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The Contributions of the Second and Third Chromosomes to Selection Response in *Drosophila melanogaster*

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Summary. A new approach was made to comparing the contributions to response of chromosomes 2 and 3 of *Drosophila* in lines selected for high and low sternopleural bristle number.

Separate response curves for chromosomes 2 and 3 were obtained from changes in the effect of standard second and third chromosomes marked *Cy* and *Mé* which were kept segregating with their wild-type homologues during selection.

Dominance interaction between marker and wild chromosomes caused some bias in estimating responses in each direction, but the amount by which the responses diverged in the two directions of selection was relatively free from bias. On a log. scale divergences of chromosomes 2 and 3 were in the ratio 1:1.7 and their heritabilities realised early in selection were 0.14 and 0.26 respectively.

There was interaction between the *Cy* and the *Mé* chromosomes which was not altered by the selection. Almost all the responses in the lines could be accounted for by addition of the responses in single chromosomes 2 and 3, chromosomes 1 and 4 making a negligible contribution.

Sampling, as a cause of variation between selection lines, was reflected in the variation between them in response in chromosomes 2 and 3.

I. Introduction

Sternopleural bristle number of *Drosophila melanogaster* has been widely used as a model to investigate the inheritance of quantitative variation. It is therefore of considerable interest to locate the source of genetic variation in this character on each of the four sets of chromosomes. In the past this problem has been approached for sternopleurals and other characters of *Drosophila* by substituting the chromosomes whole or in part into standard backgrounds for measurement. In most cases the chromosomes were sampled from lines either before or after a long period of selection (Louw 1966, Mather and Harrison 1949, Osman and Robertson 1968 and Robertson and Reeve 1953). However these measurements, because they were taken at particular points during the selection process and of chromosomes from a chosen sample of lines, give little or no information on the nature of the approach to the limit of the separate responses in each of the four sets of chromosomes. This information can throw light on differences between the sets in the numbers, effects, degrees of additivity and initial frequencies of genes affecting the character.

In this study continuous estimates were made of the contributions of the second and third chromosomes to response in lines drawn from the Kaduna population and subjected to a long period of selection for high and low sternopleural bristle number. The estimates were made from changes in the effects of the marked second chromosome *Curly* and the third chromosome *Moiré* which were maintained segregating with their wild-type homologues during selection.

II. Materials and Methods

The selection lines

Observations were made on ten selection lines referred to as the "normal" set of lines in a study of the effect of suppressing crossing-over on selection for sternopleural bristle number by McPhee and Robertson (1970). Five lines were selected for high and five for low bristle number.

The lines were established by four generations of backcrossing males bearing the second chromosome dominant marker *Cy* and the third chromosome dominant marker *Mé* (Bridges and Brehme 1944), both originating in a single male, to successively larger samples of wild-type females drawn from the Kaduna population. This population described by Clayton et al. (1957a), has been maintained at approximately 5000 flies for some 20 years. Each backcross produced four classes of offspring marked *Cy*, *Mé*, *CyMé* and Wild in approximately equal numbers.

Selection lines were reproduced each generation by mating *CyMé* males with Wild females, both sexes selected with an intensity of 10 in 25. Of the segregant classes of offspring from which parents were not selected, 10 of each sex were scored. Since the marker chromosomes were carried in males only, they were assumed to have remained unchanged apart from mutation throughout the selection period.

Selection was discontinued after 17 generations in both directions.

Estimating chromosome response

In each generation the effect of the wild-type second and third chromosomes segregating in the lines were calculated. They were estimated in two ways from differences in bristle score between the four segregants emerging from each generation. For the second chromosome the two estimates were (i) Wild — *Cy* and (ii) *Mé* — *CyMé* and for the third they were (i) Wild — *Mé* and (ii) *Cy*-*CyMé*.

Four response curves were obtained for each line, one for each of the segregants *Cy*, *Mé*, *CyMé* and Wild. As-

suming additivity chromosome effects, the advances or total changes under selection in the four segregants should be accounted for by the following equations:

$$\begin{aligned} D\ (Cy) &= D_2 + 2\,D_3 + D_{1,4} \\ D\ (M\acute{e}) &= 2D_2 + D_3 + D_{1,4} \\ D\ (CyM\acute{e}) &= D_2 + D_3 + D_{1,4} \\ D\ (\text{Wild}) &= 2D_2 + 2D_3 + D_{1,4} \end{aligned}$$

where D(*Cy*), D (*Mé*), *D* (*CyMé*) and D (Wild) are the advances between the beginning and end of selection in each of the four segregants. The average advances in single chromosomes 2 and 3 are D_2 and D_3 and $D_{1,4}$ is the advance due to chromosomes 1 and 4 and is common to all segregants.

The separate contributions of chromosomes 2 and 3 could then be calculated from paired differences between the advances of the four segregants. Thus D_2 was estimated from (i) D (Wild) — D (*Cy*) and (ii) D (*Mé*) — D (*CyMé*) and D_3 was estimated from (i) D (Wild) — D (*Mé*) and (ii) D (*Cy*) — D (*CyMé*). The consequences of dominance interaction between the markers and their wild homologues for the above method of estimating D_2 and D_3 are discussed in the Appendix.

The responses in chromosomes 1 and 4 were not measured throughout selection. However, at the end of the selection period, fifteen females were sampled from each of the ten selection lines and their chromosomes 1 and 4 substituted, using the dominant markers *Cy*, *Mé* and *ciw*, into a standard inbred stock for measurement. This stock, called "D", was described by Osman and Robertson (1968) and has a mean sternopleural bristle score of 8 which has resisted selection in both directions.

Samples of chromosomes 4 were measured singly in males possessing an otherwise "D" genotype and these in turn provided a background in which the substituted chromosomes 1 were measured.

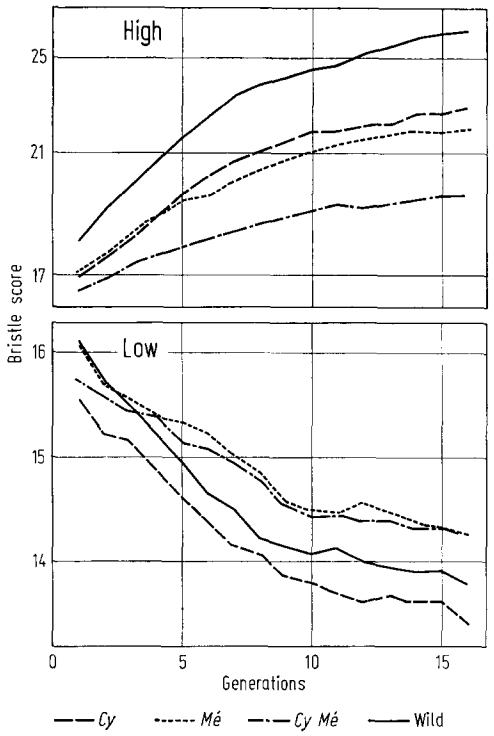

Fig. 1. Selection response measured in the four segregant classes of offspring emerging each generation. Scores of the five lines selected in each generation are pooled and are plotted on a log. scale

Scale of measurement

Since the contributions of chromosomes 2 and 3 to selection response were estimated from changes in the effects of substituting them for *Cy* and *Mé* chromosomes, a scale of measurement had to be found on which the effects of these substitutions were independent of their positions on the scale.

From studies in substituting chromosomes into different backgrounds by Louw (1966) a suitable scale for sternopleural bristle number in this population was found to be log. (S-4) where S is total bristle score. The justification for using this scale has been discussed by McPhee and Robertson (1970). In the results which follow, bristle counts were transformed to units of this scale before analysis.

III. Results

Total selection response

Changes in the mean bristle scores of the four segregant classes of offspring during 17 generations of selection are plotted on the log. scale in Figure 1. The points are three-generation moving averages of both sexes of the five lines which were selected in each direction.

At the end of the selection period responses had become considerably slower although limits may not have been reached. An increasing divergence, most marked in the high lines, occurred between the means of the four segregants as selection proceeded. By subtracting scores at the beginning from those in the three terminal generations of selection, advances were obtained for each of the four segregants. The analysis of variance of these is given in Table 1.

Table 1. *Analysis of variance of the final three generations of selection. The basic observations in the analysis were the deviations of the mean scores on the log scale of the four genotypes (Wild, Cy, Mé and CyMé) from the mean of that genotype at the start of selection*

Source of variation	Degrees of freedom	Mean square × 10^4 High	Low
Chromosome (2)	1	382.23	25.57
Chromosome (3)	1	874.63	153.12
Selection lines (L)	4	186.10	4.53
Terminal generations	2	3.98	4.54
(2) × (3)	1	0.169	2.02
(2) × L	4	22.28	7.46
(3) × L	4	28.29	3.54
(2) × (3) × L	4	8.29	5.57
Residual*	38	1.11	1.04

* Comprising interactions between terminal generations and the other sources of variation.

The most important causes of variation in selection advance between the four segregants were the main effects of the second and third chromosomes. In both directions the third accounted for a much higher proportion of the total variation than did the second. The degree to which the segregant responses diverged, varied between the lines, particularly in the high lines.

Selection advances in chromosomes 2 and 3

In the manner described, two estimates were made each generation of the effects of the marker chromosome *Cy* and *Mé*, one estimate in the presence and the other in the absence of the non-homologous marker. These were averaged over lines selected in the same direction because of the absence of interaction between chromosomes in Table 1 and three generation moving averages are plotted as responses in chromosome 2 and 3 on the log. scale $\times 10^2$ in Figure 2.

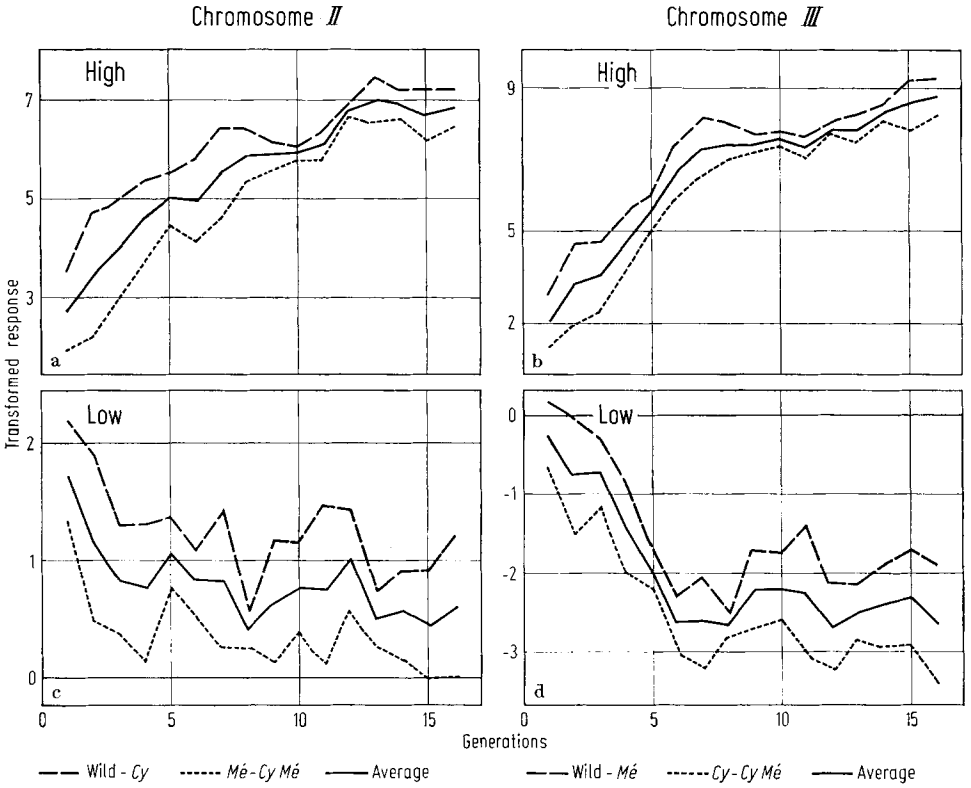

Fig. 2. Selection response in chromosomes 2 and 3 as measured by changes in the effects of the *Cy* and *Mé* chromosomes. The two estimates of response in each chromosome set are plotted separately and averaged after transforming to the scale log. (score − 4) $\times 10^2$

The striking feature of the responses is the consistent difference between the two estimates of each marker effect throughout the whole of the selection period. In all cases except the *Mé* chromosomes in the low lines, the *Cy* and *Mé* chromosomes reduced bristle score. The extent of this reduction was greater in an otherwise wild-type background than in the presence of the non-homologous marker chromosome (Figure 2a, b and c). For example, on the log. scale $\times 10^2$ the two estimates of the second chromosome effect differed at the end of selection by 2.4 units in the high lines and 3.6 units in the low lines. On the other hand, in the low lines the *Mé* chromosome increased score more in the presence of the *Cy* chromosome than in its absence (Figure 2d).

Selection advances D_2 and D_3 were calculated by averaging for each, the two available estimates of changes during the 17 generations of selection. These are given for the high and low lines separately, and summed as a divergence in both directions of selection in Table 2.

Selection advances on the third chromosome exceeded those of the second chromosome by an amount which did not differ significantly between the high and the low lines. The divergence between the high and the low advances in chromosomes 3 was 1.7 times that in chromosomes 2.

There was marked asymmetry of advance in the two directions of selection, of the same order in both sets of chromosomes. The contribution of these two chromosomes sets to advances in the high lines was 2.8 times that in the low. This compares with 1.9 the degree of asymmetry of advance in the means of the Wild segregants.

Response curves are given in Figure 3 for chromosomes 2 and 3 of the high lines. Because of their confused nature, similar responses were not drawn for the low lines.

Wide variation was evident between the lines, both in the initial effects of the two chromosome sets and in the nature of their subsequent responses to selection. Most of the response curves followed the classical asymptotic pattern, some approaching a limit faster than others e.g. the half-life of the selection

Table 2. *High and low line selection advances and divergences of chromosomes 2 and 3 (D_2 and D_3). Advances were averaged over lines and are given in units of the log. scale $\times 10^2$. Standard errors were computed from between line variances*

	D_2	D_3
High	5.05 ± 1.93	7.64 ± 2.17
Low	−1.31 ± 1.09	−3.19 ± 0.75
Divergence	6.36 ± 2.22	10.83 ± 2.29

process for chromosome 2 of line 5 was 6 generations and for the same chromosome in line 4 it was only 3 generations. An exception to the normal pattern of response was chromosome 2 of line 1 whose initial response was delayed some 5 generations.

Early responses in chromosomes 2 and 3

Responses of both chromosomes early in selection were measured by the linear regressions of their effects on cumulative selection differentials applied during the first seven generations of selection. These are referred to as realised heritabilities $h^2(2)$ and $h^2(3)$ and are compared in Table 3 with h^2 (Wild), the heritabilities realised in the means of the Wild segregants over the same period. To minimise bias in this comparison, the effects were estimated only from *Mé-CyMé* and *Cy-CyMé* respectively since these did not involve the scores of the Wild segregants. Only the heritabilities realised in the Wild means conform

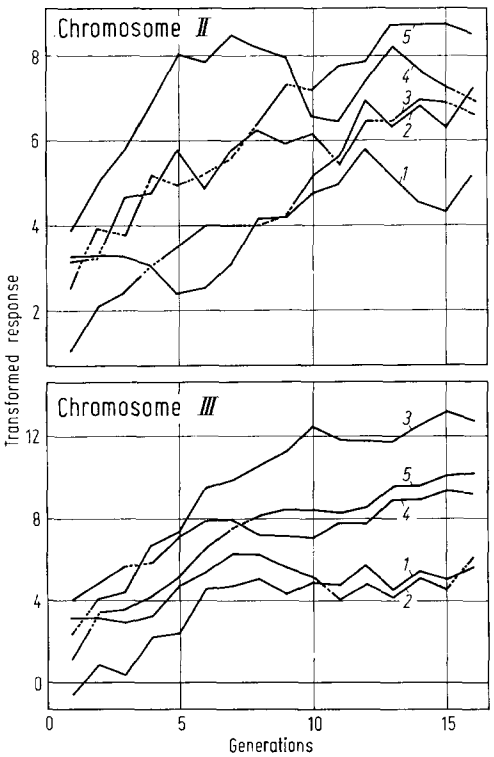

Fig. 3. Contributions to total selection response of chromosomes 2 and 3 in each of the high lines. Responses have been transformed to the log. scale as in Fig. 2

Table 3. *Realised heritabilities in the first seven generations of selection. $h^2(2)$ refers to the differential change in score of Mé and CyMé individuals, $h^2(3)$ to that of Cy and CyMé and h^2 (Wild) to the observed change in Wild individuals. Standard errors were calculated from between line variances*

	$h^2(2)$	h^2 (3)	h^2(Wild) Expected*	Observed
High	0.10 ± 0.04	0.20 ± 0.02	0.59 ± 0.07	0.53 ± 0.07
Low	0.04 ± 0.03	0.07 ± 0.02	0.23 ± 0.07	0.28 ± 0.02
Mean	0.07 ± 0.02	0.13 ± 0.01	0.41 ± 0.05	0.41 ± 0.04

* 2 (h^2 (2) + h^2 (3))

strictly with Falconer's (1953) definition of this parameter, those of chromosomes 2 and 3 relating to part of the total genetic variance only.

An expected realised heritability for the Wild means is given for comparison with the observed in Table 3. This was calculated from 2 ($h^2 2 + h^2 3$) and assumes that the whole of the response in the means of Wild segregants could be accounted for by additive gene effects on chromosomes 2 and 3.

Directional asymmetry, already observed in total selection advances, was again evident in these early responses in chromosomes 2 and 3. The degree of asymmetry was the same for both chromosome sets separately and for their combined estimate of the expected response in the Wild segregants. This was higher than the degree of asymmetry actually observed in the Wild segregants, the ratio of high to low expected being 2.5 compared with a ratio of 1.9 observed. It would seem that the effects of the marker chromosomes slightly over-estimated the high response in chromosomes 2 and 3 and hence the expected Wild response, and under-estimated the low. However, these biases tended to cancel each other since by averaging the expected h^2 (Wild) in the two directions, a value of 0.41 was obtained, the same as the average of the observed heritabilities of the Wild segregants. This also suggests that between them, additive effects on chromosomes 2 and 3 accounted for almost all the early response in the Wild means. Heritability estimates of 0.14 and 0.26 for the diploid second and third chromosome sets were obtained by doubling the values for single chromosomes in Table 3.

Selection advances in chromosomes 1 and 4

The mean bristle scores of chromosomes 1 and 4, sampled from the lines at the end of selection and substituted into a "D" background are given in Table 4.

Chromosome 4 apparently contributed nothing to selection response in either direction of selection. Chromosomes 1 on the other hand, contributed a very small amount to the divergence of the high and low lines. The difference between the substitution effect of chromosome 1 from the high and the low lines at the end of the selection period was only 0.3 ± 0.11 bristles, an almost negligible amount. This supports

Table 4. *Divergence between high and low lines in bristle effects of chromosomes 1 and 4 as measured in the isogenic "D" background at the end of the selection period*

Chromosomes	High	Low	Divergence
1	11.90	11.60	0.30 ± 0.11
4	11.01	10.85	0.16 ± 0.11

the observation from realised heritabilities that selection advance in both directions was almost entirely due to chromosomes 2 and 3.

IV. Discussion

From a comparison between the responses observed in the selection lines and those which were expected from the estimated contributions of chromosomes 2 and 3, it appears that changes in the effects of the separate chromosomes over-estimated the true responses in the high lines and under-estimated them by about the same amount in the low lines. Such bias could occur if dominance interaction existed between the marker chromosomes and their wild homologues.

Suppose that at a locus on the marker there is a low bristle score allele which is recessive to an allele for high score, then, as shown in the appendix, the change in effect of the wild chromosome under selection would overestimate response in wild segregants in the high lines and underestimate it in low lines. Thus, if the alleles carried by the marker chromosomes are on average recessive and bristle reducing, the observed pattern of estimates would be expected. The best estimate would be obtained from the divergence where some of the bias would be removed. A situation such as this, applying to bristle loci overall on chromosomes 2 and 3, could explain the directional bias in estimates of response in these two chromosome sets. It follows that the best estimate of response would be the divergence of the lines obtained by summing the responses in both directions thereby cancelling some of the bias.

In addition to this dominance interaction between marker chromosome and wild homologue, interaction between the *Cy* and the *Mé* chromosomes was clearly demonstrated. Throughout selection there was a consistent difference between the two estimates of each marker effect, the one made in the presence and the other in the absence of the non-homologous marker chromosome. This non-additive interaction between the marker chromosomes may relate to their origins which were foreign to each other and to the Kaduna population.

As a result of the constant difference between estimates of the marker chromosome effects, there was an insignificant contribution from interaction between chromosomes to the variation in selection advances of the four segregants. Further evidence of additivity both within and between the wild chromosome sets was provided in the heritabilities realised early in selection. By simple addition of the heritabilities estimated for single chromosomes 2 and 3, a value of 0.41 was obtained for the heritability expected in the Wild means. This equalled the value observed and is in good agreement with estimates reported elsewhere for the Kaduna population (Clayton et al. 1957b).

It is not possible from the data to determine precisely the cause of the difference in response between chromosomes 2 and 3. However, drawing on a model by Hill and Robertson (1966) of two linked loci each with two alleles, some of the factors shown by them to affect selection limits can be eliminated as causes of difference in response between the chromosome sets. These are the effective population size, the standardised selection differential and the phenotypic standard deviation. These three parameters are expected to have been the same for both chromosomes 2 and 3 since both were under selection in the same lines. In addition, a similarity between the chromosome sets in the degree of asymmetry of response suggests a similarity between them in the initial frequencies of their bristle alleles, being lower for high bristle alleles than for low. This leaves as possible causes of difference between chromosomes 2 and 3, the number of loci, the distance between them and the effects of genes at these loci on bristle score.

All four chromosomes have been reported by Osman and Robertson (1968) to contribute to the genetic variation in sternopleural bristle score in the Kaduna population. If the assumption is made that bristle loci are distributed uniformly over the total genome, then the expected number of loci and the distance between them should be similar for chromosomes 2 and 3 since their map lengths are about equal. This being so, all but the distribution of the locus effects between and within the two chromosome sets is eliminated.

A situation could be envisaged where the magnitude of gene effects at the loci are of the same order for both chromosome sets but selection response in chromosome 2 suffered through the important loci being more tightly linked than on chromosome 3. However, in terms of the model of Hill and Robertson, although the observed difference between chromosome sets in selection limits would have been expected under these conditions, the two-fold difference in early rates of response would not. Therefore, under the assumption of equal numbers of loci on chromosomes 2 and 3, it would follow that the difference in response of the two chromosome sets was due primarily to a difference in magnitude of the effects of genes situated at the loci on each chromosome.

The consequences of population sampling were evident in the variation of initial effects and subsequent patterns of response of chromosomes 2 and 3 in the high lines. The highest limit reached in these lines might well have been exceeded by substituting chro-

mosome 2 from line 5 and chromosome 3 from line 3 into the same stock. Discontinuities in response typical of those described by Thoday and Boam (1961) probably arose from the sudden release of genetic variability following crossing-over.

Appendix

Changes in the effects of marker chromosomes used to estimate selection advances in their wild homologues

It is proposed to estimate D_2 and D_3, the separate contributions of wild chromosomes 2 and 3 to total selection advance, from changes in the effects of the marker chromosomes *Cy* and *Mé* segregating in the selection lines. These are measured from advances observed in the means of the four segregant classes of offspring. Thus D_2 is estimated from (i) D (Wild) — D (*Cy*) and (ii) D (*Mé*) — D (*CyMé*) and D_3 from (i) D (Wild) — D (*Mé*) and (ii) D (*Cy*) — D (*CyMé*). For the purpose of explanation, the estimate of D_2 from a change in the *Cy* chromosome effect is discussed. The same reasoning however, applies in estimating D_3 from the change in the effect of the *Mé* chromosome.

Consider two bristle alleles A_1 and A_2 segregating at loci on chromosomes 2 in the selection lines. The homozygotes A_1A_1 and A_2A_2 differ by a on a scale on which the heterozygote A_1A_2 is d units above their midpoint. Suppose the bristle loci on the *Cy* chromosome segregating in the lines possess either one of the alleles A_1 or A_2. These are expected to remain unchanged throughout selection since the marker chromosomes are carried only in male parents.

Under selection, A_1 changes in frequency among the wild chromosomes from p to $p + \delta p$. Then δ (Wild), the contribution of all such loci on chromosome 2 to D (Wild), the total advance observed in the Wild segregants, can be shown to be

$$\delta\,(\text{Wild}) = \Sigma\,(a\,\delta p + 2\,d\,\delta p\,(1 - 2p - \delta p)).$$

This reduces to the familiar $\Sigma\, a\,\delta p$ in the absence of dominance.

The contribution of these loci to D (*Cy*), the total advance in the *Cy* segregants, turns out to be

$$\delta\,(Cy) = \Sigma\left(\frac{1}{2}\,a\,\delta p \pm d\,\delta p\right)$$

For loci on the *Cy* chromosome with favoured allele A_1, $-d$ is taken and $+d$ for loci with A_2.

When $d = 0$,

$$\delta\,(\text{Wild}) - 2\,\delta\,(Cy) = 0$$

and $$\delta\,(\text{Wild}) - \delta\,(Cy) = \Sigma\,\frac{1}{2}\,a\,\delta p$$

which is one half the advance in a pair of wild chromosomes 2 or alternatively, it equals the advance in a single wild chromosome 2.

The advances observed in the four segregants, D (*Cy*), D (*Mé*), D (*CyMé*) and D (Wild) are expected to differ only through differences among them in the number of wild chromosomes 2 and 3 each possesses, the chromosome 1 and 4 complement being the same for all segregants. Therefore, the two estimates of the change in effect of the *Cy* chromosome, D (Wild) — *D* (*Cy*) and D (*Mé*) — D (*CyMé*) should both equal δ (Wild) — δ (*Cy*) and this, in the absence of dominance has already been shown to equal D_2, the advance in a single wild chromosome 2. Likewise, D_3 can be estimated from D (Wild) — D (*Mé*) and D (*Cy*) — D (*CyMé*).

To illustrate the effect of dominance, suppose the alleles situated on the *Cy* chromosome are equivalent to the unfavoured A_2.

Then

$$\delta\,(\text{Wild}) - 2\,\delta\,(Cy) = \Sigma - 2\,d\,\delta p\,(p + \delta p).$$

If A_2 is dominant, $d < 0$, and

$$\delta\,(\text{Wild}) - 2\,\delta\,(Cy) > 0.$$

This means that the change in effect of the *Cy* or the *Mé* chromosome underestimates advance in its wild homologue if its bristle genes are, on average, dominant to those segregating among the wild chromosomes. Conversely, if the marker genes are recessive, the change in effect of the marker chromosome overestimates advance in its wild homologue.

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