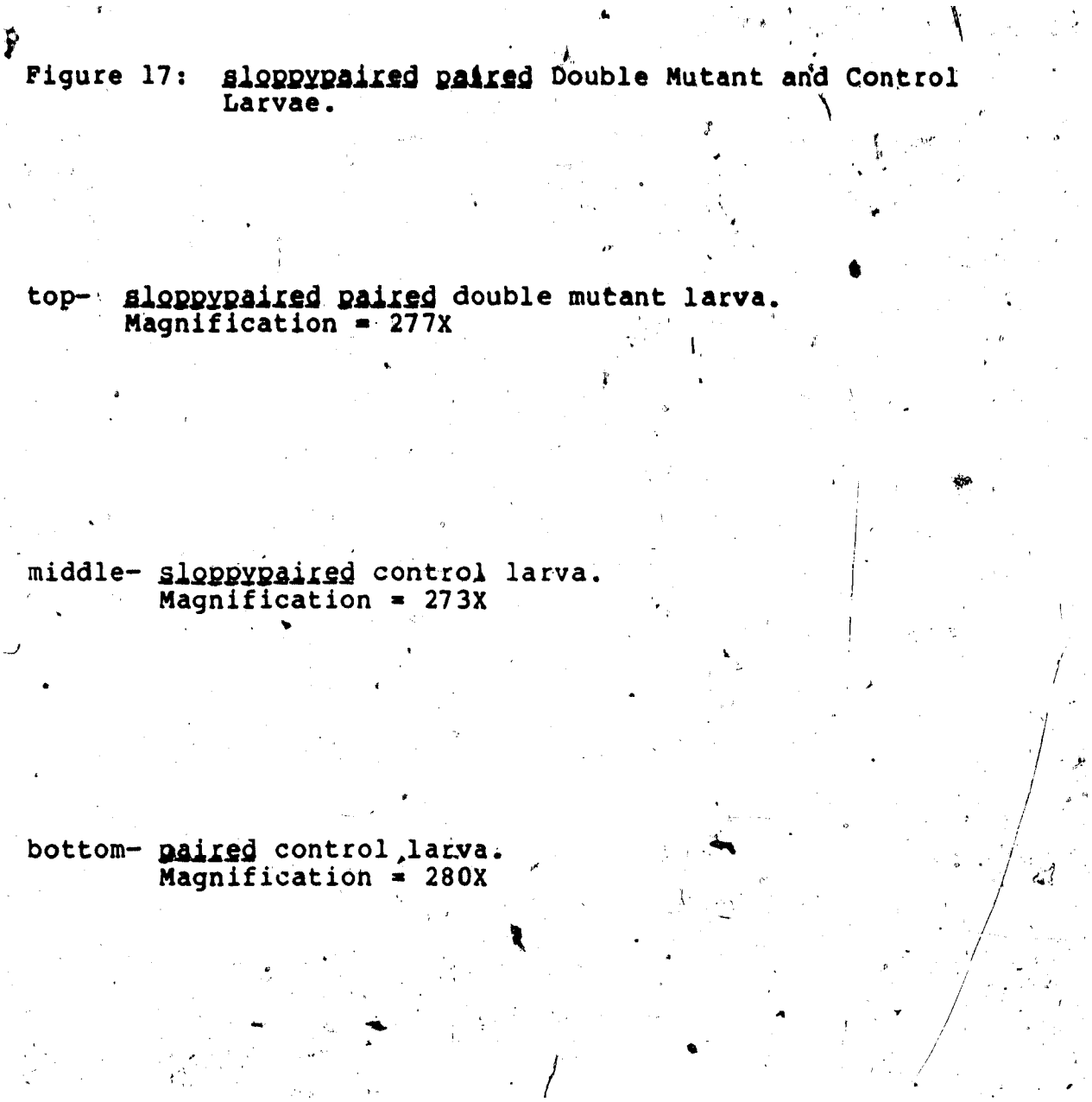
paired control (slp prd/ + prd)

The larva shown in Figure 17 (bottom) is identical in phenotype to the single mutant prd homozygotes.

11. oddskipped paired (odd prd/odd prd)

Both odd and prd are pair rule mutants in which all or part of the odd numbered abdominal segments are deleted. This double mutant larva, as shown in Figure 18 (top) is very similar to larvae homozygous for the single mutant prd. The double mutant pattern exhibits pair

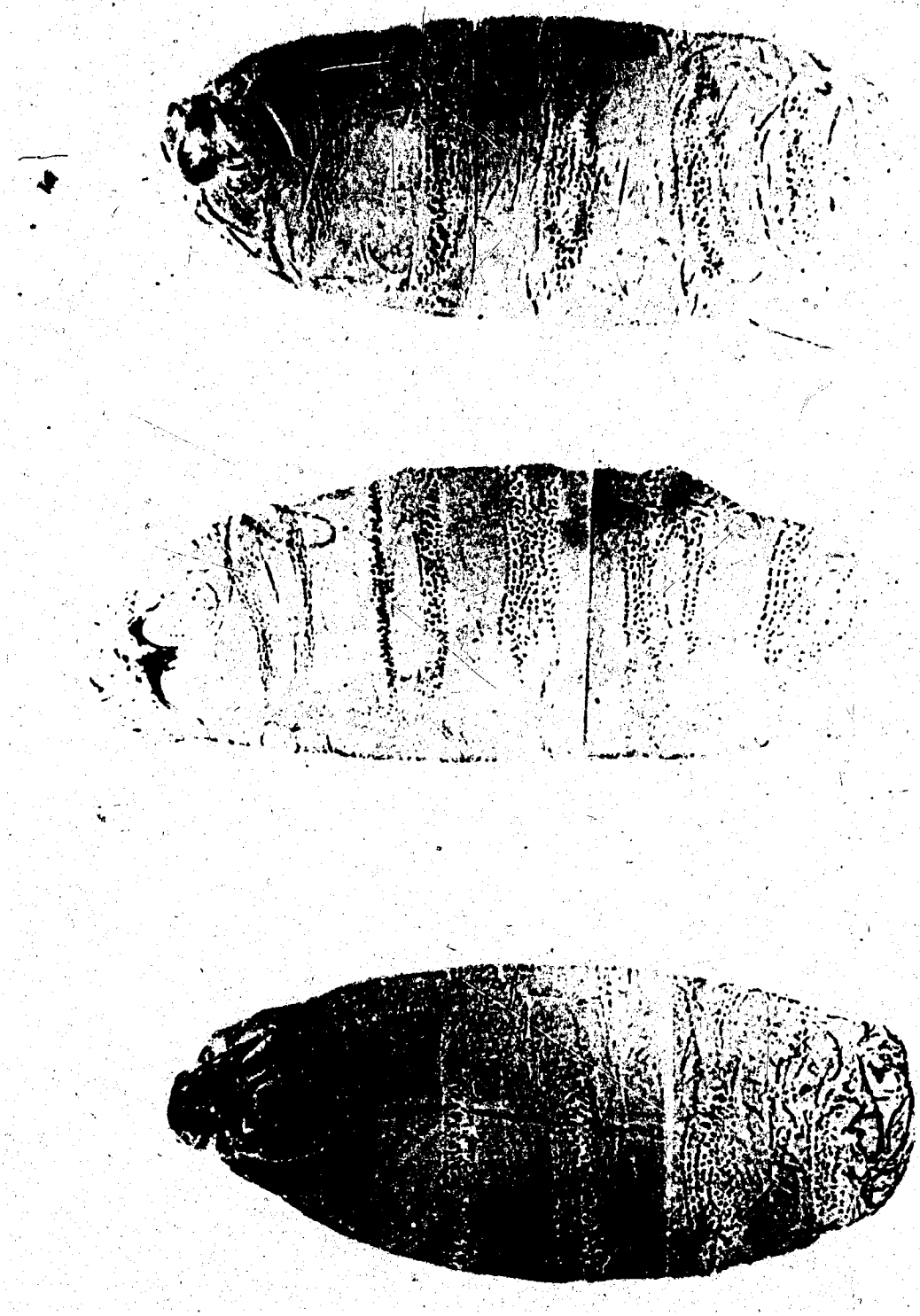
Figure 17: sloppypaired paired Double Mutant and Control Larvae.



top- sloppypaired paired double mutant larva.
Magnification = 277X

middle- sloppypaired control larva.
Magnification = 273X

bottom- paired control larva.
Magnification = 280X



rule segmentation. The large denticle belts look like composites of the anterior portion of the anterior segment's denticle belt and the posterior portion of the posterior segment's denticle belt as if the intervening material has been deleted. For example, the anterior portion of the denticle belt associated with segment A1 is now juxtaposed to the posterior portion of the denticle belt associated with segment A2. These pairwise deletions of denticle belts and naked cuticle continue along the length of the larva, resulting in the formation of four, large, abdominal denticle belts. Anterior to this, the thoracic region also exhibits a large deletion between T2 and T3, the result being closer association of portions of their denticle belts. Some of T1's belt is also present but thorough analysis of this region is difficult due to the small, fine denticles present and the similarities in the shape of T2's and T3's denticle belts.

Further examination of the double mutant larva's denticle belts reveals that the polarity of the denticles is normal in most cases. The first and fourth rows are directed anteriorly, the remainder posteriorly. Frequently, the anterior of the composite belts will be completely normal with the posterior half appearing 'crowded', as if the number of denticles present are populating a smaller region than normal. Small polarity reversal are sometimes observed at the juxtaposition of

two denticle belts.

The only apparent effect of odd on the paired phenotype is to make it weaker in the double mutant. Areas of naked cuticle are frequently observed in the middle of the composite denticle belts. This separation is never observed in the single mutant lethal larvae, (See Figure 4,bottom). Table 10 reveals that while the A1A2 unit is usually incompletely fused, more posterior units are often present without the intervening naked cuticle. Although two larvae were observed that exhibited no separation of denticle belts, in general this double mutant possesses a more wildtype denticle and segmentation pattern than that observed in either of its single mutant parent stocks. Therefore, both mutants contribute to the double mutant phenotype.

oddskipped control (odd prd/odd +)

Figure 18 (middle) illustrates the pair rule pattern exhibited by these larvae. Although a wildtype number of denticle belts are present, the odd numbered belts have pieces missing. Since the even numbered denticle belts are not affected in this way it can be concluded that the oddskipped phenotype is present but less strongly expressed than when two copies of prd^{+} are present.

The polarity of the denticles themselves is different from that observed in the single mutant odd homozygotes. Normally, the denticles in the first and fourth rows point anteriorly, the remainder posteriorly. At the

lateral edges of the denticle belts in this control the denticles are directed dorsally. A little further toward the ventral midline, they are directed ventrally. At the midline itself, the denticles exhibit normal polarity. The phenotype of these larvae and the fact that they are lethal is convincing evidence that the odd mutation is present in the double mutant.

paired control (odd prd/ + prd)

Figure 18 (bottom) illustrates the pair rule segmentation pattern observed in these larvae. As in the double mutant, pair-wise deletions are present resulting in the formation of composite denticle belts. This control differs from the single mutant prd homozygotes in that regions of naked cuticle sometimes separate the composite denticle belts. Table 11 describes the frequency of occurrence of this event.

This suggests that one entire segment is not being deleted in this control as it is in the single mutant. This is surprising considering that an odd^{+} allele is present. Apparently, it exerts a slight dominant effect when prd is homozygous.

12. sloppypaired evenskipped (slp eve/slp eve)

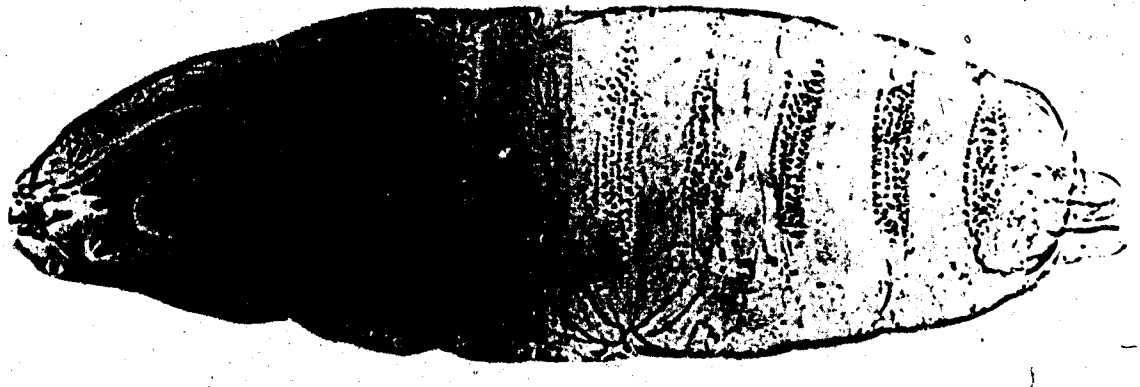
Figure 19 (top) illustrates the typical ventral pattern exhibited by these larvae. The double mutant phenotype is very similar to that of eve homozygous larvae with a few exceptions. Eve larvae normally contain the odd numbered abdominal denticle belts and the second

Figure 18: *oddskipped paired* Double Mutant and Control Larvae.

top- *oddskipped paired* double mutant larva. The phenotype in this case is less extreme than either of the single mutants.
Magnification = 271X

middle-*oddskipped* control larva.
Magnification = 304X

bottom- *paired* control larva.
Magnification = 268X



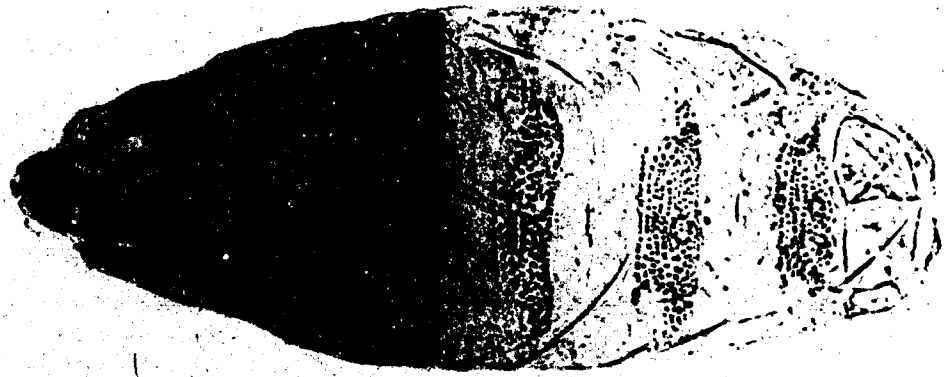


Table 10: OCCURRENCE OF INCOMPLETE FUSIONS OF DENTICLE BELTS IN *odd prd* HOMOZYGOTES

Number of larvae	Pattern Repeats Exhibiting Incomplete Fusion					
	T1	{T2-T3}	{A1-A2}	{A3-A4}	{A5-A6}	{A7-A8}
2				*		
1		X				
1			X			
3				X		
2			X	X		
3			X		X	
1			X			X
1				X	X	
2				X		X
1					X	X
1			X	X	X	
1			X	X		X
1		X	X	X	X	
1		X	X	X		X
3			X	X	X	X
5		X	X	X	X	X

'*' no incomplete fusions present

Table 11: OCCURRENCE OF INCOMPLETE FUSIONS OF DENTICLE BELTS IN ± *prd*/*odd prd* LARVAE

Number of larvae	Pattern Repeats Exhibiting Incomplete Fusions					
	T1	{T2-T3}	{A1-A2}	{A3-A4}	{A5-A6}	{A7-A8}
29				*		
2		X				
1				X		
3						X
1			X	X		

'*' no incomplete fusions present

thoracic belt. The double mutants have these belts but also sometimes differentiate A4 and A8 in addition, as demonstrated in Table 12. The denticle polarity in the odd numbered [illegible]s is normal. Usually, only pieces of A4 and A8 [illegible]esent, compl[illegible]ting analysis of their polarity. Several larvae how[illegible] contain a substantial portion of these denticle belts. When the polarity is analyzed in these larvae, there is some evidence for the presence of polarity reversals. The anterior row of denticles is directed anteriorly, the posterior row posteriorly, with the intervening denticles pointing ventrally or dorsally. If this denticle band was exhibiting normal polarity, the fourth row should point anteriorly. This is not the case.

sloppypaired control (slp eve/slp +)

A larva of this genotype is shown in Figure 19 (middle). It exhibits a typical sloppypaired phenotype in the number of denticle belts present and their polarity. The only difference between this control and larvae with two wildtype copies of the eve locus is that less cuticle appears to be deleted between denticle bands. That is, normally a pair-wise 'leaning' of denticle belts A1-A2, A3-A4, A5-A6, and A7-A8 occurs. This feature of the phenotype is less obvious in the control larvae, suggesting that their pattern is more wildtype than expected.

evenskipped control (slp eve/ + eve)

Only one larva of this genotype exhibited the expected evenskipped phenotype where all even numbered abdominal denticle belts and first and third thoracic belts are absent. A more typical example is shown in Figure 19 (bottom). Ten of 12 larvae possessed portions of A4 while 9 of 12 had pieces of A8. Table 13 details these observations. In larvae containing two wildtype copies of the slp locus none contained any denticles attributable to A4, while only 1 of 14 larvae had a few denticles associated with A8. Therefore the phenotype of evenskipped seems to be somewhat suppressed when the embryo is heterozygous at the slp locus.

13. oddskipped evenskipped (odd eve/odd eve)
25^{o} C

Figure 20 (top) illustrates an larva of this genotype exhibiting a pair rule segmentation pattern. This phenotype is very difficult to define further as the majority of larvae had only incomplete denticle belts present in the region normally occupied by A2 through A6. However, one larva was found that exhibited a normal evenskipped phenotype. That is, T2,A1,A3,A5 and A7 were present. Other larvae were similar to this but had naked cuticle between the anterior and posterior portions of one or more of their denticle belts. No larvae were observed with an oddskipped pattern. The polarity of denticles in the belts appeared normal except that small polarity reversals were sometimes observed in the

Figure 19: sloppypaired evenskipped Double Mutant and Control Larvae.

top- sloppypaired evenskipped double mutant.
Magnification = 286X

middle- sloppypaired control larva.
Magnification = 274X

bottom- evenskipped control larva with an uncharacteristically leaky phenotype.
Magnification = 275X

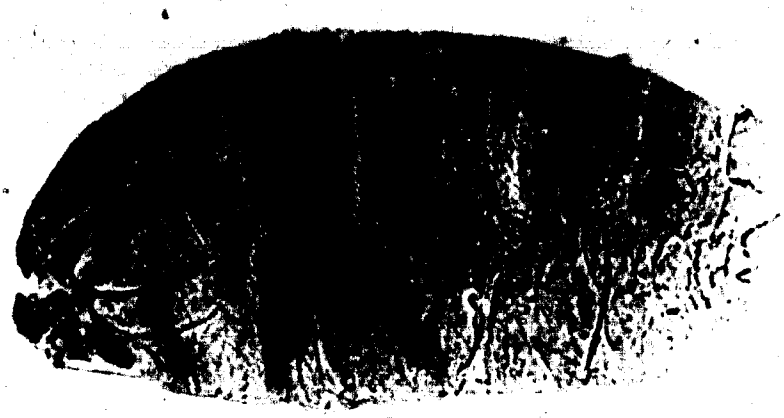


Table 12: DENTICLE BELTS PRESENT IN *slp eve* HOMOZYGOTES

Number of Larvae	Denticle Belts Present										
	T1	T2	T3	A1	A2	A3	A4	A5	A6	A7	A8
1		X		X—		X	X	X		X	
2				X		X	X	X	X	X	X
5		X		X		X	X	X		X	X
1		X		X	X	X	X	X	X	X	X

Table 13: DENTICLE BELTS PRESENT IN *slp eve*/ +*eve* LARVAE

Number of Larvae	Denticle Belts Present										
	T1	T2	T3	A1	A2	A3	A4	A5	A6	A7	A8
1		X		X		X		X		X	
2		X		X		X	X	X		X	
1		X		X		X		X		X	X
7		X		X		X	X	X		X	X
1		X	X	X		X	X	X		X	X

denticles surrounding the small area of naked cuticle.

oddskipped control (odd eve/odd +)

These larvae, as demonstrated by Figure 20 (middle), exhibit an oddskipped phenotype.

evenskipped control (odd eve/ + eve)

A larva of this genotype is shown in Figure 20 (bottom). It has a normal evenskipped phenotype.

18 °C

14. oddskipped evenskipped (odd eve/odd eve)

A larva of this genotype is shown in Figure 21 (top). Since this is the permissive temperature for the eve allele, the even numbered denticle belts should be present and normal while the odd numbered belts should display the oddskipped defects. Table 14 reveals no typical or average pattern for these larvae. No one denticle band was present and completely normal is shape at all times and none were always absent. The polarity of the denticle belts was normal. Therefore the pair rule phenotype of oddskipped is almost completely suppressed by eve at its permissive temperature, and the phenotype of evenskipped may be somewhat enhanced by odd.

oddskipped control (odd eve/odd +)

Figure 21 (middle) shows a larva of this genotype. The phenotype was in all cases normal oddskipped.

evenskipped control (odd eve/ + eve)

A larva of this genotype is illustrated in Figure 21 (bottom). As expected at this temperature, these larvae

Figure 20: oddskipped evenskipped Double Mutant and Control Larvae at 25 C.

top- oddskipped evenskipped double mutant larva.
Magnification = 274X

middle- oddskipped control larva.
Magnification = 259X

bottom- evenskipped control larva.
Magnification = 275X

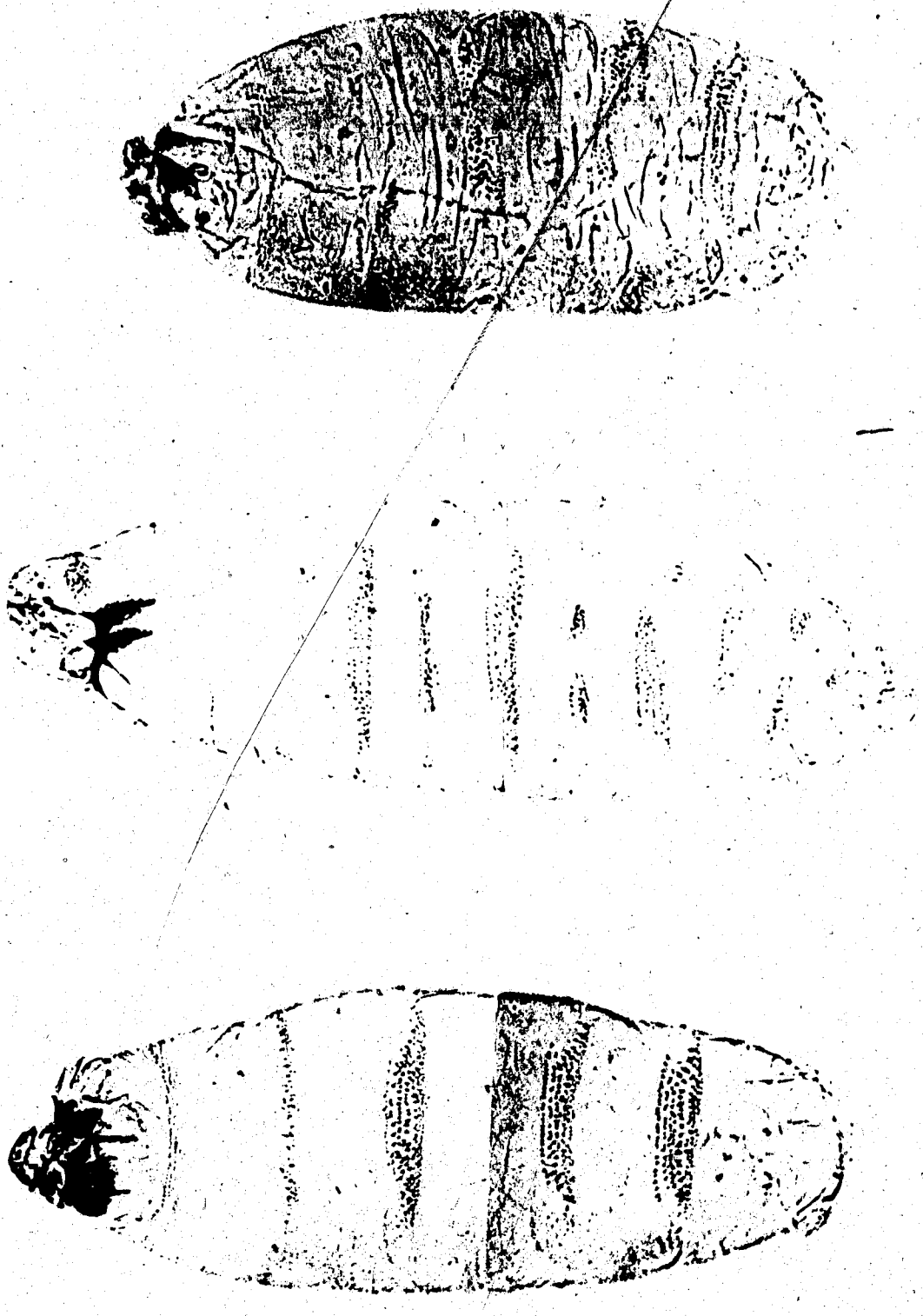


Figure 21: *oddskipped evenskipped* Double Mutant and Control Larvae at 18 C.

top- *oddskipped evenskipped* double mutant larva.
Magnification = 240X

middle- *oddskipped* control larva.
Magnification = 270X

bottom- *evenskipped* control larva.
Magnification = 303X

Table 14: DENTICLE BELTS PRESENT AT 18° IN *odd* *eve* HOMOZYGOTES

Number of Larvae	Denticle Belts Present										
	T1	T2	T3	A1	A2	A3	A4	A5	A6	A7	A8
1	X	X	X	X	X	X	X	X	X	X	X
1	X	X	X	X	X	X	X	X		X	X
1		X	X	X	X	X	X	X		X	X
1	X		X	X	X	X	X	X		X	X
1	X	X	X		X	X	X	X		X	X
1	X	X	X	X		X	X	X		X	X

appeared almost wildtype.

29 °C

15. oddskipped evenskipped (odd eve/odd eve)

As shown in Figure 22 (top), the larval phenotype can best be described in general as the fusion of any remaining portions of denticle belts. This phenotype is very variable, ranging from an almost perfect evenskipped phenotype to having denticles present only in one half of the larva. The most extreme larvae contain a region of fusions in the posterior half of the larva. These are different from fusions occurring in larvae of other genotypes as denticles are present along the lateral edges of the ventral surface and not along the midline. The denticle polarity in the denticle belts is normal with the exception that the majority of denticles in the last phenotypic class are directed ventrally.

oddskipped control (odd eve/odd +)

A larva of this genotype has a normal oddskipped pattern as shown in Figure 22 (middle).

evenskipped control (odd eve/ + eve)

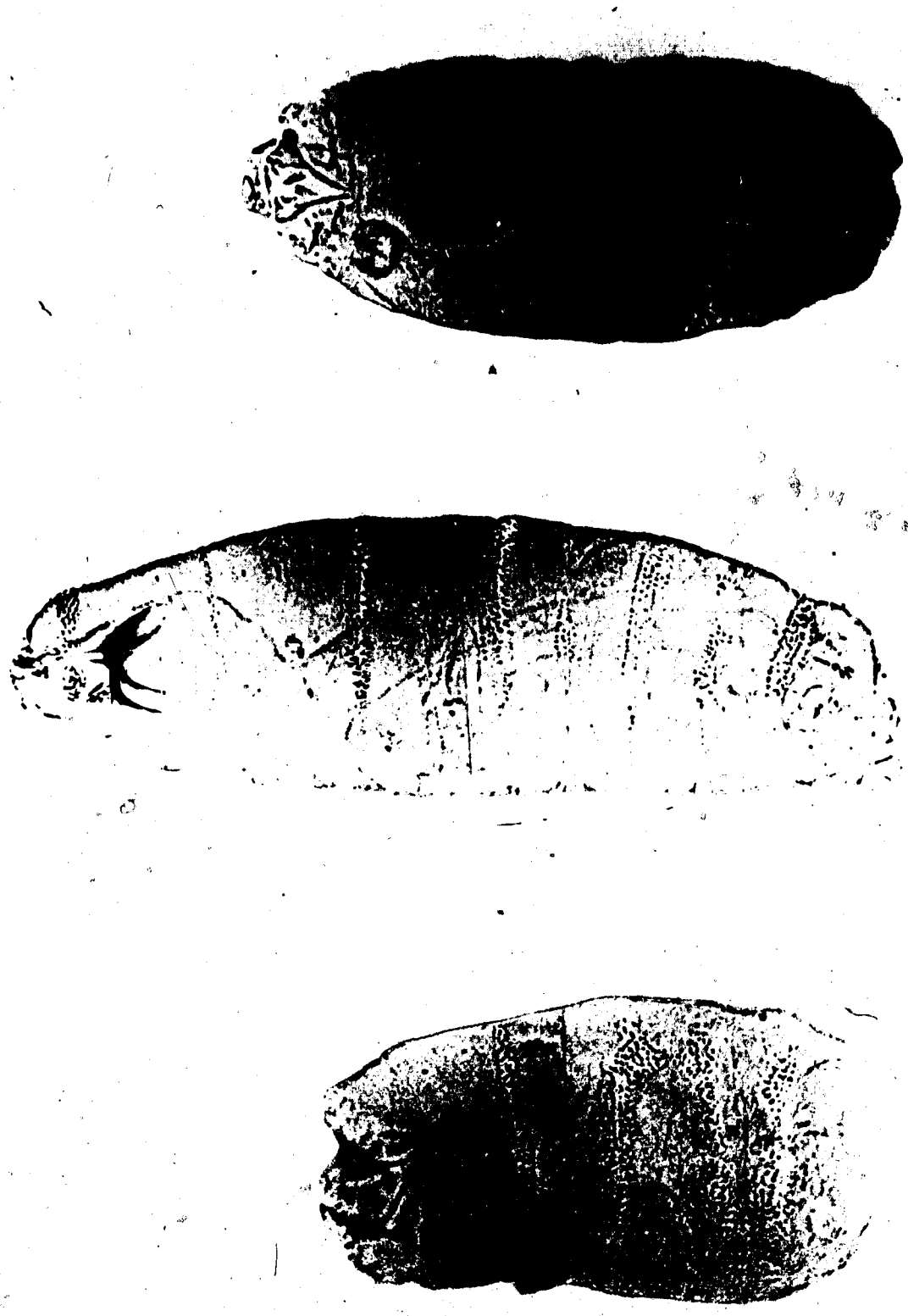
These larvae have an evenskipped phenotype as shown in Figure 22 (bottom). In some cases the four large abdominal denticle belts attempt to fuse. This is the extreme evenskipped phenotype (Nusslein-Volhard et.al., 1985).

Figure 22: oddskipped evenskipped Double Mutant and Control Larvae at 29 C.

top- oddskipped evenskipped double mutant larva.
Magnification = 275X

middle- oddskipped control larva.
Magnification = 268X

bottom- evenskipped control larva.
Magnification = 292X



16. oddskipped engrailed (odd en^{IM99} /odd en^{IM99})

A larva of this genotype is shown in Figure 23 (top). All thoracic and abdominal denticle belts, or portions of them, are present on the ventral surface. All of the denticle belts are separated by naked cuticle except A4A5 and A6A7 which, in this larva, are found together as large denticle belts. The separate belts are not of normal shape. At least one row of denticles is absent from the anterior of each belt as no anteriorly-pointing denticles are observed in this position. Further evidence of this specific defect is seen in A8. This denticle belt was never observed to participate in the formation of double sized denticle belts yet an anteriorly pointing row of denticles is not present in the anterior half of the denticle belt.

Except for the absence of the anteriormost rows, the polarity of the denticles is almost wildtype. The majority point in a posterior direction, with the exception of a few that point anteriorly. This latter class of denticles are usually scattered in an apparently random fashion throughout the denticle belt. This phenotype is significantly different from that of the en^{IM99} single mutant. The latter larvae always have pair-wise fusions of denticle belts, thus, a complex interaction between the two mutations is occurring since the double mutant repeat pattern is more wildtype than either of the single mutants.

oddskipped control (odd $\frac{IM99}{en}$ /odd +)

The larva of this genotype in Figure 23 (middle) exhibits a pair rule repeat pattern. Although the odd numbered denticle belts contain defects, in general, the larval phenotype tends to be more wildtype than normally observed in odd homozygotes. Larger pieces of the odd numbered denticle belts are present, or rather, less of the pattern is deleted when only one wildtype copy of the en locus is present. The deletions observed in these belts are not consistent. One larva may have an apparently normal A1 with A7 being all but absent, in the next larva this situation could be reversed. Denticle polarity is normal in all denticle belts examined.

engrailed control (odd $\frac{IM99}{en}$ / + $\frac{IM99}{en}$

These larvae, as evidenced by Figure 23 (bottom) exhibit an $\frac{IM99}{en}$ phenotype.

17. oddskipped engrailed (odd Df(2)en28/odd Df(2)en28)

Figure 24 (top) illustrates a larva of this genotype. The larvae exhibit an apparently wildtype number of repeat units. Each repeat contains two separate and different polarity reversals. The first type is identified by a row of small denticles which appears within it. Larger, darkly pigmented denticles surround this row. Those anterior of it point posteriorly, those posterior of it are directed anteriorly. The second type of polarity reversal is almost the opposite of the first. A small patch of naked

Figure 23: 1I99 oddskipped en Double Mutant and Control Larvae.

IM99
top- oddskipped en double mutant larva.
Magnification = 344X

middle- oddskipped control larva.
Magnification = 265X

IM99
bottom- en control larva.
Magnification = 373X

cuticle is contained within this type; the denticles anterior of the patch point anteriorly, those posterior of the patch are directed posteriorly. There are no large regions of naked cuticle within the pattern.

oddskipped control (odd Df(2)en28/odd +)

A larva of this genotype is shown in Figure 24 (middle). The larvae on this control slide exhibited a pattern that was more wildtype than that normally observed for odd homozygotes. More of the pattern of the odd numbered denticle belts is present.

engrailed control (odd Df(2)en28/ + Df(2)en28)

The larva displayed in Figure 24 (bottom) exhibits the typical Df(2)en28 phenotype.

18. wingless engrailed ($wg\ en^{IM99}$ /$wg\ en^{IM99}$)

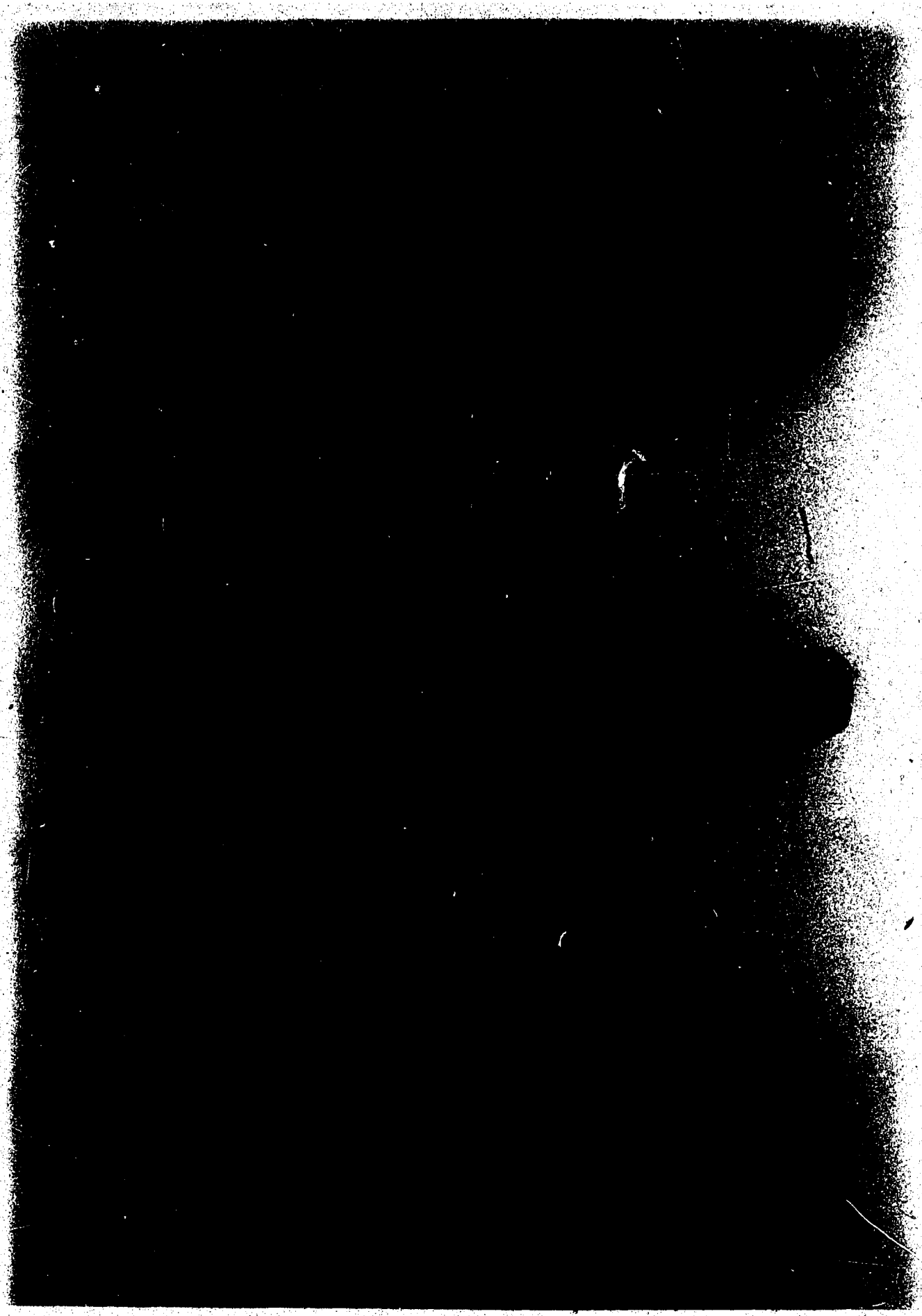
A larva of this genotype is shown in Figure 25 (top). This double mutant has only one thoracic denticle belt. The identity of this belt is ambiguous although it is very similar in appearance to the thoracic belt found in wg homozygotes. The polarity and general appearance of the denticle belts is very similar to that found in en^{IM99} homozygotes. The only observable difference between the single mutant homozygotes and the double mutant larvae is that the latter contains the 'rug of denticles' phenotype. There is very little naked cuticle present within the denticle belt pattern. This is in contrast to the en^{IM99} homozygous phenotype where large regions of naked cuticle are observed completely

Figure 24: oddskipped Df(2)en28 Double Mutant and Control Larvae.

top-oddskipped Df(2)en28 double mutant larva.
Magnification = 335X

middle- oddskipped control larva.
Magnification = 248X

bottom- Df(2)en28 control larva.
Magnification = 349X



separating denticle belts. Another feature of the phenotype which reveals it to be more similar to $\underline{en}^{IM99}$ than $\underline{wg}$ is the presence of the small patches of naked cuticle with surrounding denticles directed away from the patch's centre. This is never observed in $\underline{wg}$ homozygotes. Seven of these structures can usually be identified, although they do not occur precisely on the ventral midline they are never observed at the lateral edges of the belts.

$\underline{wingless}$ control ($\underline{wg}$ $\underline{en}^{IM99}$ /$\underline{wg}$ +)

A larva of this genotype is shown in Figure 25 (middle). These control larvae exhibit the $\underline{wingless}$ phenotype with one exception. In single mutant $\underline{wg}$ homozygotes all of the denticles in A1 are directed anteriorly. In these control larvae, although the vast majority of denticles are directed anteriorly, posteriorly pointing denticles are present at the anterior edge of the denticle belt. The remainder of the pattern is indistinguishable in phenotype from that found in single mutant $\underline{wg}$ homozygotes.

$\underline{engrailed}$ control ($\underline{wg}$ $\underline{en}^{IM99}$ /$\underline{en}^{IM99}$

Figure 25 (bottom) illustrates a larva of this genotype. These control larvae are identical in phenotype to the single mutant $\underline{en}^{IM99}$ homozygotes with one exception. The latter class of larvae contain large regions of naked cuticle separating composite denticle belts. The control larvae possess much smaller regions of

Figure 25: *wingless* ^{IM99} *en* Double Mutant and Control Larvae.

top- *wingless* ^{IM99} *en* double mutant larvae.
Magnification = 402X

middle- *wingless* control larvae.
Magnification =360X

bottom-*en* ^{IM99} control larva.
Magnification = 350X

naked cuticle. The composite denticle belts are no longer completely separated from each other, rather, secondary fusions occur with separations only at the lateral edges of the belts. Thus, the *en*^{IM99} phenotype seems to be enhanced by the *wg* allele.

19.*wingless engrailed* (*wg Df(2)en28/wg Df(2)en28*)

An illustration of this phenotype could not be provided as the denticles are very lightly pigmented and adequate photographs could not be made. These homozygotes appear to exhibit a pair rule repeat pattern similar to the *Df(2)en28* phenotype. However, as in *wg* homozygotes the denticles at the lateral edges of the ventral surface are small and point ventrally. Those in the interior of the pattern are slightly larger and demonstrate polarity similar to that observed in *Df(2)en28* homozygotes. Several embryos were discovered that initially appeared to be unfertilized eggs. At the anterior of these embryos however, a few denticles could be distinguished encircling the area where head structures are normally present. The remainder of the embryo could not be analyzed. The mounting medium used in this study was too efficient at clearing internal structures and therefore it could not be determined whether very light denticles were present on the ventral surface or whether the cuticle was naked.

wingless control *wg Df(2)en28/wg* +)

Larvae of this genotype display typical *wingless*

phenotypes.

engrailed control (wg Df(2)en28/ + Df(2)en28)

Larvae of this genotype display the Df(2)en28 homozygous phenotype except that less naked cuticle is present within the denticle pattern of the control larvae.

20. sloppypaired engrailed ($slp\ en^{IM99}$ /$slp\ en^{IM99}$)

Figure 26 (top) illustrates a larva of this genotype. The phenotype observed in a sample of these larvae was extremely variable. It ranged from those that resembled the larva in Figure 26 to larvae that could be described as approaching a wildtype denticle pattern. A phenotype like that exhibited in the figure could be described as very similar to the wingless phenotype. This larva has very little naked cuticle in the ventral denticle pattern. Polarity reversals are evident in eight repeat units in the abdominal region. The larva can be thought of as having an en^{IM99} pattern from which the naked cuticle present between A1 and A2, A3 and A4, A5 and A6, A7 and A8 has been deleted by slp.

The larvae that approached a more wildtype phenotype had naked cuticle between most denticle belts. The polarity in the belts was not normal,i.e. almost all denticles pointed posteriorly, but neither did they exhibit the polarity reversals common to larvae homozygous for the en^{IM99} allele.

sloppypaired control ($slp\ en^{IM99}$ /slp +)

The larva shown in Figure 26 (middle) is an example of these control larvae. They exhibit a typical sloppypaired phenotype.

engrailed control ($slp\ en^{IM99}$ / + en^{IM99})

Figure 26 (bottom) illustrates a larva with this genotype. It possesses a typical en^{IM99} homozygous phenotype.

21. engrailed gooseberry ($en^{IM99}\ gsb$/$en^{IM99}\ gsb$)

A larva of this genotype is shown in Figure 27 (top) and exhibits the 'rug of denticles' phenotype. No naked cuticle is present in the denticle pattern. The lateral edges of the ventral surface contain small, ventrally oriented denticles. All of the denticles are directed ventrally except those found close to the ventral midline which point anteriorly and posteriorly. The repeat pattern may be pair rule although this is quite ambiguous. The only evidence supporting this conclusion is the presence of two exceptional larvae which contain four large composite denticle belts on their ventral surfaces. The most posterior denticle belt (putatively A8) does not always fuse with the other belts.

engrailed control ($en^{IM99}\ gsb$/en^{IM99} +)

Figure 27 (middle) illustrates a larva with this genotype. It resembles en^{IM99} homozygotes except that less naked cuticle is present between the composite denticle belts. For this reason, the secondary fusions of the large belts appear to be more complete in the

Figure 26: sloppypaired IM99 en Double Mutant and Control Larvae.

top- sloppypaired IM99 en double mutant larva.
Magnification = 326X

middle- sloppypaired control larva.
Magnification = 288X

bottom- IM99 en control larva.
Magnification = 329X

control larvae.

gooseberry control (en^{IM99} gsb/ + gsb)

A larva of this genotype is shown in Figure 27 (bottom). The phenotype of these larvae closely resembles the gooseberry phenotype. This and the fact that the larvae do not hatch is convincing evidence that this mutant is present on the double mutant chromosome.

Figure 27: IM99 en gooseberry Double Mutant and Control Larvae.

top- IM99 en gooseberry double mutant larva.
Magnification = 331X

middle- IM99 en control larva.
Magnification = 344X

bottom- gooseberry control larva.
Magnification = 284X

4.DISCUSSION

The primary purpose of this study was to test several current models for the specification of insect segments which also attempt to explain how specific segmentation mutant phenotypes are produced. If a model is correct in describing the mechanisms responsible for producing single mutant phenotypes, it should by extrapolation, correctly predict the double mutant phenotypes too. The goal, then, is to work out the implications of each model, predict double mutant phenotypes, and finally compare these predictions with the actual phenotypes recorded above in the Results section.

ANALYSIS OF MODELS AND PREDICTIONS

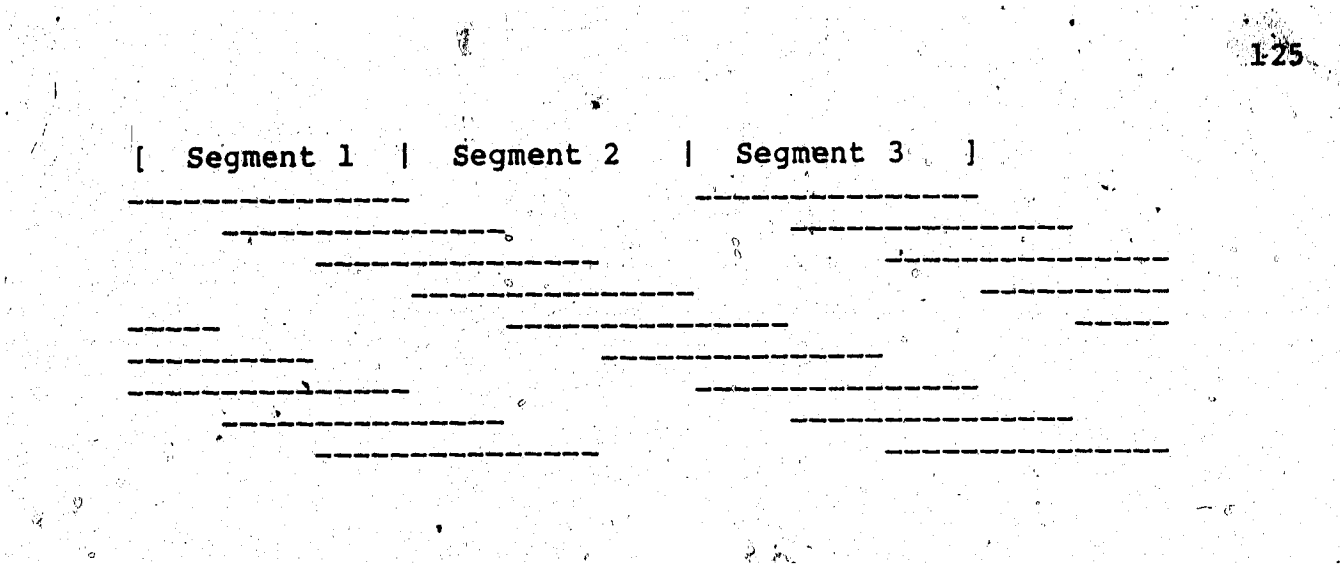
Model 1

The first model considered is based on the 'Selector Gene' concept. It was developed to explain the pair rule segmentation mutants obtained at nine different loci (Nusslein-Volhard and Wieschaus, 1980).

These genes would comprise a set of 'selector genes' necessary for determination of the normal repeated anterior-posterior sequence of pattern elements in each pair of segments. Each gene is postulated to be switched on in alternate, segment- sized domains that are all different from, but partially overlapping, those of the other eight genes (Figure 28). Thus, these 'selector' genes would be acting independently and combinatorially

Figure 28: Selector-Gene Model of Overlapping Deletions.

The dashed lines represent the active, alternate, segment-sized domains of the pair rule genes. Due to space contraints, only Segment 1 and Segment 2 are complete in this figure.


[Segment 1 | Segment 2 | Segment 3]

to determine the cell states along a pair of segments. A mutation in one of these genes might be expected to change the combinatorial codes of all the pattern elements in its 'on' domain, and none of the pattern elements in its 'off' domain. If the new codes were 'nonsense' words, this model would explain the deletion of segment-wide stripes of markers from the wildtype pattern.

Cell death in alternate segment-wide stripes has been reported in embryos of a pair rule mutant called fushi tarazu (Martinez-Arias and Lawrence, 1985) supporting this explanation. Cell death would be followed by the juxtaposition of cells with similar positions in neighbouring segments, thus reestablishing a pattern with the normal sequence of segmental elements but exactly half the normal number of segments.

Some evidence supporting this model has been garnered from analysis of transcription patterns of several pair rule genes. These gene transcripts have been found in 'stripe'-like double wavelength patterns in the early embryo (Ingham et. al., 1985, Weir and Kornberg, 1985).

Segment polarity phenotypes can be explained by invoking an additional set of selector genes whose domains are present in every wildtype repeat unit. Cell death would then juxtapose cells that have positional identities normally far apart in the segment. These

cells would be expected to intercalate according to the rules of Wright and Lawrence resulting in a duplication of the remaining pattern elements in reverse polarity.

Predictions based on Model I

If the mutants acted independently as hypothesized, the absence of an active gene product in one domain would not affect the genes active in other domains. Double mutant phenotypes could then be predicted simply by adding together the pattern deletions caused by each single mutation. Since the deletions thus generated will not, in general, be exactly one segment wide, intercalation of intermediate positional values and corresponding codes by the Wright and Lawrence rules has to be invoked to predict the resulting pattern. This is a serious limitation of this model as is does not provide an explanation for this behavior. The phenotypes predicted for deletions of increasing sizes are shown in Figure 29. Figure 30 illustrates the portions of each or alternate repeats that are thought to be deleted in the mutants used in this study.

Model II: Meinhardt

A more complex model has been proposed by Dr. H. Meinhardt (1986) and involves a hierarchy of elements controlling the final differentiated embryonic pattern (Figure 31). The model proposes that a reaction-diffusion gradient of primary information is present in the egg. This information determines four 'cardinal'

Figure 29: Repeat Patterns Produced By Progressively Larger Deletions.

This figure illustrates the expected repeat pattern when segmental deletions of varying sizes are made.

When less than half of a segmental unit is deleted, the positional values juxtaposed are very similar and therefore the deleted material is intercalated.

When half to an entire segment-sized piece is deleted, the juxtaposed values are very far apart. When the shortest route is followed for intercalation, it is through the segment boundary. This results in the formation of an ectopic boundary in reverse orientation.

The same logic applies for the remaining two deletions.

Horizontal axis- anterior-posterior axis of a segment.

Vertical axis- - refers to the angle specified by X and Y.

Dashed lines indicate the expected repeat pattern.

α
α
wildtype
polarity reversal
normal spacing
normal pattern
pair rule spacing
polarity reversal
pair rule spacing
< .5
.5–1
1–1.5
1.5–2
size of deletion (segmental units)
expected repeat pattern

Figure 30: SINGLE MUTANT PHENOTYPES AS GENERATED BY THE SELECTOR GENE MODEL

Segment Polarity Mutants	Portion of Pattern Absent
wg	cdea
ptc	bcd
gsb	cde
Pair Rule Mutants	
odd	alternate abcde
slp	alternate cdea
prd	alternate cdeab
eve	alternate eabcd

Figure 31: REPRESENTATION OF MEINHARDT'S INFORMATION SYSTEM

Level	Positional Information	Mutants Generated
A	1 gradient	maternal effect
B	cardinal regions	Gap
C	cell state pattern	Pair rule
D	segmental repeat pattern	segment polarity
E	embryonic cuticle pattern	

A	Maternal gradient
B	4 1 - - - 1 2 - - - 2 3 - - - 3 4 - - - 4 1
C	4 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1
D	S APS APS APS APS APS APS APS APS APS APS A
E	T 1|T 2|T 3|A 1|A 2|A 3|A 4|A 5|A 6|A 7|A 8|

regions in the developing embryo which activate a repeating cell state pattern described as 1-2-3-4. Each repeat of this pattern in turn generates two repeats of an S A P segmental repeat pattern. A is the anterior compartment, is induced by either 1 or 3 and corresponds to the anterior naked cuticle. P is the posterior compartment, is induced by the interaction of cell states 1+2 and 3+4, and is also composed of naked cuticle. S defines a third compartment, is induced by either 2 or 4, and forms the denticle belts. The segment boundary is surmised to form at the confrontation of P and S cells. The S:A confrontation would result in the formation of the posterior edge of the denticle belts. Kornberg (1981) proposed that segments are formed by the alternation of anterior and posterior compartments. Meinhardt pointed out that if stripes of only two states (e.g. A and P) were present, the polarity defined by the confrontation of the two states would necessarily alternate at every A:P and P:A junction. Therefore in the Meinhardt model, a third compartment was added in order to explain the constant anterior-posterior polarity of the segments. Hence the polarity reversals of segment polarity mutant phenotypes could be explained by deletion of one of the three states.

Segmentation mutant phenotypes can be generated by deleting specific pattern elements at various levels of the hierarchical system, as in Figure 31. Maternal

effect mutants such as bicaudal and dorsal might, according to Meinhardt's hypothesis, act at the level of the primary positional information gradient established in the egg by the maternal genome (Figure 31). Gap mutants would express their defect at the second level, when the 4 "cardinal regions" are established. A gap mutant would therefore delete several contiguous segments. Pair rule mutants would affect the next level of information. This level deals with the establishment of the 1-2-3-4 pattern repeats. In this way , every second segmental repeat would be absent from the final mutant cuticular pattern. For example, deleting 2 from the pattern will produce double-sized segments with an SAAP pattern. This describes the evenskipped phenotype of almost normal size denticle belts and large areas of naked cuticle (Figures 32,33). Deleting 3 could generate the paired phenotype with large composite denticle belts and smaller regions of naked cuticle (Figures 32, 33). Segment polarity mutants would express their defects at the final level of information, the S A P level. If one of these states were absent, polarity reversal phenotypes would be generated. For example, loss of A would produce the patch phenotype of S\P/S\P/S (Figures 32, 33). This pattern includes twice the normal number of segment boundaries and the polarity reversal demonstrated in the duplication of the anterior rows of the denticle belts. Polarity reversals are expected in the pattern

Figure 32: SINGLE MUTANT PHENOTYPES AS GENERATED BY MEINHARDT'S MODEL

Segment Polarity Mutants	Portion of Pattern Absent
wg	P
ptc	A
Pair Rule Mutants	
odd	4
eve	2
prd	3

Figure 33: SINGLE MUTANT PHENOTYPES AS EXPLAINED BY MEINHARDT'S MODEL

Segment Polarity Mutants

Mutant	Pattern
wg	1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1 A S A S A S A S A S A S A S A S A S A S A
ptc	1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1 PS PS PS PS PS PS PS PS PS PS P

Pair rule Mutants

Mutant	Pattern
odd	1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 APS A APS A APS A APS A APS A A T1| T3 | A2 | A4 | A6 | A8
prd	1 2 4 1 2 4 1 2 4 1 2 4 1 2 4 1 APS S APS S APS S APS S APS S A T1|T2-T3|A1-A2|A3-A4|A5-A6|A7-A8
eve	1 3 4 1 3 4 1 3 4 1 3 4 1 3 4 1 A APS A APS A APS A APS A APS A T2 | A1 | A3 | A5 | A7 |

because only two of the three required states remain.

Predictions Based on Model II

Double mutant phenotypes can be predicted by this model by deleting various combinations of cell states. If both mutants belong to the segment polarity class of mutations, the state represented by each at the S A P level is deleted. If one mutant belongs to the pair rule class and one to the segment polarity class, then not only is an element deleted from the S A P level, but one also from the 1-2-3-4 level of the system (Figures 31,32).

This model has several limitations. For this study, several double mutant phenotypes can not be predicted as the single mutant phenotypes of one pair rule mutant (slp) and one segment polarity mutant (gsb) are themselves not readily explained in terms of the Meinhardt model. For example, the deletion hypothesized to occur in slp, is smaller than , and contained within the deletion thought to describe the paired phenotype. As cell state '3' is theoretically removed by this mutation (Figure 32), only part of 3 should be removed to generate the sloppypaired phenotype. Gooseberry cannot adequately be explained because the region deleted to describe its phenotype overlaps part of both the S and A regions. Thus there are more phenotypes known than there are cell states to delete. Another limitation of this model is that although it provides a neat explanation for

polarity reversals in embryonic patterns it does not explain the polarity reversals produced in surgical experiments by intercalary regeneration (Lawrence and Wright, 1981). Therefore two different explanations are being proposed for the same aberrant pattern.

Model III: Double Wave Model For Positional Information

The third and final model considered in this study suggests a physicochemical basis for segmental positional information in the developing embryo (Russell,1985). Two separate and independent reaction-diffusion systems are postulated to specify a repeating segmental gradient of positional information along the length of the embryo, Figure 34. Each reaction-diffusion system generates sine waves of similar wavelengths but different phase. Each cell is assumed to read the ratio of the concentrations of the two components and therefore, its position in a segment (Figure 34). Each system is independent of the other and thus may vary in wavelength, equilibrium level, amplitude or phase without affecting any of these variables in the second system. Sine waves were chosen for simplicity, but any other periodic wave function ultimately produces the same results. One important stipulation of the model is that the two systems share the same period or wavelength. The value of the wavelength would specify the length of a normal segment in the embryo. In one

Figure 34: Simulation of wingless, patch and wingless patch using the Double Wave Model

Wildtype-pattern This figure demonstrates how the wildtype is simulated by this model.

a, anterior portion of the denticle belt. Small denticles are found in this location.

b, middle portion of the denticle belt. These denticles are larger than those found in 'a' and more darkly pigmented.

c, most posterior denticles in each belt. These are very small and heavily pigmented.

d, naked cuticle.

e, naked cuticle.

*, indicates the wildtype equilibrium level of the two waves in this figure and those discussed below.

The horizontal axis represents the anterior-posterior axis of the segments. Anterior is the lowest value of the gradient.

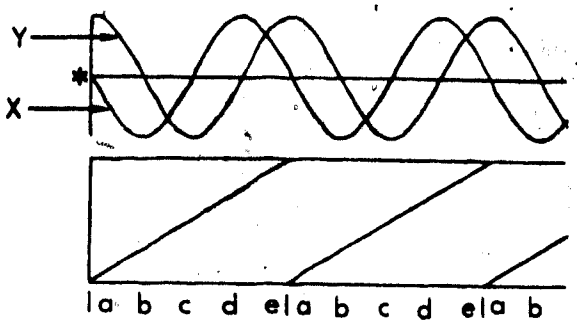
The vertical axis refers to the angle specified by X and Y.

A. This figure simulates the wingless phenotype. The highest level the peak reaches is 'b', which corresponds to large, heavily pigmented denticles. When the values increase toward 'e', normal polarity is observed. When the values decrease, back down toward 'a', a polarity reversal is observed. This is simulated by lowering the equilibrium level of wave X.

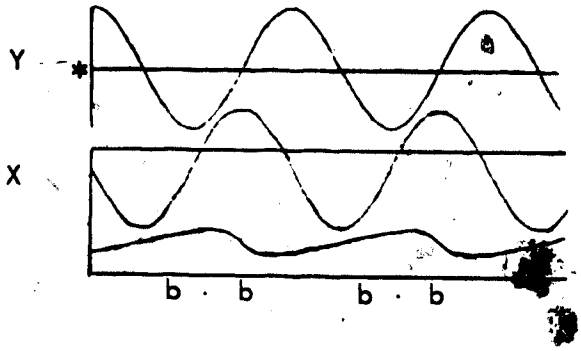
B. This simulation of the patch phenotype includes the duplication in reverse polarity of the anterior edge of the denticle belt. This results in the presence of twice the normal number of segment boundaries. The duplication in the naked cuticle is difficult to observe due to the paucity of markers in this region. However, it can be identified in the thoracic segments since it results in a duplication of the hairs in the Keilin's organs. This phenotype is simulated by raising the equilibrium value of wave Y.

C. This is a simulation of the double mutant phenotype. The curve resembles the wingless phenotype except that it does not rise as high as wingless. This predicts that the double mutant should contain smaller denticles similar to those found in the anterior portion of the denticle belts. This pattern is generated by lowering the equilibrium value of wave X and raising the equilibrium level of wave Y. For this simulation, the single mutant simulations are simply combined.

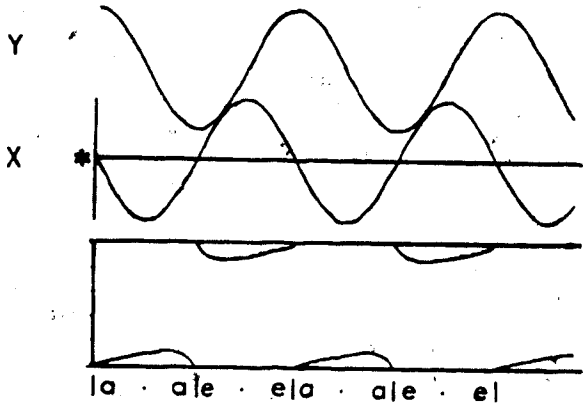
wildtype



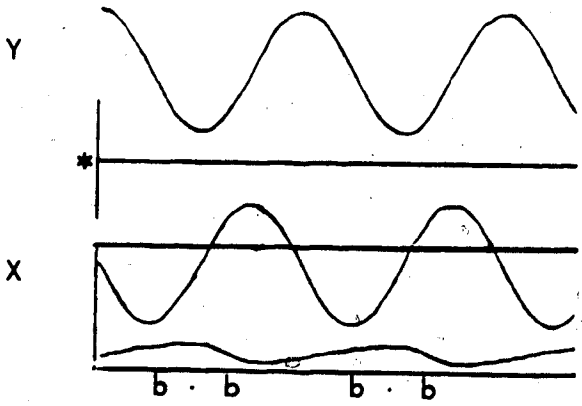
A



B



C



segmental repeat unit, each cell would have a unique value which it would share with the cell in every other repeat unit that occupies a corresponding position. This model is unique among the models suggested as it explains the Wright and Lawrence rules of regeneration as well as segmentation mutant phenotypes.

Segmentation mutant phenotypes can be simulated by altering the values of specific variables in the reaction-diffusion systems. For example, lowering the equilibrium value of one component beyond a certain threshold (Figure 34 A) simulates the wingless phenotype. It causes at least 1/2 of each segment to be replaced by a duplication of the remaining part in reversed polarity. Increasing the equilibrium level of the other component also results in a deletion, but this time the middle portion of each segment is removed and the edges are duplicated (Figure 34 B). Thus a patch phenotype can be simulated. Again, the duplicated material is in the opposite polarity. In general, all segment polarity phenotypes can be simulated with the change of a parameter to an appropriate value. Pair rule mutants can be simulated by doubling the wave lengths of both systems. This would produce segments double the normal size.

Predictions Based on Model III

Predictions of double mutant phenotypes can be generated by changing several variables at once. For

example, if the phenotype of the wingless patch double mutant is to be simulated, the equilibrium level of X is lowered while the equilibrium level of Y is raised, Figure 34 C. In this way, the simulations of single mutant phenotypes are added together to simulate the expected double mutant phenotype.

One difficulty is encountered with this approach. Sometimes, two single mutant phenotypes to be combined are explained by changing the same variable, for example, wingless and gooseberry are both simulated by lowering the equilibrium level of X. When predicting the double mutant phenotype produced by these two mutants, is the lowest and therefore more extreme mutant's X value to be used, or are the two values combined to produce a much lower value? Here I have assumed for simplicity (and for theoretical considerations) that the more extreme single mutant value will be the value of the variable in the double mutant.

The major limitation of this model is that specific pair rule mutant phenotypes with their unique deletion patterns cannot be generated without introducing another coordinate or reaction-diffusion system to specify segmental identity along the anterior-posterior axis of the embryo. Another difficulty with the model's explanation of pair rule phenotypes has been revealed by recent studies that localize the embryonic sites of transcription of several pair rule genes (Ingham et. al.

1985; Hafen et. al.,1984). These genes were found to be transcriptionally active in alternate segments in the wildtype embryo. These problems tend to suggest that the model describes rather than explains how pair rule phenotypes arise, however, the model can still be used to simulate double mutant phenotypes of the Pair rule + Segment polarity class. Since only a general pair rule pattern can be generated, not the pattern for a specific pair rule mutant, no predictions have been made using this model for double mutant combinations belonging to the Pair rule + Pair rule class.

COMPARISON OF PREDICTIONS WITH RESULTS

It is very important in all experiments to [illegible] adequate controls. This study is no exception several are included. The first is the analysis of embryos homozygous for only one segmentation mutant. Stocks that are maintained for long periods of time accumulate mutations which may modify or enhance the segmentation mutant phenotype. As well, the mutants obtained for this study were on the original, mutagenized chromosomes. For this reason as much of the original chromosome as possible was removed through recombination and replaced with a standard background from a multiply marked, homozygous viable chromosome. This ensures minimum alteration of the observed phenotype by other mutations. These single mutant embryos closely match published descriptions, (Nusslein-Volhard et. al.,1984),

and were used as controls for predicting double mutant phenotypes using the three models.

The second control includes embryos homozygous for one segmentation mutant and heterozygous for another, generated primarily to confirm by non-complementation that each putative double mutant chromosome used in the experiments really carries both mutations (Figure 2). For example, males carrying mutation 1 and 2 were back-crossed to females carrying mutation 1 alone, and also to females carrying only mutation 2. In the first case, the lethal embryos present should display the phenotype of mutation 1. In the latter case, they should exhibit the phenotype of mutation 2.

To ensure that the phenotype observed in the double mutants was due to the mutant loci and not other variable factors, two copies of each double mutant combination were independently constructed. In all cases, both constructs resulted in the same phenotype. However, only one copy of the oddskipped Df(2)en28 combination was recovered due to difficulties enumerated below.

DISCUSSION OF RESULTS IN RELATION TO EACH MODEL.

Predictions of double mutant phenotypes have been generated for each of the three models discussed above. As each model has specific limitations, particular double mutant phenotypes cannot always be simulated. When this occurs, the double mutant is discussed in relation to the models that do provide a prediction.

wingless patch

The only double mutant containing two segment polarity mutations whose phenotype is predicted by all three models is the wingless patch double mutant. Figure 35 compares the observed phenotype with those predicted by the additive-deletion model, the Meinhardt model, and the double wave model respectively. As shown in the Figure, the first model predicts that all of the pattern in each segmental repeat should be deleted. The second model predicts that mutation of the ptc locus deletes A and mutations of wg remove P from the segmental repeat pattern. As S is the only remaining element of the pattern, only medium sized denticles (level a-b) should remain, and all traces of anterior-posterior polarity should be abolished. The third model's predictions are simulated by changing the equilibrium values of the waves of each reaction-diffusion system. The value for one wave (X) is lowered while the value for the other wave (Y) is raised, (Figure 34 C). This would also produce a pattern in which only medium sized denticles participate, but a weak polarity reversal in each repeat unit should still be present. The repeat units themselves should be of the same size and in the same numbers as in wildtype. No segment boundaries should be present.

The actual phenotype of this double mutant (Figure 35) is very interesting. There is no naked cuticle present in the pattern, as predicted by two of the

Figure 35: EXPECTED AND OBSERVED DOUBLE MUTANT PHENOTYPES

Segment Polarity + Segment polarity

Mutants	Pattern	Repeat Unit Size
wg ptc		
Model I	* * *	no repeat
Model II	S S S	wildtype
Model III	b.b	wildtype
Observed	b	wildtype
wg gsb		
Model I	b.b	wildtype
Model III	b.b	wildtype
Observed	b.b	wildtype
ptc gsb		
Model I	a.a	wildtype
Model III	a.a	wildtype
Observed	a.a	wildtype
Pair rule + Segment polarity		
odd wg		
Model I	b.b	pair rule
Model II	SAA	pair rule
Model III	b.b	pair rule
Observed	b.b	pair rule
eve wg		
Model I	b.b	pair rule
Model II	SAA	pair rule
Model III	b.b	pair rule
Observed	b.b	wildtype

Figure 35: CONTINUED

Mutants	Pattern	Repeat Unit Size
wg prd		
Model I	b.b	pair rule
Model II	SAS	pair rule
Model III	b.b	pair rule
Observed	b.b	pair rule
slp wg		
Model I	b.b	wildtype
Observed	b.b	wildtype
slp ptc		
Model I	a	pair rule
Observed	a.a/e.e\a.a/e.e	pair rule
odd ptc		
Model I	a.a	pair rule
Model II	P/S\P	pair rule
Model III	a.a	pair rule
Observed	a.a/e.e	pair rule
slp ptc		
Model I	a.a	pair rule
Observed	a.a/e.e	pair rule
Pair rule + Pair rule		
odd prd		
Model I	abcde	pair rule
Model II	SAP	pair rule
Observed	abcde	pair rule

Figure 35: CONTINUED

Mutants	Pattern	Repeat Unit Size
slp eve		
Model I	abcde	pair rule
Observed	abcde	pair rule *
odd eve		
Model I	e	pair rule
Model II	A	pair rule
Observed	?	pair rule *
odd en^{IM99}		
Model I	b.b	pair rule
Model III	b.b	pair rule
Observed	weak en^{IM99}	pair rule *
odd Df(2)en28		
Model I	b.b	pair rule
Model III	b.b	pair rule
Observed	Df(2)en28	pair rule
wg l^{IM99}		
Model I	b.b	wildtype
Model III	b.b	wildtype
Observed	b.b	wildtype

* * * - no denticles or naked cuticle should be present according to the prediction.

* - double mutant is phenotypically more wildtype than expected or predicted.

? - complex phenotype, tentatively described in the Results section.

models. The pattern observed still shows evidence of a wildtype number of repeat units based on the appearance of small denticles in specific regions of the denticle pattern. Organized polarity is present in the pattern as alternate 'waves' of denticles weakly orient towards the anterior or posterior. These waves define a wildtype number of polarity repeats. Denticles toward the edge are strongly oriented towards the ventral midline. From these observations it can be deduced that the third model best predicts the actual double mutant phenotype. The predictions of the second model, Meinhardt's, are not correct as they do not allow for any remaining anterior/posterior denticle polarity in the pattern. The first model predicts that no denticles should be present, and perhaps even no naked cuticle either. However, the inherent uncertainty with which the deletion endpoints can be defined means that the single mutant deletions may not completely overlap in the double mutant pattern. A small amount of the pattern between the deletion ending at 'a' and the deletion beginning at 'b' could possibly be present. If this were the case only medium sized denticles, as observed in the anterior-most rows of denticles in the wildtype pattern, should be observed. One polarity reversal per repeat unit should also be present. The number of repeat units would be identical to that found in wildtype embryos. Therefore, Model I may also be compatible with the observations.

wingless gooseberry

As explained above, only the first and third models are capable of generating predictions for this double mutant. Figure 35 illustrates the expected and observed phenotypes. Note however, that the two models give identical predictions of the double mutant phenotype. Since the equilibrium level of X is lowered to simulate both of these segment polarity mutant phenotypes, it was assumed that the more extreme value of X (the wg value), was the appropriate level.

The actual double mutant phenotype is very similar to that of wingless. No naked cuticle is present within the denticle pattern, the denticles themselves exhibit the highly organized polarity pattern identical to that observed in wg homozygotes. Both models' predictions match the phenotype observed in the double mutant.

patch gooseberry

The actual double mutant phenotype contains no naked cuticle within the denticle pattern. The denticles are small and lightly pigmented, as found in the anterior-most rows of wildtype denticle belts.

The predictions generated by Models I and III are found in Figure 35. Both predictions appear to fit the observed phenotype.

oddskipped wingless

The actual phenotype of this double mutant may be described as a wg polarity pattern superimposed on the

leaky pair rule repeat pattern in odd, (Figure 11, top).

Models I and III correctly simulate the observed phenotype, but Model II does not (Figure 35). In this case a substantial proportion of each repeat pattern is predicted to contain naked cuticle, and the denticle polarity pattern predicted by the second model is the opposite of that observed in the embryos, Figure 35.

evenskipped wingless

The repeat pattern of this double mutant was difficult to analyse due to its complexity, however in general, it can be described as a wg-like polarity pattern superimposed on a wildtype or very weak pair rule repeat pattern.

Models I and III both predict a wg-like polarity pattern superimposed on a pair rule repeat pattern. Model II's predictions are the same as for the previous double mutant. None of the Models correctly simulates the actual phenotype (Figure 35). This may be due to an interaction between these loci in which the evenskipped pair rule phenotype is suppressed. The wg eve/ + eve control phenotype also supports this explanation. Difficulties associated with the analysis of the repeat pattern, are enumerated in the Results section.

wingless paired

This double mutant phenotype can be described as a wg-like polarity pattern with a pair rule repeat spacing.

Both Models I and III predict this result. The pattern predicted by Model II is very different and does not resemble the actual phenotype. See Figure 35 for the predictions and observed phenotype.

sloppypaired wingless

The double mutant phenotype is a wg polarity pattern in a wildtype spacing, Figure 14.

Model I is the only model that can generate predictions for this combination of mutants since the other two cannot simulate the sloppypaired phenotype. It predicts a wg polarity pattern in a wildtype spacing (Figure 35). This is what is observed.

oddskipped patch

In this case, the observed phenotype is a clear ptc-like polarity pattern with a pair rule spacing.

All three models predict this result, Figure 35.

sloppypaired patch

The double mutant phenotype consists of a beautiful ptc-like polarity pattern present in a pair rule number of repeats.

Model I predicts this result, Figure 35. Model II and III cannot generate predictions for this combination of mutants.

sloppypaired paired

The actual double mutant phenotype is very similar to the paired phenotype.

Model I correctly predicts this result, Figure 35.

oddskipped pairred

A pair rule repeat pattern similar to the sloppypaired phenotype is exhibited by this double mutant. Therefore the phenotype is a less extreme pair rule phenotype than either of the parental single mutants.

Model I predicts that since the additive deletion is 1-1.5 segmental units in size, a pair rule repeat pattern should be observed. Model II also predicts a pair rule pattern similar to the evenskipped phenotype. Therefore neither model simulates the actual phenotype completely, (Figure 35). Small polarity reversals appear to be present within the denticle pattern. Polarity reversals are expected from the experiments of Lawrence and Wright (1981) if the deletion is larger than 1.5 segmental units. But although the deletion may be large enough to produce this effect, at the same time it should cause a more extreme phenotype than either of the single mutants, not one that is less extreme, as observed.

sloppypaired evenskipped

The double mutant phenotype is observed to be very similar to the evenskipped phenotype although less extreme as 'extra' denticle belts are often present (Table 12).

Model I correctly predicts that the phenotype should be similar to eve but does not explain the appearance of the additional denticle belts (Figure 35).

oddskipped evenskipped

This double mutant was tested at three temperatures since eve^{1D19ts} is a temperature-sensitive allele. At 25°C it exhibits a pair rule repeat pattern similar to the evenskipped phenotype.

Models I and II are able to generate predictions for this combination of mutants. Since the expected additive-deletion is 1.5-2 segments wide, the first model predicts that a polarity reversal in a pair rule spacing should occur (Figure 35), but this should be in the naked cuticle and would not therefore be detectable. Model II also predicts that only naked cuticle should be present on the ventral surface of the embryo (Figure 35). Therefore, both models fail to predict the observed phenotype.

DOUBLE MUTANTS WITH ENGRAILED ALLELES

The remaining double mutant phenotypes can be predicted by Models I and III only since it is not apparent how the engrailed phenotype can be simulated by the Meinhardt model. In order to simulate the engrailed phenotype using Model III, the equilibrium level of X must be lowered and its wavelength doubled, (Russell,1985). Two alterations must be made since two separate defects are present. Two engrailed mutants were used, a weak allele, en^{IM99}, and Df(2)en28, a deficiency for the locus (Eberlein and Russell,1983).

IM99
oddskipped en

The actual phenotype of this double mutant is a weak engrailed-like pattern, containing as it does in the single mutant, a pair rule repeat pattern with a superimposed segmental deficiency. However, the phenotype in combination with odd is weaker than usual with less denticle belts fused.

Model I and III both predict that a wg-like phenotype should be present in a pair rule repeat pattern (Figure 35). So neither model is successful in predicting the observed phenotype. An interesting feature of this phenotype is that the observed deletions of pattern elements are smaller than the expected deletions.

oddskipped Df(2)en28

These double mutants display an engrailed phenotype typical of en28 with pair-wise fusions of denticle belts and (like wg) a wildtype number of clear anterior/posterior polarity reversals.

Both models' predictions are incorrect for this combination of mutants since they both generate a wg-like pattern with a pair rule number of repeats (Figure 35).

IM99
wingless en

This double mutant phenotype has a segment polarity pattern similar to an extreme wingless phenotype.

This is the phenotype predicted by both Model I and III (Figure 35).

There are three double mutant phenotypes that

cannct be compared to the predictions generated for them by Model I and Model III. These are sloppypaired en IM99, wingless Df(2)en28 and en IM99 gooseberry. The phenotypes are too complex for the repeat pattern to be unambiguously described.

DISCUSSION OF MODELS

In general, Model II predicted the least number of double mutant phenotypes correctly. All of the predictions for the Pair rule + wg double mutants are wrong, as are those for the Pair rule + Pair rule class of double mutants. Usually, the number of repeats present in the double mutant embryo is correctly predicted but the polarity pattern is not. The model therefore cannot be extrapolated to successfully predict double mutant phenotypes. This suggests that although the model can simulate single mutant phenotypes, it does not correctly describe the mechanisms by which these phenotypes are generated.

Model I correctly predicts the phenotypes for most of the double mutant combinations. The major problem for those it simulated incorrectly was that the deletions observed in the double mutant are often smaller that those predicted by the model. In the combinations of two segment polarity mutations, the wingless patch phenotype is predicted to be deficient in all pattern elements including naked cuticle. This problem is overcome, as stated above, by assuming that some material remains

between the two non-overlapping deletions. The two combinations it fails to predict phenotypes correctly for in the Pair rule + Segment polarity class are evenskipped wingless and sloppypaired wingless. The repeat patterns of these two double mutants are very difficult to analyze, as mentioned in the Results section. It is very possible that stronger alleles of these mutants would produce a pair rule pattern. Those used in the study however produce embryos with what appears to be a wildtype repeat pattern. The model also generates incorrect predictions for two combinations of mutants in the class containing two Pair rule mutants. Again, it deletes too much material as evidenced by the simulation of the oddskipped evenskipped double mutant phenotype. It predicts only naked cuticle to be present on the ventral surface, whereas denticle belts are observed.

In all, the predictions for six double mutants are incorrect as the observed phenotype contains more material than predicted by the additive-deletion model. This indicates that the deletions predicted by Model I are too large. These observations are difficult to account for if the genes are acting as 'Selector Genes'.

Model III could not generate predictions for the class of double mutants containing two Pair rule mutants. It correctly predicts the phenotype of all Pair rule + Segment polarity mutants except those that Model I also has difficulty in simulating. This may in fact be due to

the complex phenotype as their descriptions can be rather tentative. The predictions for the class containing two segment polarity mutants are all correct. In the final two double mutants which also contain an engrailed allele, it correctly simulates the wingless-like phenotype but predicts that it will occur in a Pair rule spacing. This is incorrect as a wildtype repeat pattern is present.

Models I and III generate identical predictions for all six of the double mutants containing one Pair rule mutant and one Segment polarity mutant. Only one of the six is simulated by Model II to produce the same phenotype as the other models. This is interesting since Models I and III at first sight would seem to suggest very different roles for the genes involved in the process of segmentation in the embryo. Model I implies that if a gene is mutant, the pattern differentiated by it's 'on' domain will be absent, while the pattern produced by it's 'off' domain will be unaffected by mutation. Model III deals with positional information in the embryo and how cells respond to this information. Therefore mutants might cause defects in either the specification of positional information or in how cells read and respond to this information.

There are two important similarities that may help to explain why these two models predict similar results, while Model II does not. First, in Models I and III each

gene is assumed to act independently. Therefore, one mutation is not expected to affect the expression of the other mutation. In Model II, however, if one of the cell state elements is deleted, e.g. '2', then not only is the '1234' repeat pattern affected to produce a pair rule spacing, but the polarity of the pattern elements is also affected because of the effect on the 'SAP' genes via the hierarchical system of control. Thus, the 'S' normally produced by 2 would also be absent. In many cases it is this feature of Meinhardt's system that results in the generation of incorrect polarity patterns in the correct spacing. The second feature that Models I and III share is that the genes controlling segmentation are assumed to be switched on in partially overlapping domains. In Model II, the domains of the genes are non-overlapping to the point that only three phenotypes can be generated for segment polarity mutants. These phenotypes would be quite distinct and the model can not explain a mutant that shares a portion of the same pattern with another mutant, e.g. wg and gsb.

Other reasons exist to explain why the models do not correctly predict some phenotypes. One possibility that was not anticipated when this study began was interactions between loci. The results show substantial evidence to indicate that this is assumption is not correct (Table 15).

Table 15: DOUBLE MUTANTS EXHIBITING INTERACTIONS AND DOSAGE EFFECTS

Double Mutant	Interactions	Dosage Effects
odd enIM99	X	X
odd Df(2)en28	X	X
odd prd	X	X
odd wg		X
slp eve	X	X

Interactions, Dosage Effects

When the double mutants were being generated by recombination, an oddskipped Df(2)en28 combination was very difficult to produce. These two loci are more than 50 map units apart suggesting that one half of all flies should be recombinant for the two loci. One half of these recombinants should be double mutants. Although many males of the appropriate phenotype were selected and tested, only one double mutant was identified. When embryos were mounted and analyzed, it was found that the odd control embryos exhibited a much more wildtype phenotype than the original odd stock. The double mutant itself displays what appears to be a wildtype number of repeats with no naked cuticle present. It is therefore quite possible that mutual suppression between the two loci is occurring. The presence of only one functional copy of the engrailed locus may be supressing the oddskipped mutation so that animals that are heterozygous for the former and homozygous for the latter may be viable. The selection scheme used to identify double mutants does not allow recognition of this type of interaction. The other possibility is that double mutant males are sterile. This also implies an interaction at some level between the two [illegible]. Further evidence supporting this conclusion is [illegible] when the phenotype of this double mutant is compared with that of the oddskipped en[illegible]IM99 combination. en[illegible]IM99 is an

hypomorphic engrailed allele, while Df(2)en28 is an amorph. The double mutant with the former allele exhibits a pair rule repeat but fusion between denticle belts occurs much less frequently than in the en^{IM99} control, or in the original stock. The double mutant phenotype is much more wildtype than predicted. The combination including the latter allele exhibits a wildtype repeat spacing. It is not apparent why this occurs but the effect could be described in formal terms as the suppression of odd by en. Further examination of this interaction is intended but unfortunately is beyond the scope of the present study.

Interactions between other loci were also observed. oddskipped paired double mutants exhibit a phenotype that resembles the wildtype phenotype more closely than either of the two separate mutant phenotypes. As well, the prd control embryos sometimes contain regions of naked cuticle within the composite denticle belts. This is never observed in odd^{+} prd homozygotes. This suggests that the odd allele is suppressing the paired phenotype.

Interactions that were observed in this study are noted in Table 15. Until further work has been completed, the possibility that these effects are due to modifiers present in the original mutant stocks cannot be denied. However, effects such as those observed in the odd wg/ + wg control are unlikely to be the result of modifiers. These control larvae exhibit a pair rule

effect where the odd numbered wingless denticle belts are morphologically different than the even numbered wingless belts.

The class containing two Pair rule segmentation mutants also has a member displaying dosage effects that cannot be ascribed to the presence of modifiers. Both controls of the sloppypaired evenskipped double mutant appear to be suppressed. The slp control embryos have less naked cuticle deleted from their pattern than expected on the basis of the additive-deletion model. This results in less pair-wise associations of denticle belts, or 'leaning' that is observed in A1-A2,A3-A4,A5-A6, and A7-A8. In the eve control, the denticle belts associated with segments A4 and A8 are present with a frequency never observed in the single mutant stock (Table 13).

Models I and III are most accurate in predicting double mutant phenotypes. A large number of their predictions are very similar suggesting that the positional information system proposed by Model III may simulate the mechanisms acting in Drosophila development. Although Model I does not suggest how positional information acts, it may simulate the response of mutant cells to unchanged information, or normal cells to altered information.

When Model I fails to correctly predict phenotypes it is usually because less material is deleted from the

cuticular pattern in the embryo than proposed by additive deletions of its simulated single mutant phenotypes. Recently, the transcription patterns of several segmentation mutants have been analyzed with the same result. In many cases, the expression pattern observed in wildtype embryos bears little relation to the cuticular defects present in the homozygous mutant embryos. This is true for the gap mutant Kruppel (Knipple et. al., 1985) and the pair rule mutants engrailed (O'Farrell et. al., 1985), paired (Kilcherr et. al.,1986) and hairy, where cells that do not normally express the gene are lost by cell death (Ingham et. al.,1985).

Model I relies on a combinatorial system of overlapping gene domains to explain pattern formation. This suggests that the expression of segmentation genes should be affected by mutations in the genes that control them or that establish the information they require for proper spatial expression. Carroll and Scott (1986) found that several gap and pair rule loci can affect the expression of fushi tarazu, (ftz) a pair rule gene. Embryos mutant for this gene lack the denticle belts from T2, A1, A3, A5, and A7. Struhl (1985) placed the coding region of this gene under the control of a Drosophila heat shock promoter and found that when it was indiscriminately expressed, an 'anti-ftz' phenotype resulted. This was not a completely reciprocal effect

however; analysis of the phenotype showed that there were regions of the pattern that were deleted in both ftz and heat shocked embryos, and other areas that were unaffected in both. A similar result was obtained for the mutant runt (Gergen and Wieschaus, 1986). Increasing the number of wildtype copies of this gene resulted in a near reciprocal phenotype. Again, some cells were affected both by the lack of runt product, and its excess. These results would appear to disprove the selector gene theory since a direct relationship between a gene's domain and the defect its absence produces are postulated by it. There is no doubt that some form of combinatorial encoding system is present in the embryo, but the simplistic relationships that have been hypothesized to exist are probably wrong.

Model II was not very effective at predicting double mutant phenotypes; however it cannot be discarded in its entirety. The major limitation of this model is the hierarchical relationship between the pair rule mutants and expression of segment polarity. It is quite likely that a gene hierarchy exists in development. The temperature sensitive point of wg for example is quite late in embryogenesis (M.Auld, pers. comm.). This suggests that it is expressed much later than other genes which affect the initiation or response to positional information in the embryo and may perhaps be controlled by them. A direct relationship such as that suggested by

Meinhardt does not seem likely considering how ineffective this model was in predicting double mutant phenotypes, especially the pair rule + segment polarity combinations.

Model III, the double wave model was interesting in that it was the only one that commented on actual processes occurring in development. It was quite efficient at predicting double mutant phenotypes. This suggests that although the model cannot describe certain phenotypes,e.g. different pair rule mutants, its general premise on the form that positional information takes; reaction-diffusion systems, may be correct. This is supported by recent molecular evidence that indicates that several maternal gene products form gradients of concentration in the pre-blastoderm embryo,(Mlodzik et.al.,1985). It is probable that this occurs through reaction-diffusion systems in the egg; systems such as these are able to occur spontaneously in in vitro systems (Welsh et. al., 1983). One possible component of the information system may be dorsal, a maternal effect mutant that produces embryos with no ventrally derived structures.

The dorsalized progeny of this mutant can be partially rescued by injection of wildtype cytoplasm. This results in the formation of structures never observed in uninjected controls (Santamaria and Nusslein-Volhard, 1983). Preconditions with interesting

ramifications exist. For example, the rescued response is restricted to the site of injection which must be on the ventral, not dorsal side of the mutant embryos. Although the cytoplasm is equally effective at rescue when taken from cleavage stage embryos, when the donor cytoplasm is withdrawn from embryos in syncytial blastoderm, ventral cytoplasm is twice as effective at rescuing mutant embryos as cytoplasm from the dorsal side (Santamaria and Nusslein-Volhard, 1983). This maternal gene product therefore must be responsible for specifying information integral to the formation of ventral structures in the embryo.

Another maternal effect gene which exhibits interesting transcription patterns supporting the existence of information gradients is caudal. The maternal transcripts, when localized in the pre-blastoderm embryos are found to form a concentration gradient along the anterior-posterior axis of the egg (Mlodzik et. al., 1985). A mutant phenotype for this gene has yet to be identified but if a concentration gradient of its transcripts exists in the embryo, it is possible that the gene product(s) could specify information necessary for the formation of posterior structures.

An inclusive theory of development requires features from all three models. Positional information is known to exist and therefore the model must account for global

coordinate systems and the processes which inform cells of their fates. The original purpose of this study was to determine which of the three models best simulated double mutant phenotypes in hopes that this would shed light on some of the mechanisms controlling pattern formation in Drosophila. When the double mutants were analyzed it was found that all of the models contained elements that were useful when considering development.

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