A PHENOGENETIC STUDY OF THE LOZENGE PSEUDOALLELES IN DROSOPHILA MELANOGASTER. II. EFFECTS ON THE DEVELOPMENT OF TARSAL CLAWS IN HETEROZYGOTES[1]

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CONSIDERABLE attention has been drawn to the various cases of apparent multiple alleles which upon critical examination have been found to exh bit a low order of recombination, as if they occupy spatially separable, adjacent gene loci. Such mutants, termed pseudoalleles, exhibit what have been considered to be the physiological criteria of allelism; namely 1) they exhibit similar phenotypic effects, and 2) their heterozygotes exhibit dominance of one or the other member or intermediate phenotypes. In addition, position effects are noted in several cases, as follows: if one considers a pair of pseudoallelic loci, a^+ and b^+, and their respective mutant alleles, a and b, the coupling or *cis* heterozygote, a^+b^+/ab, appears wild type, while the repulsion or *trans* heterozygote, a^+b/ab^+, exhibits the mutant phenotype. Considerable speculation, disagreement, and confusion exists concerning the interpretations to be given to these observations.

The confusion may be avoided if it is recognized that the disagreement is concerned with two quite distinct problems: 1) The observations associated with pseudoallelism have led to interpretations regarding the *structural organization of the hereditary material.*

Three views have been offered: (a) The chromosome is the fundamental unit of heredity. This interpretation proposes that the chromosome is a physical continuum whose intact organization is necessary to exhibit the normal phenotype. Mutations are believed to be due to rearrangements within this organization. Such rearrangements exhibit the separable behavior of genes when subjected to a breeding experiment (GOLDSCHMIDT 1938, 1951). (b) The chromosome consists of a linear arrangement of separable segments. Each segment consists of a grouping of subsegments exhibiting a low order of recombination (PONTECORVO 1953). (c) The genetic material consists of discrete units which are separable on the basis of recombination. This view suggests that pseudoalleles are duplicates which may have arisen by unequal crossing over (LEWIS 1951; GREEN 1955).

2) The second area of disagreement concerns the nature of the *physiological mechanisms* underlying the phenotypic and position effects associated with pseudoallelic loci. (a) One hypothesis proposes that the entire segment acts as a physiological unit. Such regions may exist in alternative forms, subject to both internal

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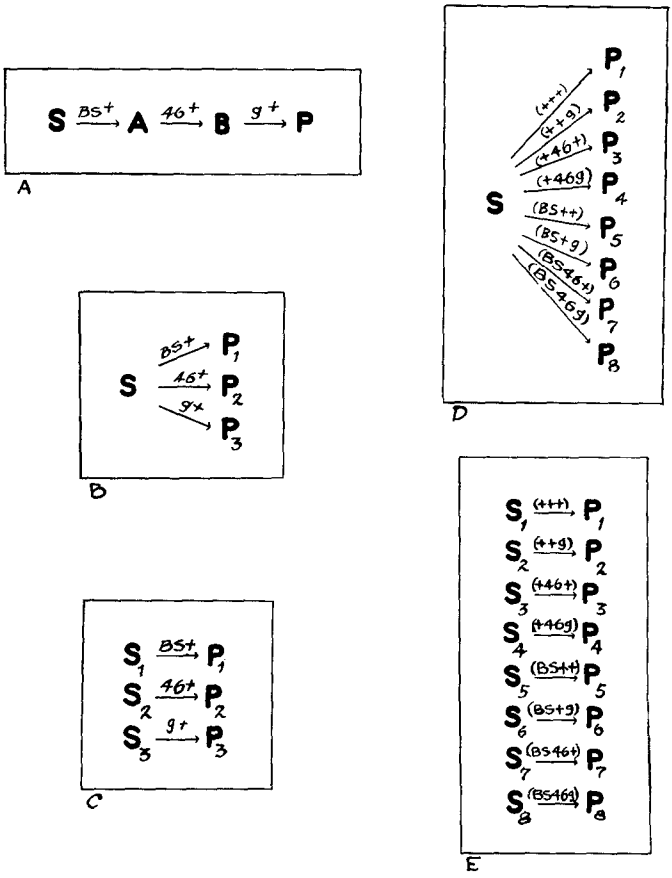

FIGURE 1.—Alternative models for the physiological action of the lozenge pseudoalleles. S is substrate; P is morphogenic product; A and B are intermediates. S_1, S_2, · · · S_n are different, although possibly related, substrates. P_1, P_2, · · · P_n differ either quantitatively or in specificity.

and external recombination, and exhibit all of the physiological properties of alleles (fig. 1, D and E). According to this view, pseudoallelic position effects are merely the reflection of the dominance relations which obtain among physiological alternatives (GOLDSCHMIDT 1951; PONTECORVO 1953). (b) Pseudoallelic loci are concerned with successive steps in a sequence of reactions which may be localized to the site of the genes in the chromosome (fig. 1, A; LEWIS 1951). (c) Pseudoallelic loci are physiological duplicates concerned with the conversion of the same or similar substrate(s) into the same or similar product(s) (fig. 1, B and C; CHOVNICK and FOX 1953).

It should be noted that these positions have been restated to emphasize that they fall into two groups, one concerned with the structure and the other concerned with the function of the hereditary material. When originally elaborated by the various workers concerned, such a separation was not, in fact, maintained. For example, in the development of his views regarding the structure of the hereditary material,

GOLDSCHMIDT draws structural conclusions from physiological observations. On the other hand, GREEN (1955) defines the structural unit in terms of recombination, but maintains that the structural unit is also a functional unit. It has been previously suggested that the investigation of each of these separate problems involves a different set of operations, and that each may be resolved only by evidence obtained from the appropriate operations (CHOVNICK and FOX 1953; BARISH and FOX 1956). The results of the present investigation, a study of the phenotypic effects of pseudoalleles, therefore have direct bearing only upon questions concerning the physiological mechanisms of pseudoallelism. It is recognized that at the primary level of action of the genetic material no such separation can be sensibly maintained; at this level, regardless of the mechanisms involved, structure and function must be intimately correlated. The point to be made is that the congruence of the results of breeding experiments, the principal source along with cytology, of evidence regarding structure, with the results of physiological investigations, the principal source of evidence regarding function, cannot be assumed, and that the operations necessary to identify the primary level of action are not yet available.

The three lozenge pseudoalleles in *Drosophila melanogaster*, which are the subjects of the present work, represent a particularly favorable series for study (GREEN and GREEN 1949). A large number of genotypic 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 associated with abnormalities of genital disc derivatives (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 (CHOVNICK and LEFKOWITZ 1956).

The latter effects offer, in particular, a system amenable to quantitative evaluation, and exhibit the position effect. The two major structures of the tarsus are the claws and pulvilli. Both structures are affected by the lozenge mutants. The claw

TABLE 1

*Claw size estimates of homozygous genotypes**

Genotype	Mean	Variance	n	$s^2_{\bar{x}}$
+ + +/+ + +	10.34	5.10	91	.056
+ + *g*/+ + *g*	8.91	2.44	162	.015
+ 46 +/+ 46 +	8.25	4.89	143	.034
+ 46 *g*/+ 46 *g*	7.19	5.06	119	.043
BS + +/*BS* + +‡	4.83	6.34	161	.040
BS + *g*/*BS* + *g*‡	4.43	5.48	174	.033
BS 46 +/*BS* 46 +‡	1.07	4.86†	81	.091
BS 46 *g*/*BS* 46 *g*‡	0.00	4.86†	98	.105

* Taken from CHOVNICK and LEFKOWITZ (1956).

† Estimated as the average variance of the other distributions.

‡ Mean, variance, and variance of mean corrected for truncation by the methods of IPSEN (1949).

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. A position effect is evidenced in that coupling heterozygotes are wild type, while repulsion heterozygotes exhibit mutant claws.

All homozygous combinations of wild and mutant pseudoalleles of lozenge (*BS*, 46, and *g*) were previously examined with respect to their effects on the development of the tarsal claws (CHOVNICK and LEFKOWITZ 1956). The mean claw sizes of the various genotypes, expressed in terms of a series of grades, were arrayed in a cumulative fashion (table 1). Depending upon the assumptions made, the observations were consistent with any of the interpretations outlined in figure 1. This being the case, variation in length of tarsal claws associated with positional change of the lozenge pseudoalleles in heterozygotes was studied, and the results are reported in this communication.

MATERIALS AND METHODS

The materials and methods used in this study have been described in the previous report (CHOVNICK and LEFKOWITZ 1956). Eight coisogenic strains differing essentially in the possession of one or another of eight combinations of wild and mutant pseudoalleles of lozenge (*BS*, 46, and *g*) and balanced over a *ClB* chromosome from a single source, were used in matings to produce all offspring described in this report. Observations were restricted to females. Reciprocal crosses were made in each case. No significant differences were found between reciprocal cross offspring, and the means and variances discussed represent pooled results. Several of the distributions exhibited truncation at the lower end of the scale. Correction of mean and variance estimates of such distributions followed the previously described procedure.

RESULTS AND CONCLUSIONS

Dominance effects

The five models portrayed in figure 1 fall into two groups. Three of the models (A, B, and C) are based on the suggestion that the action of each of the lozenge loci is independent of the other two, except as they may be related with respect to substrate specificity. The remaining models (D and E) are based on the supposition that the entire lozenge segment, including all three loci, acts as a single unit of action. An attempt to distinguish between these two alternative groups may be made by examining the dominance effects exhibited in the present data.

If one were to assume that the unit of physiological action with respect to tarsal development is the entire lozenge segment, then the various combinations of wild and mutant pseudoalleles would comprise the alternative forms of this unit. Each alternative form, according to this view, is designated by a combination of wild type and mutant alleles at three points in the segment. Eight alternative forms are so designated, and these may be arranged in the following serial order, according to the degree of their effects in homozygotes: + + +, + + *g*, + 46 +, + 46 *g*, *BS* + +, *BS* + *g*, *BS* 46 +, *BS* 46 *g* (table 1). If this view is correct, examination of dominance effects in heterozygotes involving all combinations of these eight al-

TABLE 2

Claw size estimates of heterozygous genotypes

Genotype	Mean	Variance	n	$\frac{s^2}{\bar{x}}$
+ + *g*/+ 46 +	9.18	2.41	154	0.016
+ + *g*/+ 46 *g*	9.11	3.82	82	0.047
+ + *g*/*BS* + +	8.77	2.82	102	0.028
+ + *g*/*BS* + *g*	8.75	2.81	94	0.030
+ + *g*/*BS* 46 +	8.97	3.62	120	0.030
+ + *g*/*BS* 46 *g*	8.85	3.16	131	0.024
+ 46 +/+ 46 *g*	8.08	5.96	143	0.042
+ 46 +/*BS* + +	8.01	3.44	95	0.036
+ 46 +/*BS* + *g*	7.18	4.10	108	0.038
+ 46 +/*BS* 46 +	7.04	4.49	108	0.042
+ 46 +/*BS* 46 *g*	6.56	4.29	140	0.031
+ 46 *g*/*BS* + +	7.01	6.73	112	0.060
+ 46 *g*/*BS* + *g*	7.09	4.77	143	0.033
+ 46 *g*/*BS* 46 +	5.38	4.05	119	0.034
+ 46 *g*/*BS* 46 *g*	5.10	2.99	98	0.031
BS + +/*BS* + *g**	4.70	3.91	163	0.025
BS + +/*BS* 46 +*	3.21	3.51	119	0.032
BS + +/*BS* 46 *g**	1.75	5.41	112	0.072
BS + *g*/*BS* 46 +*	3.23	2.71	128	0.022
BS + *g*/*BS* 46 *g**	1.58	6.41	109	0.094
BS 46 +/*BS* 46 *g**	−2.62	4.09†	83	0.187

* Mean, variance, and variance of mean corrected for truncation by the methods of IPSEN (1949).

† Estimated as the average variance of the other distributions.

ternatives would reveal a pattern resembling those commonly associated with allelic interaction.

All heterozygotes possessing one chromosome carrying + + + lozenge region have been reported to exhibit the wild phenotype (GREEN and GREEN 1949). Our observations support this conclusion. They have large, pigmented claws, well developed pulvilli, and wild type eyes. Thus, the highest member of the above series is completely dominant over all other members of the series. All other combinations of the various alternatives exhibit mutant phenotypes, but an examination of the data discloses dominance in several cases. Thus, the next member of the series, + + *g*, exhibits complete dominance over all lower members of the series (table 2). The + 46 + segment exhibits complete dominance over the next two combinations, + 46 *g* and *BS* + +, but shows intermediate effects with lower combinations in the series (table 2). Similarly, the + 46 *g* segment exhibits complete dominance over the next two, *BS* + + and *BS* + *g*, and intermediate effects with lower combinations. All remaining combinations show intermediate effects. Clearly, the interaction

pattern is reminiscent of allelic interaction. With respect to dominance relationships, therefore, these data are consistent with the view that the entire segment operates as a physiological unit.

By way of contrast, an attempt to interpret these data in terms of the hypothesis that each of the lozenge loci is an independently operating physiological unit leads to inconsistencies. In heterozygotes involving a + + + segment, all three mutants are completely recessive to their respective wild alleles. When, however, + + g/ + + *g* is compared with the remaining heterozygotes possessing a + + *g* segment (table 2), *g* appears to be dominant to g^+. Similar difficulties are encountered with respect to *BS* and 46. Such inconsistencies should be called position effects, but to do so would impose an additional problem upon those which already exist.

The simplest conclusion to be drawn is that the lozenge loci do not function as independent physiological units, clearly ruling out the hypothesis represented in figure 1C. Kinetic considerations (WRIGHT 1941; STRAUSS 1955), relating to substrate utilization, might be developed to reconcile the hypotheses portrayed in A and B with the data, but only at the expense of considerable complexity. It would seem simpler to conclude that the entire segment operates as an integrated unit in tarsal development (fig. 1, D and E). Each of the alternative segments, as specified by a particular combination of wild and mutant alleles at three points, exhibits a unified function.

As pointed out by GOLDSCHMIDT (1951), this view possesses the added advantage of dissolving the problems posed by the apparent position effects exhibited by pseudoalleles. Such effects are now seen as reflections of the dominance relationships which exist among the segmental alternatives. No special hypothesis is needed to explain them, since they are to be expected under the conventional views regarding dominance.

Mode of action of the lozenge segments

Accepting the conclusion that the lozenge segment acts as a physiological unit, it now becomes possible to inquire into the mode of action of the various alternatives with respect to each other in individuals possessing two X chromosomes. This question is most easily examined in those cases not obscured by dominance. More specifically, in the case of those segments which do not exhibit dominance, do the segments present in such individuals act in an additive fashion on the chosen scale of measurement, or are they non-additive in effect?

The model which has been proposed (fig. 1, D and E) assumes that each lozenge segment mediates the rate of conversion of a substrate into a morphogenic product which quantitatively determines claw size. The truncation at the lower end of the scale, and cumulative effects in homozygotes, previously led to the following view: (1) A minimum quantity of product is necessary before a claw can be produced. (2) After this threshold concentration is reached, claw size is directly related to the amount of product available. By correcting for the threshold at the lower end of the scale, the mean claw sizes of the homozygous genotypes may be taken as estimates of the amount of morphogenic product attributed to the genotype (CHOVNICK and LEFKOWITZ 1956). According to this view, each of the lozenge segments is

concerned with the amount of morphogenic product made available during the critical point of tarsal development.

The hypothesis of additive action of the segments may be subjected to test by using it to predict mean claw sizes for the various heterozygotes from the observed means of the homozygotes. Assuming that the lozenge segments operate additively, the mean claw size of any homozygous genotype represents an estimate of twice the morphogenic product contribution attributable to one segment. Thus, $P_{BS++} = \frac{1}{2}\bar{x}_{BS++/BS++}$, and this value may be used as an estimate of the morphogenic product contribution of one X chromosome carrying the combination of lozenge pseudoalleles $BS + +$. In this fashion, estimates may be generated for chromosomes bearing all combinations of wild and mutant pseudoalleles of lozenge which do not exhibit dominance. Prediction of the mean claw size for any heterozygous genotype is achieved as follows:

$$\bar{x}_{BS++/BS\,46+} = P_{BS++/BS\,46+} = P_{BS++} + P_{BS\,46+} \tag{1}$$

and

$$\bar{x}_{BS++/BS\,46+} = \tfrac{1}{2}\bar{x}_{BS++/BS++} + \tfrac{1}{2}\bar{x}_{BS\,46+/BS\,46+} \tag{2}$$

Comparison of predicted and observed values is made by examination of the test statistic, c, obtained as the ratio of the difference between observed and expected values to the standard error of the difference. For the genotype $BS + +/BS\ 46 +$, the test statistic c, may be obtained as the absolute value of (3).

$$\frac{\bar{x}_{BS++/BS\,46+} - \frac{1}{2}\bar{x}_{BS++/BS++} - \frac{1}{2}\bar{x}_{BS\,46+/BS\,46+}}{\sqrt{s^2_{\bar{x}_{BS++/BS\,46+}} + \frac{1}{4}s^2_{\bar{x}_{BS++/BS++}} + \frac{1}{4}s^2_{\bar{x}_{BS\,46+/BS\,46+}}}} \tag{3}$$

Since the dominance effects preclude accurate estimation of the contribution of segments $+ + +$, $+ + g$, $+ 46\ g$ by this method, heterozygotes involving these segments are omitted from consideration. The remaining genotypes may be tested, and are included in the upper portion of table 5. With one exception, all of the means observed do not differ significantly from the values expected on the hypothesis of additive action of lozenge segments. The one exceptional case is that of $BS\ 46 +/BS\ 46 +$ and $BS\ 46\ g/BS\ 46\ g$. Examination of the distribution of claw values for these genotypes reveals that very few flies of these groups possess claws (CHOVNICK and LEFKOWITZ 1956). Since that portion of the distribution above the truncation point represents so few flies, small sampling fluctuations would have profound effect upon the estimate of the mean adjusted for truncation. Moreover, the observed distribution of the heterozygote $BS\ 46 +/BS\ 46\ g$ falls in this same category. These three genotypes had the lowest means in the entire study. For these cases, the estimate of variance was taken as the average variance of the other distributions, and the error incurred in the estimation of adjusted means is not afforded by the sampling variance estimates.

It is concluded that the lozenge segments carry out their functions in an additive fashion on the chosen scale. This conclusion stands in contrast to that previously reached with respect to the individual lozenge loci, whose effects in homozygotes were observed not to be additive on the same scale (CHOVNICK and LEFKOWITZ

1956). The hypothesis that the lozenge segments as a whole operate as physiological units in tarsal development is thus strengthened.

Additive action of lozenge segments and dominance effects

The preceding analysis omits consideration of those segments which exhibit dominance. Dominance effects in mutant heterozygotes, however, must be considered in any interpretation of the mode of action of lozenge in tarsal development. The model which has been proposed (fig. 1, D and E) assumes that each lozenge segment mediates the rate of conversion of a substrate into a product which quantitatively determines claw size. As demonstrated in the previous section, such action, in heterozygotes which do not exhibit dominance, is additive on the chosen scale.

The observation of dominance effects leads to an extension of this interpretation. If additivity is assumed to be characteristic of even those segments which exhibit dominance, it is possible to develop a consistent interpretation from the considerations summarized graphically in figure 2. The horizontal axis in this figure represents the scale of variation in morphogenic product made available by the genotype. Within any one genotype, individual variation in amount of this product is attributable to environmental sources. T_0 represents the threshold below which the amount of product is insufficient to result in claw development. The location of the T_0 threshold is described in a previous report (CHOVNICK and LEFKOWITZ 1956).

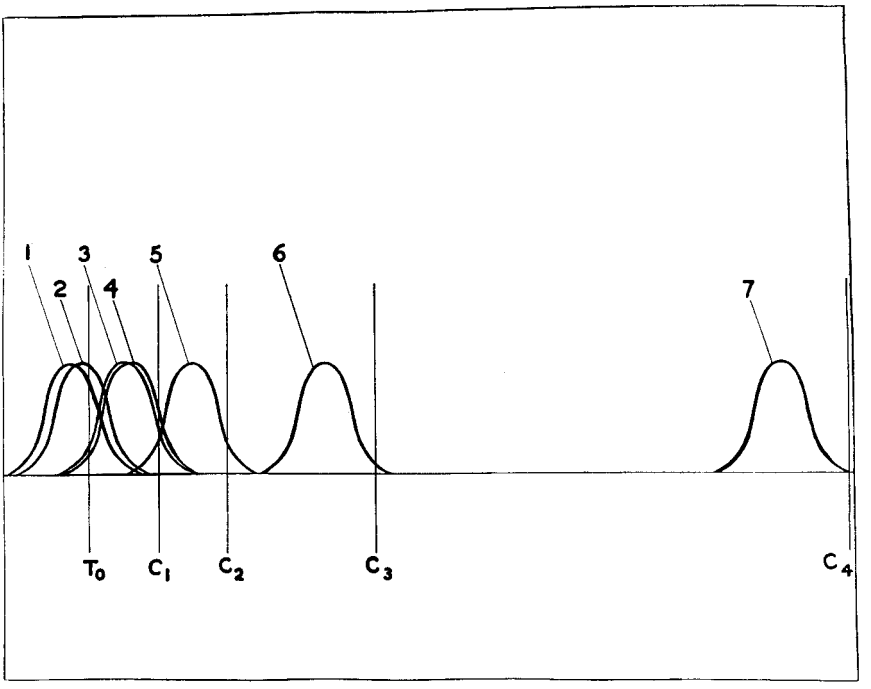

FIGURE 2.—Diagrammatic representation of the amount of morphogenic product conditioned by lozenge genotypes, plotted on an additive scale. Position of threshold and ceilings indicated. (1) *BS* 46 *g*/*BS* 46 *g*, (2) *BS* 46 +/*BS* 46 +, (3) *BS* + *g*/*BS* + *g*, (4) *BS* + +/*BS* + +, (5) + 46 *g*/+ 46 *g*, (6) + 46 +/+ 46 +, (7) + + *g*/+ + *g*.

The observed distributions of claw sizes for the homozygous genotypes *BS* 46 *g*/*BS* 46 *g* and *BS* 46 +/*BS* 46 + indicate that the mean amount of morphogenic product for each falls below the T_0 threshold (table 1). Similarly, the mean amount of morphogenic product attributable to each of the homozygous genotypes *BS* + *g*/*BS* + *g* and *BS* + +/*BS* + + would be located to the right of the T_0 threshold. Numerical values could be assigned to the mean amounts of morphogenic product corresponding to the mean claw size estimates for the various genotypes: *BS* 46 *g*/*BS* 46 *g*, 0.00; *BS* 46 +/*BS* 46 +, 1.07; T_0 threshold, 1.56; *BS* + *g*/*BS* + *g*, 4.43; *BS* + +/*BS* + +, 4.83.

The four chromosomal segments thus far considered do not exhibit dominance. The observed mean claw sizes of the various heterozygous combinations of these segments would therefore correspond to the amount of product expected if these segments operate in an additive fashion, as demonstrated in the previous section. Thus, the mean amount of product for each of these heterozygous combinations could be plotted on the same scale as that already used for the homozygotes. While they have been omitted from the diagram for purposes of simplicity, their values are as follows (predicted means from table 5): *BS* 46 +/*BS* 46 *g*, 0.54; *BS* + *g*/*BS* 46 *g*, 2.22; *BS* + +/*BS* 46 *g*, 2.42; *BS* + *g*/*BS* 46 +, 2.75; *BS* + +/*BS* 46 +, 2.95; *BS* + +/*BS* + *g*, 4.63.

If mean claw size is always a direct measure of the amount of morphogenic product made available by the genotype, the next homozygous genotype, + 46 *g*/+ 46 *g*, would be located at 7.19 on this same scale. Furthermore, if this segment operates in an additive fashion in heterozygotes with the previous segments, the mean amount of morphogenic product in each heterozygote should be intermediate between the corresponding homozygotes. The observed claw sizes of such heterozygotes would then be expected to exhibit intermediate values. In point of fact, the + 46 *g* segment exhibits dominance over the *BS* + + and *BS* + *g* segments; i.e., the observed mean claw values are not different from that of the + 46 *g*/+ 46 *g* homozygote. If it is nevertheless assumed that additivity still operates, these observations must mean that a ceiling exists, C_1, which is subject to at least two possible interpretations: (1) Further increase in amount of morphogenic product takes place after the critical period for tarsal development, and thus leads to no increase in claw size. (2) Substrate limitation prevents production of morphogenic product over this ceiling. Under either circumstance, the genotype + 46 *g*/+ 46 *g* must be capable of conditioning the production of an amount of morphogenic product well over this ceiling, since one dose of + 46 *g* is sufficient to carry heterozygotes with *BS* + + or *BS* + *g* over the ceiling.

Since the C_1 ceiling is that point at which further increase in morphogenic product leads to no further increase in claw size, this point must be located at 7.19 on the scale. It is then possible to estimate $P_{+\ 46\ g}$, the morphogenic product contribution of one dose of + 46 *g*, in the following manner. If one dose of *BS* + *g* contributes 2.22 units of morphogenic product, i.e., one half of the mean claw value of *BS* + *g*/*BS* + *g*, then one dose of + 46 *g* must contribute at least 4.97 such units if the mean product value of + 46 *g*/*BS* + *g* is to exceed the C_1 ceiling. Thus the homozygote + 46 *g*/+ 46 *g* must be capable of producing an amount of morphogenic product no less than 9.94 units on the scale (2 × 4.97).

As a check on this interpretation, it is possible to predict the amounts of morphogenic product expected in the heterozygotes + 46 *g*/*BS* 46 + and + 46 *g*/*BS* 46 *g*. Such predictions would be based upon the additivity of the *BS* 46 + and *BS* 46 *g* contributions as estimated in the previous section, with the + 46 *g* contribution as estimated in the previous paragraph. For the + 46 *g*/*BS* 46 + genotype, the predicted amount of morphogenic product would be 5.51 (4.97 + 0.54). For the + 46 *g*/*BS* 46 *g* heterozygote, the predicted amount of morphogenic product would be 4.97 (4.97 + 0.00). Since these values fall below the C_1 level, they would be expected to correspond to the observed claw values. This expectation is fulfilled (table 5).

For those genotypes which fall below a ceiling, comparison of expected and observed claw values is accomplished by examination of the test statistic, *c*. Computation of *c* is modified from that presented in the previous section to account for the existence of the ceiling. Thus, for the heterozygote, *BS* 46 *g*/+ 46 *g*, the expected amount of morphogenic product $P_{BS\ 46\ g/+\ 46\ g} = P_{BS\ 46\ g} + P_{+\ 46\ g}$. For the segment, *BS* 46 *g*, which does not exhibit ceiling effects, $P_{BS\ 46\ g} = \frac{1}{2}\bar{x}_{BS\ 46\ g/BS\ 46\ g}$. However, for the segment, + 46 *g* which does exhibit a ceiling,

$$P_{+\ 46\ g} = \bar{x}_{+\ 46\ g/+\ 46\ g} - \tfrac{1}{2}\bar{x}_{BS+\ g/BS+\ g}. \tag{4}$$

Thus,

$$P_{BS\ 46\ g/+\ 46\ g} = \tfrac{1}{2}\bar{x}_{BS\ 46\ g/BS\ 46\ g} + \bar{x}_{+\ 46\ g/+\ 46\ g} - \tfrac{1}{2}\bar{x}_{BS+\ g/BS+\ g}. \tag{5}$$

Since the estimate, $P_{BS\ 46\ g/+\ 46\ g}$, falls below the C_1 ceiling, then

$$P_{BS\ 46\ g/+\ 46\ g} = \bar{x}_{BS\ 46\ g/+\ 46\ g} = \tfrac{1}{2}\bar{x}_{BS\ 46\ g/BS\ 46\ g} + \bar{x}_{+\ 46\ g/+\ 46\ g} - \tfrac{1}{2}\bar{x}_{BS+\ g/BS+\ g}. \tag{6}$$

The test statistic, *c*, then takes the form of (7).

$$\frac{\bar{x}_{BS\ 46\ g/+\ 46\ g} - \frac{1}{2}\bar{x}_{BS\ 46\ g/BS\ 46\ g} - \bar{x}_{+\ 46\ g/+\ 46\ g} + \frac{1}{2}\bar{x}_{BS+\ g/BS+\ g}}{\sqrt{s^2_{\bar{x}_{BS\ 46\ g/+\ 46\ g}} + \frac{1}{4}s^2_{\bar{x}_{BS\ 46\ g/BS\ 46\ g}} + s^2_{\bar{x}_{+\ 46\ g/+\ 46\ g}} + \frac{1}{4}s^2_{\bar{x}_{BS+\ g/BS+\ g}}}} \tag{7}$$

Continued treatment of the remaining genotypes along this line provides complete accounting for their phenotypes. This treatment requires the assumption of additional ceilings in order to account for the various dominance effects (C_2, C_3, C_4), but additivity of the contributions of the various segments to the amount of morphogenic product in each genotype extends over the whole scale. Further evidence that this is the case is obtained from the observation that the observed variance of each genotype is in no way correlated with its mean claw size (tables 1 and 2). The position of each ceiling and the manner in which it was estimated is given in table 3. The contribution of morphogenic product of each of the eight lozenge segments and the source of its estimation is given in table 4. Table 5 contains the following items: the predicted amounts of morphogenic product (*P*) conditioned by all genotypes, calculated in the manner indicated above; the expected claw value for each genotype, taking into consideration ceiling effects; the observed claw values for all genotypes; and the test statistic, *c*, for the differences between expected and observed

TABLE 3

Scale position of ceilings

Ceiling	Scale position	Source of estimate
C_1	7.19	+ 46 *g*/+ 46 *g*
C_2	8.25	+ 46 +/+ 46 +
C_3	8.91	+ + *g*/+ + *g*
C_4	10.34	+ + +/+ + +

TABLE 4

Morphogenic product contributed by lozenge segments

Segment	Contribution	Source of estimate
+ + +	10.34	+ + +/+ + +, because of complete dominance
+ + *g*	8.91	+ + *g*/+ + *g*, because of complete dominance
+ 46 +	5.83	$C_2 - (BS + +)$
+ 46 *g*	4.97	$C_1 - (BS + g)$
BS + +	2.42	$\frac{1}{2}$ (*BS* + +/*BS* + +)
BS + *g*	2.22	$\frac{1}{2}$ (*BS* + *g*/*BS* + *g*)
BS 46 +	0.54	$\frac{1}{2}$ (*BS* 46 +/*BS* 46 +)
BS 46 *g*	0.00	$\frac{1}{2}$ (*BS* 46 *g*/*BS* 46 *g*)

claw values. Calculation of c is not justified for those genotypes which served as sources of information for the estimation of ceiling positions or segmental contributions.

For those heterozygotes with predicted amounts of morphogenic product (P) which fall at or above ceiling levels, the expected claw values are estimated to be equal to that of the homozygote in the series. That this is indeed the case may be seen from comparison of the heterozygote samples with the homozygote by test of the hypothesis that they are drawn from the same population. Such a test takes the form of an analysis of variance and values of F are indicated in table 5.

In table 5, the 36 lozenge genotypes have been arranged in five groups. The first group contains the genotypes for the lowest segments in the series, i.e. those exhibiting no dominance. In this group, a linear relationship exists between the predicted amount of morphogenic product (P) and the observed claw values (fig. 3). The second group contains the + 46 *g*/+ 46 *g* homozygote, and heterozygotes of + 46 *g* with lower members of the segmental series. Here a linear relationship between P and observed claw values is exhibited up to the C_1 ceiling, where an abrupt change of slope occurs so that the observed claw values never exceed that ceiling (7.19). Indeed, these first two groups could be combined into a single group exhibiting a linear relationship between P and observed claw values up to C_1, where an abrupt change in slope would occur. The third group of genotypes in table 5 are those involving + 46 +, homozygous or heterozygous with lower members of the series. Here again, a linear relationship between P and observed claw values is exhibited up to the C_2 ceiling, where an abrupt change of slope occurs so that the observed claw values never exceed that ceiling (8.25). All genotypes involving + +

TABLE 5

Morphogenic product, and estimated and observed claw values of lozenge genotypes

Genotype	*P*	Claw values		*c**	*F*
		Expected	Observed		
BS 46 *g*/*BS* 46 *g*	0.00	0.00	0.00		
BS 46 *g*/*BS* 46 +	0.54	0.54	−2.62	6.49	
BS 46 +/*BS* 46 +	1.07	1.07	1.07		
BS 46 *g*/*BS* + *g*	2.22	2.22	1.58	1.73	
BS 46 *g*/*BS* + +	2.41	2.41	1.75	2.02	
BS 46 +/*BS* + *g*	2.75	2.75	3.23	2.09	
BS 46 +/*BS* + +	2.95	2.95	3.21	1.12	
BS + *g*/*BS* + *g*	4.43	4.43	4.43		
BS + *g*/*BS* + +	4.63	4.63	4.70	0.33	
BS + +/*BS* + +	4.83	4.83	4.83		
BS 46 *g*/+ 46 *g*	4.97	4.97	5.10	0.40	
BS 46 +/+ 46 *g*	5.51	5.51	5.38	0.43	
BS + *g*/+ 46 *g*	7.19	7.19	7.09		$F = 0.183$
BS + +/+ 46 *g*	7.38	7.19	7.01		*d.f.* = 2, 371
+ 46 *g*/+ 46 *g*	9.94	7.19	7.19		
BS 46 *g*/+ 46 +	5.83	5.83	6.56	2.30	
BS 46 +/+ 46 +	6.37	6.37	7.04	2.03	
BS + g/+ 46 +	8.05	8.05	7.18	2.90	
BS + +/+ 46 +	8.25	8.25	8.01		$F = 0.913$
+ 46 *g*/+ 46 +	10.80	8.25	8.08		*d.f.* = 2, 378
+ 46 +/+ 46 +	11.66	8.25	8.25		
BS 46 *g*/+ + *g*	8.91	8.91	8.85		
BS 46 +/+ + *g*	9.45	8.91	8.97		
BS + *g*/+ + *g*	11.13	8.91	8.75		
BS + +/+ + *g*	11.33	8.91	8.77		
+ 46 *g*/+ + *g*	13.88	8.91	9.11		$F = 0.415$
+ 46 +/+ + *g*	14.74	8.91	9.18		*d.f.* = 6, 838
+ + *g*/+ + *g*	17.82	8.91	8.91		
BS 46 *g*/+ + +	10.34	10.34			
BS 46 +/+ + +	10.88	10.34			
BS + *g*/+ + +	12.56	10.34			
BS + +/+ + +	12.76	10.34			
+ 46 *g*/+ + +	15.31	10.34			
+ 46 +/+ + +	16.17	10.34			
+ + *g*/+ + +	19.25	10.34			
+ + +/+ + +	20.68	10.34	10.34		

* P ($c \geq 2.58$) = .01.

g are associated with *P* values exceeding the C_3 ceiling, and observed claw values do not deviate significantly from 8.91, the value of C_3. All genotypes involving + + + exhibit a similar relationship to C_4. Observed means for the heterozygotes involving + + + are not included in table 5. Dominance of the + + + segment over all

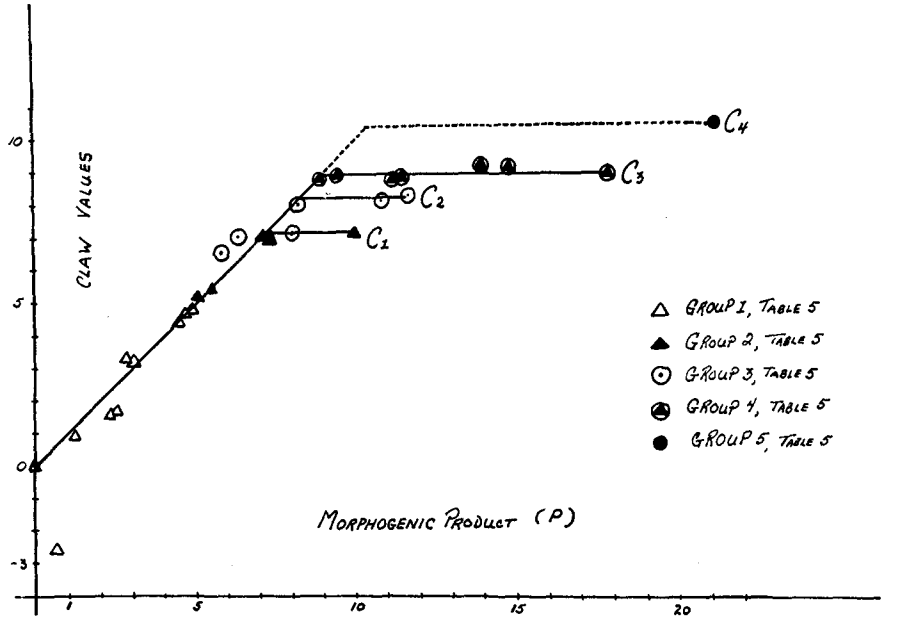

FIGURE 3.—Relationship between observed claw value and predicted morphogenic product for all homozygous and heterozygous lozenge genotypes.

lower members of the series had been reported previously (GREEN and GREEN 1949). Examination of small samples of these heterozygotes supported this view, and a detailed analysis of this group of heterozyogtes was omitted.

As WRIGHT (1941) has demonstrated on the basis of enzyme kinetics, allelic series are expected to exhibit relationships such as are observed for the lozenge segmental alternatives if the effects are enzymatically mediated as in the following model:

gene or segment
↓
enzyme
↓
$S \rightarrow P \rightarrow$ Observed phenotype

In terms of this model, the following assumptions have been made in the calculation of predicted amounts of morphogenic product (P): 1) the existence of a steady state in the conversion of S to P over a specific interval of time during development, 2) the production by the alternative segments of differing amounts of enzyme rather than of enzymes differing in specificity, and 3) strict proportionality of the rate of production of P to concentration of enzyme. Under these circumstances, the predicted amounts of morphogenic product may be equated to the concentration of enzyme conditioned by each alternative segment, and the observed claw values may be equated to the rate of conversion of S to P. Figure 3 could therefore be taken as portraying the relationship between enzyme concentration and the rate of conversion

of S to P, and a departure from linearity in this relationship could result from departures from any of the assumptions listed above.

Slight differences in the specificity of the enzyme produced by alternative segments could result in differences in its Michaelis constant (K_s). WRIGHT (1941) has examined two limiting cases of such changes, i.e. when K_s becomes very large (approaching ∞) and when K_s becomes very small (approaching 0). In both cases, a ceiling in the rate of production of P results from substrate limitation. In the former case (K_s very large), the approach to the ceiling is asymptotic, while in the latter (K_s very small) it is linear. The data for Groups 1 and 2 (fig. 3) appear to approximate those expected when K_s is very small, but those for the other groups are insufficiently extensive to allow a decision as to whether changes of this sort are involved.

Alternatively, slight differences in enzyme specificity could result in the production from a single substrate of morphogenic products which differ in their specificity for claw production (fig. 1D). A ceiling to the rates of the alternative reactions would be imposed by substrate limitation, and would be independent of the K_s of the alternative enzymes, but at the phenotypic level several different ceilings could result from the differing specificities of the alternative morphogenic products. This could give rise to a situation such as is observed.

Larger differences in enzyme specificity could result in the utilization of different substrates by the different lozenge segments (fig. 1E). In each case a ceiling would arise from substrate limitation, but the level of the ceiling would vary from substrate to substrate. A situation such as that observed would readily result.

Finally, substitution of the alternative segments might result in changes of a different sort, i.e. in the duration of the interval of time during which S is converted into P. The multiple ceilings would then reflect time limitations to the production of morphogenic product, the specific details of which could be fitted to the observed results.

It thus becomes impossible to distinguish between figures 1D and 1E, or to specify further detail on the basis of these data. Indeed, the actual state of affairs may well consist of a combination of the conditions just discussed.

DISCUSSION

While the present data are most simply and consistently explained by the hypothesis that the entire lozenge segment acts as a physiological unit, it is not necessary to suppose that this is the only physiological mechanism associated with pseudoallelism. In the case of the vermilion pseudoalleles, the point has already been made that the entire segment appears to act as a physiological unit at one phenotypic level, but that at a second level the individual loci exhibit physiological unity, while at still another level each locus appears to consist of two or more physiological units (BARISH and FOX 1956). We would venture to suggest that each case of pseudoallelism deserves separate analysis and might well exhibit unique physiological properties.

From the most general point of view, however, the hypothesis that the entire pseudoalleic segment operates as a physiological unit would seem to bring the greatest degree of order to the existing data. In the first place, no physiological properties

need be assumed other than those usually attributed to alleles. Secondly, pseudoallelic position effects are dismissed as special problems, and become simply reflections of dominance relationships. In this connection, a more special difficulty is also dispelled, namely, the apparent contradiction between the position effects exhibited by the lozenge and vermilion pseudoalleles on morphological traits and their lack of position effects on antigenic specificity (CHOVNICK and FOX 1953; BARISH and FOX 1956). This disparity may now be seen as another example of differing dominance relationships among pleiotropic effects of particular genes. A further special problem which is greatly simplified by this point of view is that posed by the relationships of the vermilion mutants to their non-allelic suppressor (GREEN 1954, 1955). The complexity of these relationships (BARISH and FOX 1956) becomes easily explicable if the unit whose physiological action is affected by the suppressor is the entire vermilion segment. Finally, the so-called "transvection effects" which have been reported in the bithorax case (LEWIS 1954a, 1954b, 1955) become, on this view, true position effects resulting from rearrangement, rather than special physiological consequences of pseudoallelism.

From one point of view, the hypothesis represented in figure 1A might be considered a special case of the view just presented, i.e., as a particular mechanism by means of which a pseudoallelic segment could exhibit an apparently unified action. Such a view would require the following assumptions: 1) a linear sequence of reactions; 2) that this sequence is localized to the immediate vicinity of the chromosome; 3) that each locus in the segment is a discrete functional unit; 4) that each step in the sequence is controlled by one locus. Aside from the fact that evidence is available that suggests interchromosomal interaction in certain cases (CHOVNICK and FOX 1953; LEWIS 1954b and 1955) and non-linearity in others (BARISH and FOX 1956), the complexity of this view is not conducive to its acceptance.

It should be repeated that from an operational point of view, the conclusion that pseudoallelic segments may act as integrated physiological units has no necessary implications for questions related to the structural organization of the hereditary material. Discussion of the evidences which do bear on this question would be inappropriate in the present paper.

SUMMARY

In a study of the physiology of psueodallelism, the effects on the development of tarsal claws of a series of eight different lozenge segments (+ + +, + + *g*, + 46 +, + 46 *g*, *BS* + +, *BS* + *g*, *BS* 46 +, *BS* 46 *g*) have been examined in all heterozygous combinations. The interaction effects in heterozygotes lead to the conclusion that the entire lozenge segment operates as an integrated physiological unit. The pseudoallelic position effects are seen as reflections of the dominance relations which exist among the segmental alternatives. Additivity of the action of the lozenge segments is indicated. Threshold and ceiling effects suggest several alternative schemes of action of the various lozenge segments. In all cases, the various alternative lozenge segments are concerned with the production of morphogenic products which may differ in specificity, but which exert quantitative effects on tarsal development by affecting a common developmental process.

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