

Genetic regulation and pattern formation: A study of the *yellow* locus in *Drosophila melanogaster*

BY WILLIAM G. NASH AND RHODA J. YARKIN*

*Department of Biological Sciences, The George Washington University,
Washington, D.C. 20006*

(Received 11 February 1974)

SUMMARY

Many of the *yellow* alleles found in *Drosophila melanogaster* result in a unique pattern of phenotypic expression. These patterns follow the morphologically distinct cell types of the cuticle, so that for one allele all the bristles of the head and thorax might be mutant, while most of the fly appears wild type. A comparison of many different *y* mutants demonstrates that the *yellow* phenotype is expressed independently in most if not all the different cell types which form the cuticle. Control of this expression appears to reside at the *yellow* locus itself.

1. INTRODUCTION

The mechanism(s) controlling differential gene activity in higher organisms remains obscure. Lack of information about this critical aspect of development exists primarily due to the difficulty of genetically modifying a differentiated phenotype with respect to its spatial, quantitative and temporal expression. The numerous mutant alleles of the *yellow* (*y-1:0.0*) locus provide a system amenable to this kind of genetic manipulation.

The obvious visible effect of the *y* mutant is an altered pigmentation of the adult body cuticle and its derivative structures. The altered yellowish pigment appears to be a qualitatively different form of the wild-type black melanin (Waddington, 1941). At least 75 alleles of the *y* locus have been described. We have divided these mutants into two phenotypic classes. Type 1 mutants result in a completely mutant expression in all cell types which form the adult cuticle. Type 2 mutants have some cell types of the cuticle partially or fully wild type.

This paper presents a detailed phenotypic analysis of these type 2 alleles. The genetic evidence suggests that a regulatory portion of the *y* gene responds to a different signal(s) present in each cell type which forms the cuticle. The nature of this response appears to control the spatial and qualitative pattern of the *y* gene activity.

* Present address: Laboratory of General and Comparative Biochemistry, National Institute of Mental Health, Bethesda, Maryland 20014.

2. METHODS AND MATERIALS

(i) *Genetic Stocks*

Three alleles of *yellow* used in this study originated in the laboratories of W. G. Nash at The University of Michigan (1969) or at The George Washington University (1970–2). These alleles (y^M , y^{M1} , y^{M2}) while arising spontaneously, had a rather involved origin which will be described elsewhere. A complete description of their phenotypes will be presented in the results. The alleles y^1 , y^2 , y^{2s} , y^{3d} , y^{3P} , y^{td} , and y^{59b} were obtained from The California Institute of Technology, Pasadena, California. The alleles y^4 , y^{bq} , y^{S1} , y^{34c} , y^{62a} , and y^{18CH1} were obtained from The Bowling Green State University, Bowling Green, Ohio. The remaining alleles, y^{bab} , y^{59c} , and y^{bt} were kindly supplied by Drs G. D. Hanks, C. W. Clancy and L. Sandler. The alleles y^{bab} , y^{bq} and y^{18CH1} are not described in Lindsley & Grell (1967). Hanks & Newlin (1969) discovered the spontaneous allele y^{bab} . The alleles y^{bq} and y^{18CH1} originated in H. J. Muller's laboratory before 1950 (I. I. Oster, personal communication).

(ii) *Examination and classification of the y alleles*

Five regions of the adult body cuticle and seven derivative structures were examined in 1-day-old adults (Fig. 1). Regions of the body cuticle, which are pigmented in specific areas, are phenotypically distinct and on this basis are considered to be formed by different cell types. For example, the abdominal cuticle can be divided into banded and non-banded regions. The bristles, arista, sex combs and tarsal claws are examples of derivative cuticle structures. Mouth-parts and microsetae of third-instar larvae were also examined. All observations were performed on isolated structures at $\times 25$ and $\times 50$ with a Wild (M 5) stereo dissecting microscope.

The degree to which the y alleles departed from the wild type was determined visually, since no quantitative method for measuring the variations in pigmentation of the cuticle was available. For each mutant different levels of pigment expression in the 14 parts of the larval and adult cuticle, subsequently referred to as characters, were ranked on a scale from 0 to 5; the fully wild-type expression is indicated by a score of 5.

To make the classification as accurate as possible standards for each character ranging from 0 to 5 were arranged on microscope slides. The tissue was covered with a thin layer of diluted Permunt and a cover-slip was placed on top. All of the cell types of each allele were mounted on separate slides. In this way each cell type could be accurately ranked and compared to the same cell type of any other allele. The adult abdominal and thoracic cuticle were separated from the underlying tissue with Dumont no. 5 forceps prior to mounting. Larval mouth-parts were also separated from the surrounding tissue before mounting. The microsetae were prepared by removing a portion of the ventral cuticle of the third instar larvae and cleaning off the underlying tissue.

For detailed observation of the isolated parts, five randomly chosen male and female flies of each allele were used. The flies were raised at 25 °C and examined

24 h after eclosion. Variation between flies of a given genotype was not usually observed, although some alleles did show a range of expression in one or more of the characters and are described in the Results section. All the *y* stocks used in this study have been extensively outcrossed to test for effects of genetic background on the expression of the yellow phenotype.

3. RESULTS

(i) The effect of genetic background on *y* phenotypic expression

All possible pairwise combinations of type 1 and 2 *y* mutants were crossed and the progeny examined. The F_1 progenies were mated and the F_2 males examined for

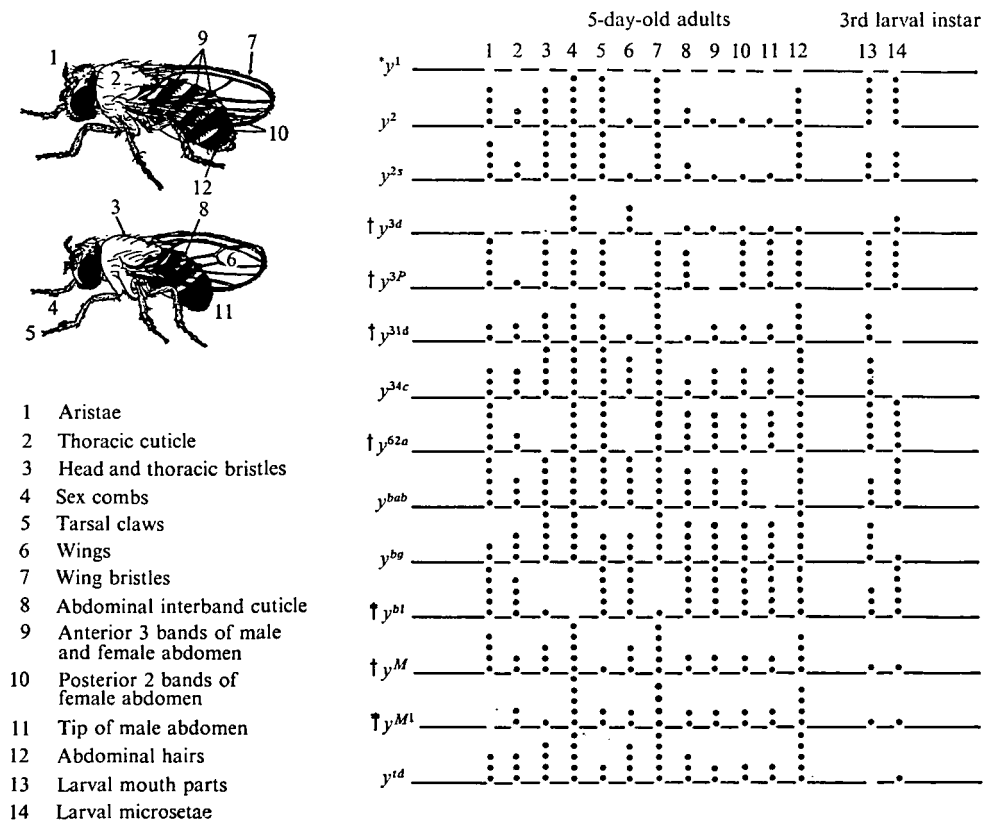


Fig. 1. The patterns of pigmentation of various yellow alleles are illustrated. The height of the bar is directly proportional to the amount of normal pigment found in the cuticular structures. The numbers at the top of the bar graph correspond to the cuticle structures described above. Many of the alleles shown in this figure variegate for some cell types. In these cases the rank shown for a cell type is the predominant colour state observed in that cell type.

* y^{M2} , y^{18CH1} , y^{59c} are all phenotypically like y^1 .

† alleles which variegate for some cell types.

‡ alleles which variegate for some cell types and also show significant variation in bristle pigmentation with outcrossing (y^{bl}) or in wing and bristle pigmentation in combination with *rosy* and *maroon-like* (y^{M1}).

phenotypic modifications. This mixing of the genetic backgrounds of the different *y* alleles did not result in any phenotypic conversion of one allele toward another. The *y^{bl}* progeny of outcrossed *y^{bl}* female parents often showed different degrees of yellow expression in the bristles. These progeny were not phenotypically converted to any other allele however. Independent crosses have shown that *y^{M1}* in combination with *rosy* (*ry-3:52.4*) or *maroon-like* (*ma-l-1:64.8*) has its wings and bristles lightened. The other type-2 *y* alleles were not affected by these mutants.

(ii) *Independent phenotypic expression of type-2 y mutants*

Examination of the type-2 *y* mutants in Fig. 1 reveals that at least 12 characters of the male and female adult cuticle show independence in the expression of the *yellow* phenotype. Independent expression refers to cases where the presence of normal or mutant pigment in one character does not predict the colour state in any other character. For example, in *y^{62a}* the tarsal claws are wild type and the head and thoracic bristles are mutant. In a second allele *y^{td}* just the reverse is found. The tarsal claws are almost completely mutant and the head and thoracic bristles are nearly wild type. With the exception of characters 8 and 10, if all possible pairwise combinations of characters among the *y* mutants in Fig. 1 are examined, examples can be found where the characters examined express opposite colour states. In addition to the 12 adult cell types both the mouth-parts and microsetae of the larvae demonstrate this independence of expression (see Plate 1).

(iii) *Non-random expression of colour states among the 14 characters*

Even a casual examination of Fig. 1 gives the impression of certain correlations in the colour states of several characters. These correlations can be demonstrated by constructing a table where each character is compared with every other character (Table 1). Pairs of characters that are equal or differ by only one unit in their colour ranking are considered very similar. Using this index of similarity, characters 4 (sex combs), 7 (wing bristles) and 12 (abdominal hairs) which comprise group A in the Table show a pronounced correlation of colour states. On the average these characters, when compared in all pairwise combinations (4×7 , 4×12 , 7×12), are in a similar colour state in 11.7 of the 13 type-2 *y* alleles shown in Fig. 1. All possible pairwise combinations of characters 2 (thoracic cuticle), 8 (abdominal interband cuticle), 9 (anterior three bands of male and female abdomen), 10 (posterior two bands of female abdomen) and 11 (tip of male abdomen) which comprise group B show a similar 11.6 colour-state correlation. If characters from group A are compared to characters from group B a definite negative correlation is observed. The average correlation for all A \times B comparisons is 4.0 whereas the mean similarity between two characters, obtained by averaging all the values in Table 1, is 7.3.

(iv) *Modes of expression of the y alleles*

The different characters of the cuticle of a single allele often express complex patterns of colour states. Flies carrying the *y^{3d}* allele have wings which are in an



The wing and wing bristles of the alleles y^2 and y^{bl} express opposite colour states. In y^2 the wing bristles are black (wild type) and the wing is yellow (mutant). In y^{bl} the wing is grey (wild type) and the wing bristles are yellow (mutant).

intermediate colour state and sex combs which are almost wild type. With the exception of the abdominal hairs which variegate for the normal and mutant colour state the remaining characters are nearly or fully mutant. In the same allele, therefore, one character can be fully mutant, a second intermediate, a third nearly or fully wild type and a fourth exhibit variegation.

Table 1. *Genotypic similarity rating for each pair of characters**

Charac- ter	Character†												
	2	3	4	5	6	7	8	9	10	11	12	13	14
1	5	9	6	10	7	7	8	7	8	7	8	8	8
2	—	5	0	7	9	1	11‡	12	11	10	2	9	4
3	—	—	8	8	5	9	7	6	7	6	10	7	5
4	—	—	—	7	4	12§	4	3	4	3	11	6	4
5	—	—	—	—	5	7	9	9	10	9	9	9	8
6	—	—	—	—	—	3	9	10	9	8	5	3	5
7	—	—	—	—	—	—	5	4	5	4	12	6	4
8	—	—	—	—	—	—	—	12	13	12	6	8	9
9	—	—	—	—	—	—	—	—	12	11	7	8	7
10	—	—	—	—	—	—	—	—	—	12	6	9	8
11	—	—	—	—	—	—	—	—	—	—	5	8	9
12	—	—	—	—	—	—	—	—	—	—	—	7	6
13	—	—	—	—	—	—	—	—	—	—	—	—	7

* Two characters with the same or a one rank difference are said to be similar. The body of the table gives the number of genotypes judged similar, on this basis, for each pair of characters.

† The character designations 1-14 are the same as in Fig. 1.

§ Group A = characters 4, 7, 12: average 4 × 7, 4 × 12, 7 × 12, score is 11.7.

‡ Group B = characters 2, 8, 9, 10, 11: average 2 × 8, 2 × 9, etc., score is 11.6.

Average A × B score is 4.0.

Character variegation occurs in alleles which have arisen spontaneously and by crossing over criteria appear to be in structurally unmodified chromosomes. y^{3d} and y^M belong to this category. Character variegation also occurs in y alleles contained within structurally modified X chromosomes. y^{3P} , y^{31d} , y^{62a} and y^{bl} belong to this category. Variegation expressed by the above y alleles is distinct from classical position effect variegation. When the y^+ gene is translocated to the Y chromosome the resulting variegation is suppressed by the addition of extra Y chromosomes and the amount of variegation varies greatly depending on which sex contributes the y^+ duplication to the progeny (Fowle & Nash, 1974). The alleles shown in Fig. 1 are not affected by the presence of extra Y chromosomes or by the sex of the parent which contributes the y allele to the progeny.

(v) *Parallel between pattern of type-2 y mutants and melanin pigmentation*

The pattern of expression of type-2 y mutants is restricted to the boundaries defined by normal melanin pigmentation. For example, if one thoracic bristle is yellow then all such bristles are yellow with the exception of those alleles which

variegate. The pattern of melanin pigmentation largely follows natural morphological boundaries. The darkly pigmented derivative cuticle structures such as bristles, sex combs, claws and so forth are interrupted by the lightly pigmented body cuticle. y^{3P} is a particularly interesting example of the parallel between y phenotypic expression and the pattern of melanin pigmentation. y^{3P} males and females have normal wild-type melanin pigmentation on the last two abdominal bands. The first three bands of both sexes are mutant. In this case expression of the y phenotype parallels the difference in the pattern of tyrosinase activity in the first three and last two bands of the adult male.

4. DISCUSSION

Among the 13 type-2 y mutants, examples can be found where each cuticle character expresses a colour state opposite to any other character. In this sense the y phenotype is expressed independently in each character. It is clear, however, that with respect to colour state certain correlations exist between groups of characters. Characters in groups A and B show a positive within-group correlation and a negative between-group correlation. Since group B characters are all part of the cuticle it is not surprising to find that they are developmentally correlated. The characters of group A, however, differ from one another in morphology, locations and the time of pigmentation, so that in these structures the mechanism of developmental control is not based on any obvious spatial or temporal relationship. We suggest that type-2 mutants may be affecting an underlying control mechanism in the expression of the y phenotype, such that mutations which result in a disfunction of y^+ in one specific set of characters often result, simultaneously, in its normal function in another specific set of characters. Type-2 mutants could be affecting that portion of the y gene which determines its pattern of activity in the cell types which form the cuticle. This suggestion is based partly on the similarity found to exist between the patterns of expression in *scute* (*sc-1.0:0*) and y alleles.

Mutations of the *sc* gene do not alter the placement of bristles on the head and thorax, but only control whether or not a particular set of bristles will be formed (Dubinin, 1933; Stern, 1954, 1968). Like *sc*, y does not alter the placement of pigmentation in the cuticle but only determines whether it will be yellow or black independently in each cell type which forms the cuticle.

Stern considers genes like *sc* to be responding, in the formation of bristles, to a prepattern already established by earlier acting genes. The prepattern consists of unique signals at each potential bristle site (Stern, 1968). The gene normally responds to all these signals but can be modified by mutation to respond to any combination of these signals. One could change the word prepattern to the newer concept of positional information (Wolpert, 1969) and not alter the analogy between the *sc* and y systems.

It seems contradictory that the y gene, which normally functions in all the cells which form the cuticle, should be activated uniquely in each cell type. We feel that this paradoxical behaviour can be explained by considering the probable function

of the *y* gene. It has been demonstrated that *y* is not the structural gene for tyrosinase since *y* flies have tyrosinase activity (Graubard, 1933; Mitchell, Weber & Schaar, 1967). A deficiency of the *y* gene is viable and results in a type-1 *y* phenotype (Muller, 1932). Waddington (1941) has concluded that the pigment in *yellow* flies is a qualitatively different form of the normal black melanin. This conclusion is further supported by the observation that the time course of pigment deposition is identical in *y*¹ and wild-type flies (W. G. Nash, unpublished observations). The product of the *y* gene apparently only ensures that the melanin synthesized is deposited in its black rather than yellow colour state.

If the function of the *y* gene is linked to the production of melanin in its dark colour state, it follows that the activity of the tyrosinase gene and the *y* gene must be co-ordinated in time, space and quantity. This assumption is supported by preliminary findings that the period of sensitivity for three EMS induced temperature-sensitive *y* mutants occur just prior to the appearance of melanin in the cuticle (Nash, 1974). Further support for this idea comes from the observations that the pattern of expression of type-2 mutants exactly parallels the pattern of melanin deposition in the cuticle. For example, in type-2 *y* mutants cases are found where the band is mutant and the interband is normal, but no examples are found where part of the band or interband is mutant while the remainder is normal. We propose that the *y* gene behaves like a pattern forming responder gene because it is using the same signal system used to turn on the tyrosinase gene. This would ensure that the function of the *y* gene would be co-ordinated with the function of the tyrosinase gene which probably is activated differentially throughout the cuticle.

The authors would like to thank Dr Carl Merrill of the N.I.M.H. Bethesda, Maryland, for graciously offering the facilities of his laboratory, Dr Tom Friedhman, Hesus Padilla and Jack Fowle for helping in the preparation of the manuscript, and Penny Ziegler who patiently looked at so many flies in order to isolate new *yellow* mutants. We gratefully acknowledge Dr E. C. R. Reeve's contribution in suggesting the use of Table 1 and pointing out the correlation between group A and B characters. This research was partially supported by an NIH Biomedical Sciences Support Grant.

REFERENCES

- DUBININ, N. P. (1933). Step-allelomorphism in *Drosophila melanogaster*. *Journal of Genetics* **27**, 443-464.
- FOWLE, J. & NASH, W. G. (1974). Manuscript in preparation.
- GRAUBARD, M. A. (1933). Tyrosinase in mutants of *Drosophila melanogaster*. *Journal of Genetics* **27**, 199-218.
- HANKS, G. D. & NEWLIN, L. (1969). New mutants. *Drosophila Information Service* **44**, 47.
- LINDSLEY, D. L. & GRELL, E. H. (1967). Genetic variations of *Drosophila melanogaster*. *Carnegie Institution of Washington Publ. no. 627*.
- MITCHELL, H. K., WEBER, V. M. & SCHAAR, G. (1967). Phenoloxidase characteristics in mutants of *Drosophila melanogaster*. *Genetics* **57**, 357-368.
- MULLER, H. J. (1932). A viable two gene deficiency. *Journal of Heredity* **26**, 469-478.
- NASH, W. G. (1974). Manuscript in preparation.
- STERN, C. (1954). Two or three bristles. *American Scientist* **42**, 213-247.

- STERN, C. (1968). Developmental genetics of patterns. In *Genetic Mosaics and Other Essays*, pp. 130–173. Cambridge, Mass.: Harvard University Press.
- WADDINGTON, C. H. (1941). Body colour genes in *Drosophila*. *Proceedings of the Zoological Society of London A III*, 173–180.
- WOLPERT, L. (1969). Positional information and the spatial pattern of cellular differentiation. *Journal of Theoretical Biology* **25**, 1–47.