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D. P. Furman · S. N. Rodin · T. A. Kozhemiakina

Transposable elements and the penetrance of quantitative characters in *Drosophila melanogaster*

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Abstract We wanted to determine whether there is a correlation between the quantitative character, the penetrance of the loss of humeral bristles in scute lines, and the distribution of transposable genetic elements in their genomes. We derived 18 isogenic lines with penetrance ranging between 2.8% and 92.0% from six mutant lines. The localization of the transposable elements (TEs) P, mdg1, Dm412, copia, gypsy and B104 was determined in all isogenic derivatives by in situ hybridization. The total number of the TE sites over all lines was 180. A comparison of the distribution of the TEs in the isogenic lines revealed the location of sites typical of lines with similar penetrance, no matter which parental line was involved. The results obtained suggest that such typical sites appear to tag the genome regions where the polygenes affecting the character in question are most likely to be found.

Key words *Drosophila melanogaster* · Scute locus · Penetrance · Polygenes · Transposable elements

Introduction

Biometrical genetics assumes the polygenic control of quantitative traits (Mather and Jinks 1982). As a rule, polygenic loci have no striking phenotypic effects or known protein products; therefore traditional direct molecular-genetic methods cannot provide for the quick and easy detection of such loci.

Progress in the study of the transposable elements (TEs) of the genome followed progress in the study of a range of genes displaying major phenotypic effects (qualitative characters). TEs tag the gene into which they have been inserted. Hence they appear to be the best available means for the identification, isolation and further study of genes at the molecular level (Bingham et al. 1981). It is likely that TEs will find a use in the mapping and further molecular and genetic analysis of the loci controlling quantitative characters.

Correlations between the localization of various TEs on *Drosophila* chromosomes and such quantitative traits as viability, fitness, effectiveness of the response to selection, and some others, has already been reported (Biemont et al. 1985; Mackay 1985; Biemont and Terzian 1988; Eanes et al. 1988; Lai and Mackay 1990; Shrimpton et al. 1990; Kaidanov et al. 1991; Mackay et al. 1992; Ratner and Vasilyeva 1992). These correlations imply that TEs can affect a character by interacting with neighbouring polygenes so that the polygenes can be eventually revealed (Mackay 1984, 1985). Such TEs are regarded as tagging polygenes and the problem of finding a "polygene-trait" correlation naturally becomes a problem of finding a "TE localization-trait" correlation.

We wanted to determine whether penetrance related to the scute mutants of *Drosophila melanogaster* correlates in any way with the genomic distribution of TEs. The scute gene (1–0.0) is the major gene concerned with the formation of large bristles (macrochaetes) and is composed of a series of step alleles. Mutants may differ qualitatively, which inidicates that they may have specific subsets of macrochaetes missing, as well as quantitatively – scute is a mutation with incomplete penetrance which implies that any bristle is missing from mutant flies at some fixed probability. Qualitative differences between the scute alleles have been shown to depend on changes in the structural organization of the locus (Campuzano et al. 1985; Ruiz-Gomes and Modolell 1987); the incomplete penetrance phenomenon is not yet explained. Polygenic control of bristle number in *Drosophila* was reported by Fraser et al. (1965), Mackay (1984), Shrimpton et al. (1990) and Thompson et al. (1991). Variation for penetrance related to the scute mutants also appears to depend on polygenes which we hope to reveal with TE insertions.

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D. P. Furman (✉) · S. N. Rodin · T. A. Kozhemiakina
The Institue of Cytology and Genetics, Novosibirsk-90, Russia

Two points regarding polygenes should not be neglected. Firstly, polygenes produce relatively small phenotypic effects; secondly, the effects can be counterbalanced. Therefore, it will be easier to define ensembles of polygenes with additive or synergistic effects than to define separate polygenes. To increase the probability of finding such ensembles it is necessary to minimize the background within-stock variation, to cover more TEs by the study and then to search and account for the properties typical of groups of TE localization sites.

We derived a total of 18 isogenic stocks from six scute stocks. In the original lines, the penetrance related to the loss of humerals was different. The isogenization allowed us to obtain genetically uniform stocks and to minimize polymorphism with respect to the distribution of TEs within any isogenic stock. Then we analyzed and compared the collective distribution of *P*, mdg1, Dm412, copia, gypsy and B104 transposable elements. The result was that we were able to identify clusters of TE localization sites typical of the isogenic stocks with similar penetrance, regardless of origin. We suggest that such typical sites should be indicative of the genomic regions where polygenic loci affect bristle number in the scute mutants.

Materials and methods

Drosophila stocks and crosses

We used six scute mutant stocks, namely sc^{34P}, sc^{55P}, sc^{59P}, sc^{146P}, sc^{147P} and sc^{133P}. All these mutants had been induced several years ago in the P–M system of hybrid dysgenesis (Furman 1993, 1994; Furman et al. 1993). Each stock was derived from one mutant male. The stocks were maintained by mass mating.

From one to six isogenic stocks (total of 18) were derived from these parental mutant stocks. Isogenization was performed by a standard genetic procedure with the use of the Basc; Cy/Pm; D/Sb balancer stock (Lindsley and Grell 1968). Each isogenic line comes from one random female of the original mutant stock. The stocks have been maintained at +25 °C.

Penetrance (the percentage probability of a bristle missing) was assessed on a sample of 100 males of each genotype.

In situ hybridization

In situ hybridization was routine (Gall and Pardue 1971) with ^{3}H-labelled probes containing the DNA of P, mdg1, Dm412, copia, gypsy and B104.

At least four preparations, with at least ten nuclei in each, were analyzed within each stock. Hybridization sites were identified after the Bridges maps (Lefevre 1976).

Since 17C is a cloning site of the P element, the label in this site following hybridization of the P element was taken into account only as an internal control of the effectiveness of hybridization.

In situ hybridization with P, mdg1, Dm412, copia, gypsy and B104 showed that there was no within-line polymorphism for their sites of localization. The individuals, assayed within each isogenic stock had identical TE distributions, at least in relation to specific salivary chromosome bands.

Calculation of similarity indices and construction of similarity matrices and a similarity tree for the isogenic stocks

A site was thought occupied, if it was labelled following hybridization with at least one of the TEs in question. The resulting distribution of labelled sites was termed the "collective distribution".

The similarity of stocks as regards TE localization was assessed by calculation of the similarity index $S = 2N/N_1 + N_2$, where N_1 and N_2 are the numbers of localization sites in the pairs of stocks under comparison, and N is the number of the localization sites that are common to these stocks (Hunt et al. 1984). The similarity index may take values from 0 (no common localization sites) to 1 (identical distribution of sites).

The calculated similarity indices were arranged into a similarity matrix as follows: we set out the stocks in order of increasing penetrance and then put the similarity index for each pair of stocks into the corresponding cell.

A similarity tree was constructed using the UPGMA technique (Zharkikh et al. 1991) by calculating the distances between the isogenic lines. The measure employed was the number of different hybridization sites for each pair of stocks. In contrast to phylogenetic trees, the figures on the edges are irrelevant.

Only the distribution of informative sites of TE localizations were used in calculating the similarity indices and in constructing the similarity tree: the sites unique for a stock, or those occurring in all stocks, fell short of consideration.

Results

Figures for penetrance in the parental and isogenic stocks are shown in Table 1. The frequencies of humeral bristles missing in the isogenic stocks range from 2.8% to 92.0%, which is more than in the parental stocks (from 19.8% to 89.5%). The character values form an almost continuous row. Mutants with strongly different penetrance yeilded isogenic stockes with similar penetrance. Otherwise, the original mutant lines with similar penetrance gave isogenic lines with very different penetrance (see, for example the sc^{55P-9}–sc^{133P-3} and sc^{55P-9}–sc^{59P-10} pairs). Is there an association between penetrance in the isogenic stocks and the distribution of TEs in their genomes? Were this to be the case then the TE distributions would be alike in stocks with similar penetrance, irrespective of their origin.

This supposition was verified by using data on the collective distribution of TEs of all 18 isogenic stocks. The total number of localization sites was 180, of which 48 were not informative. Based on the distribution of the 132 informative cases we created a similarity matrix for the isogenic stocks (Table 2, lower triangle).

As can be seen, it is the stocks with similar penetrance that have a high similarity index. The more the stocks differ in penetrance, the more they differ in TE distribution. The strongest discrepancies are principally related either to pairs, one of whose components is a derivative of sc^{34P} (for instance, stocks 7–8), or to pairs of common origin (e.g. stocks 6–16, 6–17 etc.). The discrepancies relating to sc^{34P} will be discussed below. Common origin is crucial, apparently, because such stocks share a lot of sites, "parental" sites, that make the main contribution to the value of the similarity index. This is why some overestimation of the index takes place for such lines.

The collective properties of the stocks are better illustrated after qualitative analysis of the data from Table 2. In order to present the matrix of similarity indices in graphic form we set the threshold *s* for the similarity indices at 0.54, which is an averaged value of

Table 1 Penetrance in males from parental and isogenic stocks

No.		Stocks	Humeral bristles			Post-vertical bristles		
			h^1	h^2	Mean	pv^1	pv^2	Mean
	Parental	sc^{34P}	22.5	17.0	19.8	54.0	64.0	59.0
		sc^{55P}	24.0	34.0	29.0	–	–	–
		sc^{59P}	28.5	40.5	34.5	–	–	–
		sc^{146P}	52.5	50.5	51.5	–	–	–
		sc^{147P}	80.0	77.5	78.8	–	–	–
		sc^{133P}	90.0	89.0	89.5	–	–	–
1	Isogenic	sc^{59P-5}	2.5	3.0	2.8	–	–	–
2		sc^{34P-16}	10.5	11.5	11.0	74.0	77.0	75.5
3		sc^{59P-8}	12.5	18.0	15.2	–	–	–
4		sc^{59P-4}	21.0	23.0	22.0	–	–	–
5		sc^{55P-9}	22.0	27.5	24.8	–	–	–
6		sc^{133P-3}	31.0	28.5	29.8	–	–	–
7		sc^{147P-7}	41.5	39.5	40.0	–	–	–
8		sc^{34P-22}	43.0	43.0	43.0	–	–	–
9		sc^{34P-10}	47.5	47.0	47.2	58.0	43.0	50.5
10		sc^{133P-1}	59.0	59.0	59.0	–	–	–
11		$sc^{133P-10}$	61.0	64.0	62.5	–	–	–
12		sc^{146P-2}	63.0	63.5	63.2	–	–	–
13		sc^{146P-8}	72.0	69.5	70.8	–	–	–
14		sc^{59P-10}	72.0	72.0	72.0	–	–	–
15		$sc^{133P-11}$	72.0	73.5	72.8	–	–	–
16		$sc^{133P-16}$	72.5	74.5	73.5	–	–	–
17		$sc^{133P-18}$	79.5	79.5	79.5	–	–	–
18		$sc^{147P-14}$	92.0	92.0	92.0	–	–	–

Table 2 The matrix of similarity of isogenic stocks based on the distribution of TEs from six families (below diagonal) and the matrix of binary values of similarity indices (above diagonal). Designations as in Table 1. Solids bars for $s > 0.54$; blank bars for $s < 0.54$

Stocks	Stocks																	
	9	2	8	1	3	4	5	6	7	10	11	12	13	14	15	16	17	18
	9	■	■	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
		2	■	□	□	□	54	□	□	□	□	□	□	□	□	□	□	□
			8	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
1	1			1	■	■	■	54	■	□	□	□	□	□	□	□	□	□
2	51	2			3	■	■	□	□	□	□	□	□	□	□	□	□	□
3	73	53	3			4	■	■	□	□	■	□	□	■	□	□	□	□
4	71	46	87	4			5	■	□	54	■	□	□	■	54	■	54	□
5	65	54	58	66	5			6	■	■	■	□	■	■	■	■	■	■
6	54	53	52	55	66	6		s	7	■	■	■	□	□	□	□	□	■
7	56	34	48	50	49	56	7		t	10	■	■	■	54	■	■	■	■
8	52	74	47	48	45	51	44	8		o	11	■	■	■	■	■	■	■
9	48	67	38	38	40	46	42	81	9		c	12	■	■	□	■	□	■
10	48	44	46	51	54	57	57	52	43	10		k	13	■	■	■	■	■
11	47	43	52	56	56	68	57	47	50	71	11		s	14	■	■	■	■
12	50	46	48	46	45	53	57	48	53	60	62	12			15	■	■	■
13	44	48	42	49	48	58	51	49	49	56	60	65	13			16	■	■
14	49	44	50	59	56	57	51	45	48	54	66	59	59	14			17	■
15	44	40	46	49	54	62	48	44	43	57	71	51	66	63	15			18
16	47	47	45	50	55	64	52	51	46	59	68	55	74	68	76	16		
17	47	37	45	44	54	68	50	45	39	60	70	50	60	64	80	71	17	
18	39	40	42	44	41	55	64	46	45	62	75	56	64	67	69	64	74	18

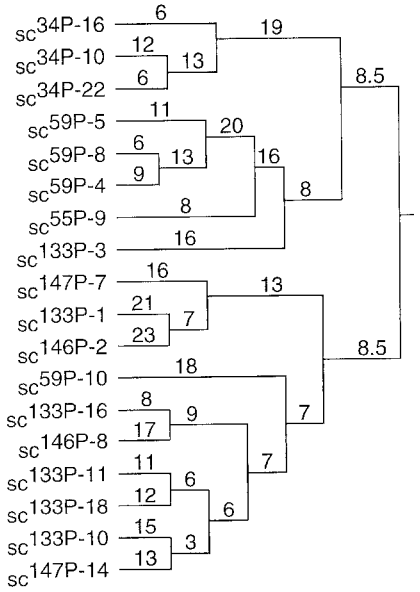
the similarity index over the matrix, and replaced their particular values by binary estimates: 1 (all $s > 0.54$) or 0 (all $s < 0.54$). Then we rearranged the columns so as to aggregate zeros and unities into groups (upper triangle of Table 2). The matrix clearly comprises three clusters of stocks. The first consists of the sc^{34P} derivatives, the second of $sc^{59P-5} - sc^{133P-3}$ stocks (penetrance up to 40%), the third, the most strongly "populated", comprises $sc^{147P-7} - sc^{147P-14}$ stocks (penetrance 40% or more). The third and second are partly overlapping, i.e. it is possible to speak of a subcluster of stocks with intermediate penetrance.

The stocks of the second and third clusters have similar penetrance within their respective clusters. The

sc^{34P} derivatives form a cluster of their own, which implies that these stocks are somewhat different from the others. What sort of difference is this? The sc^{34P-16} and sc^{34P-10} stocks differ from the others since both h^{1-2} and pv^{1-2} are missing. The sc^{34P-22} stock is a special case. The post-verticals are now all present, the percentage loss of humeral is 43% (Table 1). This explains why sc^{34P-22} should have occurred in the vicinity of sc^{147P-7} and why irremovable discontinuities would have arisen in the third cluster. Therefore, there is a difference in principle between sc^{34P-22} stocks and its immediate neighbourhood. The case of the group of sc^{34P} stocks is distinctive, apparently, because the lesion conferred on the major gene by the mutation is different in character to that in the other alleles, and because there is an additional set of polygenes related to the post-verticals in the stocks of this group. Thus, sc^{34P-22} served an internal control of the effectiveness of the method of classification of the mutants and favored the hypothesis of an association between penetrance and TE distribution.

We checked the correlation by constructing a similarity tree for the isogenic lines (Fig. 1). The structure of the tree involves as many clusters of lines as does the similarity matrix. The sc^{34P} derivatives are in a cluster of their own. One cluster is formed by $sc^{59P-5} - sc^{133P-3}$ (penetrance not exceeding 40%) and one is formed by $sc^{147P-7} - sc^{147P-14}$ (penetrance 40% and over). The order of lines on the tree vertices is the same as that given in Table 1 with an accuracy of 3–4 permutations; these are all within-cluster permutations. Since the procedure reveals an order in relation to clusters, such a correspondence is quite satisfactory.

Fig. 1 The tree of similarity for isogenic lines on the basis of the collective distribution of the mdg1, Dm412, copia, B104, gypsy, P elements. Constructed by the UPGMA method (Zharkikh et al. 1991). Designations as in Table 1



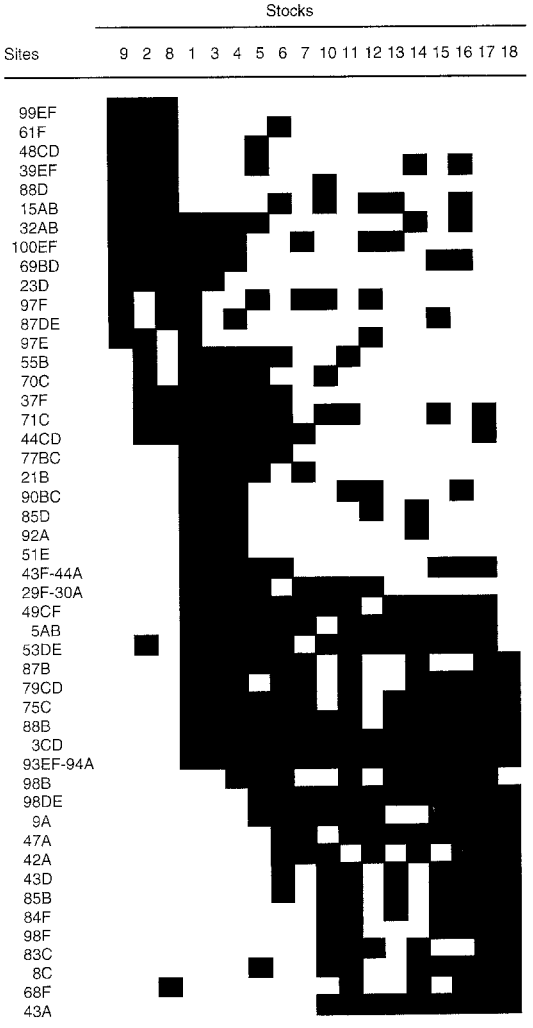
It was also of importance to determine whether there are clusters of hybridization sites related to the clusters of isogenic stocks. We left the stocks in the order of increasing penetrance untouched, but rearranged the hybridization sites so that these were maintained as tightly as possible each mutant cluster. A representation of this construction is given in Fig. 2.

The one-hundred-and-thirty-two hybridization sites are arranged as follows. Twenty of them form three clusters that may be associated with the blocks of isogenic stocks from the similarity matrix and the similarity tree. The first consists of sites characteristic of the isogenic stocks of sc^{34P} (sites 99EF–15AB); the second (sites 77BC–51E) belongs to stocks with a penetrance less than 40%; the third (sites 84F–43A) is mostly related to stocks with a penetrance of more than 40%.

Twenty eight sites are present at two neighbouring clusters of stocks, namely 32AB–44CD are at the first and second, with 43F–44A–42A at the second and third.

As to the other 84, nothing special can be said. They display no specific collective properties, nor are these so

Fig. 2 Clusters of TE hybridization sites, corresponding to the clusters of isogenic lines revealed in the similarity matrix and tree (explained in the text). Designations as in Table 1



significant as to make up clusters. Therefore, they are of little consequence to the structure of the table. Analysis of a larger number of isogenic stocks will perhaps allow new sites to be revealed and/or the known sites to be better classified.

The results reported here suggest that lines with similar penetrance are similar in the genomic distribution of TEs and provide some evidence that there are at least three groups of polygenes that are related to penetrance.

Discussion

Because only the cooperative effort of a group of polygenes may produce a perceptible effect on a quantitative character, the investigator faces a dilemma. On the one hand, it is necessary to cover as many sites of localization as possible (by involving more TEs in the analysis) in order to raise the probability of identifying all the relevant loci. On the other hand, it is a matter of special skill to detect such relevant sites in the large pool of inserts persisting in the genome. There must be comparatively few such sites and these can be easily overlooked in the multitude of irrelevant sites (Shrimpton et al. 1990). This is what we tried to take into account while looking for a "penetrance of scute – TE distribution" correlation.

To begin with, the parental genomes have not been modified in any way. The search for correlation was based only on the within-line genotypic variation in penetrance and the TE distribution which had been revealed with the help of the isogenic derivatives. Consequently, minimization of polymorphism for the localization sites within the isogenic stocks was achieved, which facilitated the search.

With the six TEs we covered 180 localization sites. A comparison of the patterns of the collective distribution of TEs in the isogenic stocks helped both to reveal the relevant sites of TE localization and to identify clusters (ensembles) of sites typical of the groups of isogenic stocks of different origin yet similar in penetrance. The number of the typical sites appears to be somewhat overestimated and can be more precisely specified by surveying a larger number of isogenic stocks and TEs. We assume that these are the genomic regions that contain polygenes related to penetrance. These were few, which is consistent with data on some other quantitative characters; for example, the number of sternopleurals (Thompson et al. 1991 and references therein).

Although these clusters provide a rather conventional classification, when combined with other data they may be useful for further attempts at detecting polygenes and individualizing their effects. Consider the group of isogenic derivaties of sc^{34P}: in one of three stocks, namely sc^{34P-22}, only humerals were missing, whereas the post-verticals were all represented. At the same time, it is this stock that has a label at sites 26F and 21D, which associates it with most of the rest of the stocks with missing humerals. On the other hand, it is only in sc^{34P-22} that 97E stays unlabelled, whereas it is otherwise in the two other related stocks. It is likely that polygenes modifying penetrance for post-verticals are located in these regions and produce antagonistic effects.

It has been shown that the number of certain bristles, or of their groups, is modified by different series of polygenes (Fraser et al. 1965; Mackay 1985; Thompson et al. 1991). What provides indirect support for this is the special situation of the group of sc^{34P} derivatives which form a separate cluster in the structure of the similarity matrix and tree; so too does the fact that there are extremely few hybridization sites which belong to both the first and third clusters.

The available data on the molecular-genetic organization of the AS–C region permit some assumptions to be made on how polygenes affect penetrance. It has been shown that the formation of bristles is governed by the expression of a transcript of the achaete-scute complex, and the pattern of bristles on the fly body seems to involve a set of cis-operating regulators, concentrated in untranscribable regions of the gene (Leynes et al. 1989). We may assume that gene expression and hence penetrance, is modified by the interaction of the regulatory regions of the complex with the polygenes located in various parts of the genome; in the present case on chromosomes 2 and 3.

Thus, a comparative analysis of the distribution of TEs in the isogenic lines may be useful at the initial stage of studying the polygenic system, when nothing is known about the number of localization of its polygenes, since it helps to reveal the genomic regions where polygenes are most likely to occur. The corresponding TEs can be helpful for a structural and functional analysis of the genomic regions and in the understanding of the regulatory mechanisms of the interaction between the polygenes and the major genes of character forming systems.

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