A PHENOGENETIC STUDY OF THE LOZENGE PSEUDOALLELES IN DROSOPHILA MELANOGASTER.

I. EFFECTS ON THE DEVELOPMENT OF THE TARSAL CLAWS IN HOMOZYGOTES[1]

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REEXAMINATION of a number of multiple allelic loci have uncovered cases of closely linked pseudoalleles exhibiting, at least superficially, the physiological characteristics of alleles. In several of these cases, a comparison of coupling and repulsion heterozygotes has revealed a position effect. The coupling heterozygotes appear as wild type, while the repulsion heterozygotes appear mutant (LEWIS 1951).

In considering the chromosomal distribution of genes with respect to their functions, pseudoalleles clearly represent deviations from randomness. Two general interpretations have been proposed in explanation of these deviations. One view suggests that refinement of procedures has led to an exposure reflecting upon the structure of the hereditary material (GOLDSCHMIDT 1951; PONTECORVO 1953; STADLER 1954). This view holds that the chromosome is composed of physiologically differentiated regions. Such regions may exist in alternative forms, subject to both internal and external recombination, and exhibit all of the physiological properties of alleles. According to this view, the position effect is simply a reflection of the dominance relations between alleles.

An alternative interpretation of pseudoallelism suggests that such loci represent exceptional cases of evolutionary intermediates (LEWIS 1951; GREEN 1954). This hypothesis proposes that pseudoalleles arose as duplications of single genes, and are in process of physiological and spatial distinction. In the process of physiological differentiation, such duplicates come to control adjacent steps in a biosynthetic process (LEWIS 1951, 1952). Finally, aberrational processes accompanied or followed by further physiological differentiation would lead to the establishment of a random distribution of these genes. It is proposed that such a mechanism represents a major path by which the number of genes may be increased.

The position effects of pseudoalleles are explained by the further hypothesis that the reaction sequence controlled by these genes proceeds independently on each of the homologous chromosomes. In the coupling heterozygote, the reaction path is blocked on one chromosome. However, sufficient product is formed on the homologous chromosome to yield a wild type individual. In the mutant repulsion heterozygote, the reactions are blocked on both chromosomes by the presence of mutant genes. A further modification of this hypothesis permits some diffusion of intermediates between homologous chromosomes (LEWIS 1954).

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Thus, two hypotheses are proposed in explanation of the phenomenon of pseudoallelism and associated position effects. This paper presents one aspect of a program of studies designed to contribute evidence towards the resolution of this problem.

The three lozenge pseudoalleles in *Drosophila melanogaster* represent a particularly favorable series for study (GREEN and GREEN 1949). A large number of possible genic combinations are available. Pleiotropy is exhibited and several variables may be studied. The mutant phenotype includes abnormalities in the external shape and pigmentation of the eye (GOTTSCHEWSKI 1936; OLIVER 1947; GREEN 1948); abnormalities in the various structural components of the ommatidia (CLAYTON 1952); infertility of females (OLIVER and GREEN 1944; ANDERSON 1945); antigenic changes (CHOVNICK and FOX 1953); and abnormalities in the structure of the distal segment of the leg, the tarsus (CUMMINGS 1946; CHOVNICK 1953).

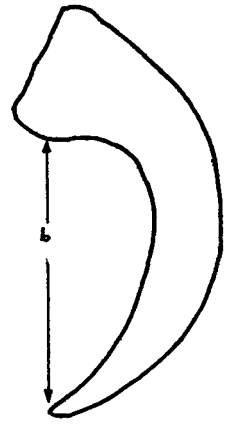


FIGURE 1.—Diagram of a normal shaped tarsal claw. (b) Represents straight line measurement used as estimate of claw size.

The two major structures of the tarsus are the claws and pulvilli. Both structures are affected by the lozenge mutants. The claw anomalies range from a reduction in claw pigmentation through reduction in size of claws to complete absence of claws. The effect on the pulvillus, a glandular structure, appears to parallel the claw anomaly.

The structural changes in tarsal claws accompanying change in dosage and position of the lozenge pseudoalleles were studied. The dosage study in homozygotes is reported in this paper. Claw effects accompanying changes in position are to be reported separately.

MATERIALS AND METHODS

Flies were cultured in half-pint milk bottles on the standard corn meal–molasses–agar medium fortified with yeast extract and seeded with living yeast prior to use. The cultures were maintained at 27°C in a constant temperature incubator. Each culture was started by the addition of two pairs of males and virgin females. Parents

were removed on the seventh day, and only the first 10 to 20 mutant females to emerge were examined.

Examination of heterozygotes and comparison with homozygotes was considered essential for the study. Since lozenge is sex-linked (only female heterozygotes can be obtained), the entire study was confined to females, removing possible sex-influenced effects in such comparisons. The data collected on any one genotype was accumulated over a period of time, and includes a minimum of seven replicates.

Legs were removed, placed on albuminized slides, and the tarsal claws were examined under the microscope. The measurement *b* (fig. 1) was made with a Bausch and Lomb filar micrometer eyepiece at 537.5 magnifications. A number of difficulties in the measurement will be discussed below. It should be noted that possible subjective influence in the measuring procedure was removed in the following fashion: Matings were coded and individuals to be examined were recognized by code number. The slides were prepared, recorded and randomized. The labor was divided between the authors in such a fashion that any pair of legs under observation could not be related to any other pair, or to any genotype, without conscious effort.

DERIVATION OF STRAINS

In studying the effects of genic substitution at specific loci, the ideal procedure would vary only the genes of interest. Although such a procedure is technically impossible, an approach to this ideal was attempted.

A series of seven stocks possessing the sex-linked pseudoalleles of lozenge; *BS*, *46*, and *g*, in all possible combinations were made available by M. M. GREEN. These stocks were subjected to a series of crosses designed to render them coisogenic with a highly inbred wild strain of the Oregon R-I series of J. SCHULTZ.

In the following description of the derivation of experimental strains, the symbol, *lz*, will be used to represent the lozenge regions. The derivation of all strains was accomplished in two steps: (1) The transfer of the lozenge combinations from diverse X chromosomes to the Oregon R-I series X chromosome. This step was accomplished by use of marker genes closely linked to lozenge, and recovering crossovers from heterozygous females. (2) Substitution of Oregon R autosomes was accomplished by use of dominant marked inversions. During this step, the Oregon R X chromosome was introduced into each strain (figs. 2 and 3).

In this fashion six balanced strains were established, each essentially differing from the inbred Oregon R strain only with respect to the lozenge region of their X chromosomes. The following lozenge combinations were established in the described derivation: *BS46g*, *BS46+*, *BS+g*, *BS++*, *+46+*, *++g*. The Oregon R strain served as wild type. The combination *+46g* used in this study was derived by crossing over in heterozygotes *+46+/++g* possessing the isogenic background.

Following their derivation, these *ClB* balanced strains were maintained by brother-sister mating. A number of possible sources of deviation from coisogenicity are inherent to the procedure and should be noted: (1) The X chromosomes of the stocks may differ with respect to the 12 crossover unit segment between singed and vermilion which includes the lozenge region. However, X chromosomal crossovers outside of

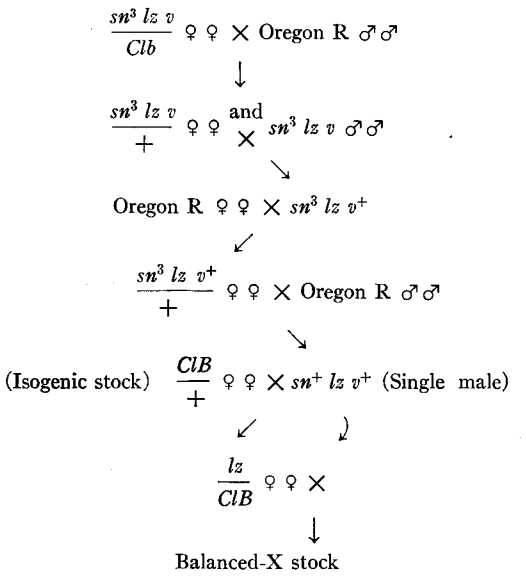


FIGURE 2.—Transfer to common X-chromosome.

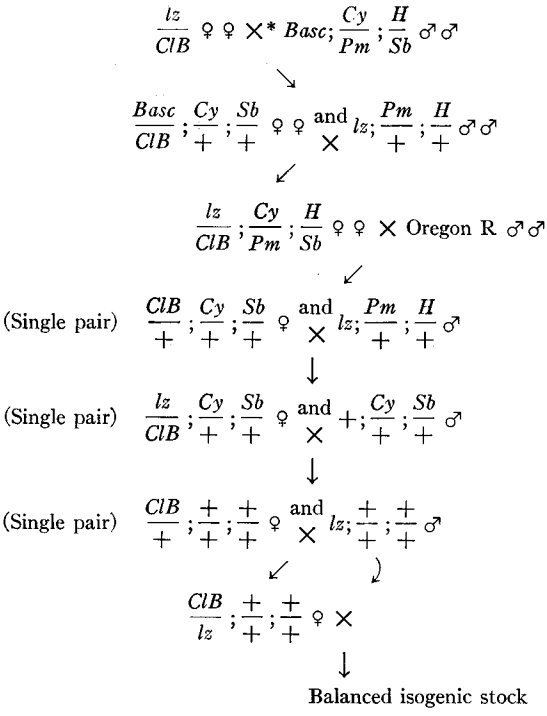


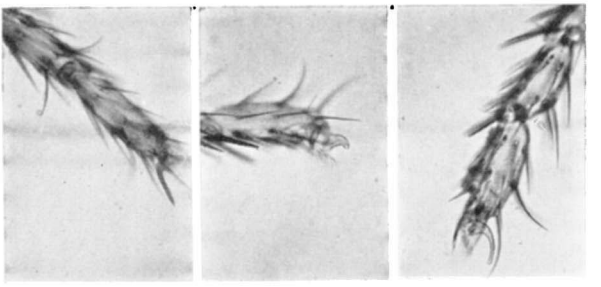
FIGURE 3.—Substitution of Oregon-R Autosomes and Y Chromosome

*Complete formula: *sc*^{sl}*B In–S* w^a *sc*^{8}; *Cy/Pm*, *ds*^{33k}; *H/In*(3*R*)*Mo Sb sr.*

this region may lead to other differences. (2) Heterogeneity of autosomes (2 and 3) may have resulted from crossing over between Oregon R chromosomes and "marker" chromosomes during the derivations, particularly in the unsupressed regions of these chromosomes. (3) The fourth chromosome was not controlled during the derivation. However, during the derivation there were four outcrosses to Oregon R rendering it probable that the mutant stocks possess Oregon R fourth chromosomes. (4) Spontaneous mutation with visible effect since the derivation has been noted and selected out. No control over non-visible mutation is possible.

CLAW MEASUREMENTS

Preliminary attempts to measure the size of tarsal claws met with several problems. Due to the small sizes involved, the claws must be measured under the compound microscope. When a leg is placed on a slide, it is impossible to orient the claw



A B C

PLATE 1.—A, B, and C shaped tarsal claws, 450×.

in precisely the same position each time. Since positions may vary from claw to claw, some measures would be direct estimates of the straight line distance *b* (fig. 1), and others would be estimates of various functions of *b*. Assuming all claws in all genotypes to be equally capable of such variation in position, the error incurred would not be significant. Moreover, a further difficulty arises when it is noted that claw shape may vary. Three distinct categories of claw shape were recognized. The three categories were labeled A, B, and C (plate I). Group C claws are the normal curved claws, and are also the most common type found in this study. Group A claws are straight, and group B represents a heterogeneous class of abnormally shaped claws. The latter group share in common the feature of being, almost exclusively, very short claws. It should be understood that these categories were fixed arbitrarily. Considerable intergrading does occur, particularly between the classes A and C.

The problem of claw position variation leads to a further difficulty. Certain of the positions preclude measurement of the claw length. Attempt to adjust the position of any claw would, for the sake of consistency, force adjustment of all claw positions. Such a laborious procedure would seriously limit sample size. Assuming that the probability of any claw to be unmeasurable is a constant, regardless of genotype, omitting such claws from the data would incur no sizable error. Time would be more profitably spent in increasing sample size.

The third problem concerns itself with the number of claws on any one individual. Does an adequate estimate of the effect per fly require measurement of the twelve claws per fly?

A preliminary study was made to gather information bearing on the following questions: To what extent does variation in claw shape lead to error in estimation of the effect of genotype on claw size? Does omission of unmeasurable claws lead to a similar error? Does an adequate estimate of the effect per fly require examination of all legs of each fly?

At the time of these experiments, the genotype *+46g* was in process of derivation, and was consequently omitted. All six legs were examined, claws were measured, classified as to shape. Measurements were recorded in terms of units on the scale of the filar micrometer (37.1 units = 0.01 mm at 537.5 magnifications). Claws were subsequently graded as follows: 0–50 (1), 51–75 (2), 76–100 (3), and increasing by units of 25. The largest claws ever found fell in the category 176–200 (7). Since the wild type possesses two claws per leg, two claws were expected on each leg. A missing claw was graded zero. A claw value per leg was obtained by summing the values for the four claws on each pair of legs and dividing by two.

Examination of individuals of the various genotypes revealed that two of the genotypes, *BS46+* and *BS46g*, rarely possess claws (table 1). Considering only those genotypes with appreciable incidences of claws, it is noted that the distribution of abnormal claw types (A and B) from leg to leg is not random. Only 0.21 of these claws were on the third legs, and the frequency increases from posterior to anterior (chi square = 10.1, d.f. = 2). Furthermore, the pooled frequencies of abnormal claws among the genotypes are quite low. With one exception, this value is not higher than 0.045. The exceptional case is that of *BS+g* where 0.127 of the claws examined were of abnormal shape, and almost all of these were small claws of type B. However, the incidence of abnormal claws in this genotype does not markedly influence the estimate of the mean claw value per leg (table 2). In spite of the fact that most of the abnormal claws in this genotype were on the first and second legs, estimates of the mean using the first, second, or third legs are not significantly different. In

TABLE 1

Distribution of claw shapes, unmeasurables, and missing claws on legs of homozygotes

Homozygous genotype	Claw types												Claw shapes			% Abnormal claw shapes	0 claws	UNM claws	% UNM claws	No. sites with claws
	A			B			UNM			0										
	Leg			Leg			Leg			Leg										
	1	2	3	1	2	3	1	2	3	1	2	3	A	B	C					
+++	2	1	1	0	0	0	43	27	35	0	0	2	4	0	585	0.68	2	105	17.8	589
++g	4	2	0	0	0	0	26	23	18	18	14	14	6	0	393	1.50	46	67	16.8	399
+46+	8	4	0	1	0	1	13	16	18	19	15	7	12	2	291	4.50	41	47	15.4	305
BS++	2	1	0	3	1	3	11	8	7	62	60	69	3	7	208	4.50	191	26	11.9	218
BS+g	1	0	0	11	7	4	5	6	6	42	49	48	1	22	157	12.70	139	17	9.4	180
BS46+	1	1	1	0	1	3	0	1	0	125	125	124	3	4	5	58.30	492	1	8.3	12
BS46g	1	0	0	1	0	1	0	1	0	166	166	167	1	2	3	50.00	498	1	16.6	6

TABLE 2

Estimates of mean claw value/leg for first, second, and third legs of homozygotes

Homozygous genotype	Leg			F	d.f.
	1	2	3		
+++	8.35	8.92	9.32	10.80	2,147
++g	6.73	7.39	7.76	4.76	2,111
+46+	6.29	7.23	8.01	5.65	2, 87
BS++	3.98	4.19	3.67	0.63	2, 102
BS+g*	3.87	3.43	4.33	1.30	2, 78
BS46+	0.12	0.10	0.14		
BS46g	0.10	0.08	0.05		

* Excluding B claws: 4.07, 3.61, 3.95.

addition, estimate of the genotype mean excluding abnormal claws, leads to no appreciable change. From these observations, it is seen that variation in claw shape does not constitute a serious problem for estimation of claw length. No apparent error is introduced by including the abnormal claws. Restriction of observations to the third pair of legs would remove most of the abnormal claw shapes, and further reduce any error in estimation of claw size.

Distribution of unmeasurable claws (UNM) and missing claws from leg to leg shows no deviation from randomness (table 1). However, the frequency of unmeasurable claws decreases as the genotype mean decreases. This difference, a maximum of approximately nine percent, will tend to reduce the estimates of the mean per genotype, and the reduction will increase with decreasing means. However, the error incurred will not alter the relative positions of the various genotypes. In the absence of evidence that omission of unmeasurables would lead to a significant differential error in estimating means, this procedure was followed in the remainder of the study.

From consideration of the distribution of variant claw shapes, it was found that restriction of study to the third pair of legs would considerably reduce error from this source. Further evidence supporting such a restriction is seen in table 2. The mean claw values of +++ for the three leg pairs increase from anterior to posterior. With substitution of mutant alleles, the gradient effect disappears in the genotypes with small claws. Thus the third legs appear to be more sensitive to mutant gene substitution. Further implications of the gradient effect are discussed below. The remainder of the study was restricted to estimates based upon third leg measurements.

HOMOZYGOTE ANALYSIS

AUERBACH (1936) has described the development of the thoracic appendages of *Drosophila melangoaster*. These observations indicate that the period of greatest developmental activity occurs earlier in the first leg buds than in the second, and the third leg buds lag far behind. The observation (table 2) of a claw size gradient in the wild type disappearing with increased mutant gene substitution supports the following interpretations of gene action.

TABLE 3

Means and variances of third legs of homozygotes

Homozygous genotype	Direct estimates		Corrected estimates		Adjusted mean	Sampling variance	*n*
	Mean	Variance	Mean	Variance			
+++	8.78	5.10	—	—	10.34	0.056	91
++g	7.35	2.44	—	—	8.91	0.015	162
+46+	6.69	4.89	—	—	8.25	0.034	143
+46g	5.63	5.06	—	—	7.19	0.043	119
BS++	3.38	5.36	3.27	6.34	4.83	0.040	161
BS+g	3.00	4.51	2.88	5.48	4.44	0.033	174
BS46+	0.65	0.23	−0.49	4.86	1.07	0.0086	81
BS46g	0.31	0.22	−1.56	4.86	0.00	0.012	98

Both wild and mutant pseudoalleles contribute towards the conversion of a substrate to a product involved in claw production. The different alleles control the rate at which the substrate is converted into product. The genotype, +++, may be considered as most efficient in this conversion. Claw size on any leg results as a function of substrate availability at a critical point in development, and differs for each leg. However, with decreasing efficiency of substrate conversion, the gradient effect would be expected to diminish (++*g* and +*46*+). Continued decrease in efficiency of this conversion would lead to a gradient below the level of resolution (*BS*++ and *BS*+*g*).

An alternative interpretation would consider the lozenge mutants as inhibitors of claw development. ANDERS (1948) observations on the effects of lz^{cl} in the presence of the *tra* gene suggest that the lozenge effect is quite late in development. In the presence of the wild pseudoalleles of lozenge, substrate availability at periods of maximum developmental activity lead to the gradient effect. The lozenge mutants, acting late in development, lead to the production of an inhibitor, or divert the substrate into another path. Such action asserts its greatest influence on later developing legs, and leads to a depression of the gradient in claw size.

Table 3 contains a summary of the data collected on tarsal claws of the third legs of homozygotes. Histograms of the distribution of claw values per fly for the various genotypes are given in figure 4.

Complete distributions are found for those genotypes possessing larger claws; +++, ++*g*, +*46*+, and +*46g*. With decreasing means, the genotype distributions present an increasing degree of truncation at the zero value as exhibited by *BS*++, *BS*+*g*, *BS46*+, and *BS46g*. On either of the schemes of gene action described above, the truncation may be attributed to the operation of a developmental threshold. Assume a certain minimal quantity of gene product is necessary for claw development. Below this level, no claw is produced. Above threshold, the mean claw value represents an estimate of the average amount of product attributable to the genotype. Variation within any one genotype may be attributed to environmental factors.

Estimation of means and variances for the complete distributions were made

directly from the data. However, direct estimation of the means of the truncated distributions would lead to error increasing with degree of truncation. Such estimates would be higher than the true means. Estimates of the variance of truncated distributions would be lower than the true variance. Following the method of IPSEN (1949), estimates of the parameters of the truncated distributions were obtained. In two of the distributions, *BS++* and *BS+g*, only small portions of the distribution

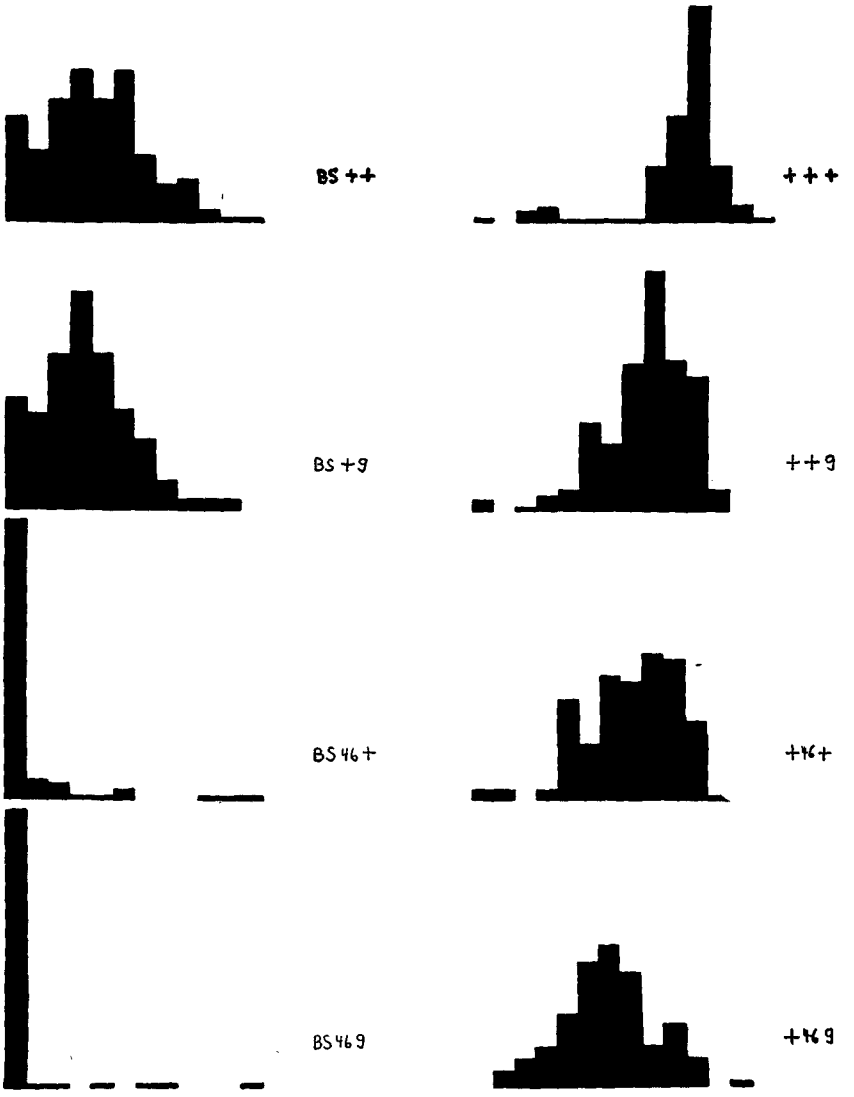


FIGURE 4.—Frequency histograms of third leg claw values/fly for the indicated genotypes. Horizontal axis indicates claw values/fly: 0, 0.1–1.0, 1.1–2.0, 2.1–3.0, 3.1–4.0, 4.1–5.0, 5.1–6.0, 6.1–7.0, 7.1–8.0, 8.1–9.0, 9.1–10.0, 10.1–11.0, 11.1–12.0. Vertical axis indicates number of flies in each category. Zero column in *BS46+* and *BS46g* distributions should be higher than indicated.

fall below the threshold. Truncated means and variances were estimated, and used to obtain estimates of the true means and variances (table 3, Corrected Estimates). In the distributions, *BS46+* and *BS46g*, only small portions of the distributions fall above the truncation point. Estimation of variance of these distributions from the small samples above the truncation point would lead to sizable error. Assuming that variation in all genotypes is due to random fluctuations in common environmental factors, the variance should be independent of the mean. No relationship between mean and variance is apparent in these data. The average variance of all distributions was used as an estimate of variance for the extremely truncated distributions. Using the truncated means and the average variance estimate of the true variance, the true means of the truncated *BS46+* and *BS46g* were obtained (table 3, corrected estimates). To remove negative means, the *BS46g* mean was set at zero, and all other means adjusted by adding 1.56 (table 3, adjusted means). The adjusted means represent estimates of the amount of gene product attributable to the genotype. These estimates have been corrected for the threshold at the truncation point and rated on a positive scale.

Estimates of the sampling variance of the mean were obtained directly for the complete distributions. The method of IPSEN (1949) was used to estimate the sampling variance of the means for the truncated distributions (table 3, sampling variance). The adjusted means associated with homozygous genic substitution separately and in compounds reveal a cumulative effect suggesting additivity of action of the lozenge loci. A test for additivity of gene action, making use of the adjusted means and their sampling variances, is provided by HOUSE (1953). A modification of this method, when applied to these data, rules out the hypothesis of additivity.

The cumulative effects seen in the present data are consistent with two categories of mechanisms of gene action. One possibility views the lozenge loci as physiological duplicates, each locus operating upon the same or similar substrate(s), and producing the same or similar product(s). The different alleles at these loci may control the rates of the reaction, or the product specificity for claw production, and thus govern the relative contribution of each locus to claw size. Although such a mechanism of gene action would predict an additive effect, deviations from additivity due to factors external to the lozenge loci are possible.

An alternative hypothesis suggests that these loci control successive steps in a sequence of reactions (LEWIS 1951; GREEN 1954). These data would support such a view, if it is further assumed that the various alleles at these loci control the rates of the individual reactions. Information concerning the possible localization of the reaction sequence to the site of the genes in the chromosomes may be derived from an examination of heterozygotes.

DISCUSSION

The spatial distinction, physiological similarities, and position effects exhibited by pseudoallelic loci have been interpreted in two general fashions. One view supported by LEWIS (1951) and GREEN (1954) consists of three features: (1) pseudoalleles represent duplications of genic material, (2) physiological differentiation, associated with or following upon duplication, leads to the association of such loci with successive

and distinct steps in a sequence of reactions, and (3) the reactions occur localized to the site of the genes in the chromosomes.

GREEN (1954) in a study of a series of vermilion mutants reported that they could be classed into two groups on the basis of interaction with a non-allelic suppressor of vermilion. One group of mutants, including v^1, exhibit wild type eyes in the presence of the suppressor, and consequently are labeled v^s mutants. Another group, including v^{36f}, are vermilion in compounds with the suppressor, and are labeled v^u mutants. It was further found that v^1 and v^{36f} are not alleles, but closely linked pseudoalleles. On the basis of these observations, GREEN suggested that all v^s mustants are alleles of v^1, and all v^u mutants are alleles of v^{36f}. BARISH and FOX (1955) reported that v^{48a}, an unsuppressed mutant, exhibited recombination with v^{36f} and not with v^1. These observations indicate that v^{48a}, an unsuppressed vermilion, is allelic to v^1, a suppressed mutant. Further evidence of overlapping physiological effects is indicated from a study of the antigenic effects of the vermilion pseudoalleles (BARISH and FOX 1955). It was noted that the mutants v^1, v^{36f}, and v^{48a} share, in common, the antigenic component V-1. The mutants v^{36f} and v^{48a} possess a component, V-2, which is absent in v^1 mutants. A third component, V-3, possessed by v^{48a}, is absent in v^1 and v^{36f} homozygotes. These observations on allelic relations and overlapping physiological effects of the vermilion mutants do not support the view that pseudoallelic loci control separate and distinct steps in a sequence of reactions.

CHOVNICK and FOX (1953) found evidence supporting an interchromosomal interaction in the production of an antigenic component associated with the lozenge pseudoalleles. The component, L-3, was found to be present in both coupling and repulsion heterozygotes involving the lozenge mutant pseudoalleles *BS* and *46*, and not present in either homozygote. Such evidence is not consistent with the third aspect of the hypothesis suggesting gene action localized to the chromosomes. In contrast to the position effects associated with morphological effects of pseudoallelic loci in Drosophila, no position effect is seen in antigenic specificities associated with either the vermilion mutants (BARISH and FOX 1955) or the lozenge mutants (CHOVNICK and FOX 1953). This apparent discrepancy suggests that the observations are being made on different levels of gene action. Priority with respect to validity of conclusions concerning the primary action of these genes awaits further study. The mechanism of gene action, in any case, should be capable of explaining all of the observations.

The antigenic effects of the lozenge pseudoalleles permitted two categories of mechanisms of gene action: (1) the loci were each associated with successive steps in a sequence of reactions and (2) the loci were concerned with conversion of the same or similar substrate(s) into the same or similar product(s). The present observations, concerned with effects of homozygous substitution of mutant pseudoalleles of lozenge upon the development of tarsal claws, are wholly consistent with the observations on antigenic effects. In the study of antigenic effects, it was noted that two components, L-1 and L-2, were associated with both of the mutants *BS* and *46*, while only the L-1 component could be detected in the presence of *g*. In considering the effects on the development of tarsal claws, homozygous substitution of *g* results in a lesser depression of claw size than does either *BS* or *46*. A number of possibilities exist for cor-

relating the antigenic and morphologic effects, but it is too soon for more than speculation.

A second interpretation given to the effects associated with pseudoallelic loci suggests that chromosomes consist of physiologically differentiated regions. These regions or units may exist in alternative forms, and are subject to both internal and external recombination. Since these differential regions are the physiological units of heredity, all of the physiological attributes of genes should refer to them. Indeed, the position effects associated with pseudoalleles are to be expected as examples of the physiological relations between alleles (GOLDSCHMIDT 1951).

In comparing the LEWIS hypothesis with that of GOLDSCHMIDT, there appears a similarity between the two views. Omitting the duplication and evolutionary intermediate aspect of the LEWIS hypothesis, both envision a similar structure to the hereditary material. The LEWIS hypothesis then resolves itself as a specific case of the GOLDSCHMIDT concept of the hereditary material. It should be expected that different physiological regions or units may exhibit different internal activities, one type being similar to that proposed by LEWIS. Moreover, in some cases, the gene defined on the operation of a breeding experiment may correspond to the physiological unit, while in others, it may be only part of such a unit.

The position that pseudoalleles are duplicates and evolutionary intermediates is based upon the association of several cases of pseudoallelic loci with salivary chromosome doublet structures. BRIDGES (1935) suggested that such structures may be single band duplications that arose by unequal crossing over. This view implies that the chromosomal regions occupied by pseudoallelic loci constitute special cases, and do not reflect upon the organization of most of the hereditary material. However, an examination of the revised map of the salivary gland X chromosome of *Drosophila melanogaster* (BRIDGES 1938; BRIDGES and BREHME 1944) reveals that 65% of the bands are associated with doublet structures. Adjacent single bands with similar morphology, and single bands adjacent to and morphologically similar to bands associated in doublets, may be interpreted as duplications which have become somewhat further differentiated. Inclusion of such bands in the count of doublet bands suggests that the pseudoallelic organization is the rule rather than the exception.

Assuming the duplication aspect of the LEWIS hypothesis to be correct, a major path for the production of new genetic material is seen. Unequal crossing over associated with or followed by some physiological differentiation leads to a chromosome organized into physiological units, each unit internally coordinated, and in control of some process. One type of internal coordination may be that of a linear sequence of reactions as proposed by LEWIS. Other types of internal organization should be expected. Further physical separation and differentiation would take place over a long period of time. At any one time, the pseudoallelic organization would represent a typical organization of the hereditary material.

No revision of experimental approach is warranted from the above consideration. The basic tool in any study of heredity is the breeding experiment, the smallest common denominator of which is the unit of recombination. However, in attempting to understand the manner in which heredity asserts itself in cell physiology, studies of the physiological units and their internal organization will be of some value.

Question has been raised as to the criteria for defining such physiological units (LEWIS 1954). The physiological unit, like the breeding unit is an operational one. It is suggested that the operational approach be followed in each case. Pseudoallelism defines the empiric unit until further definition is justified.

SUMMARY

A series of coisogenic strains of *Drosophila melanogaster* were derived. These strains differ essentially in constitution of the lozenge region of the X chromosome. All homozygous combinations of mutant and wild pseudoalleles of lozenge (*BS*, *46*, and *g*) were examined with respect to their effects on the development of the tarsal claws. A cumulative, but not additive, effect was noted suggesting two categories of mechanisms of gene action: (1) all of the loci are physiological duplicates and (2) the loci control the rates of successive steps in a sequence of reactions. Implications of these results for interpretation of pseudoallelism are discussed.

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LITERATURE CITED

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