THE GENETIC RELATIONSHIP OF TWO QUANTITATIVE CHARACTERS IN *DROSOPHILA MELANOGASTER*. I. RESPONSES TO SELECTION AND WHOLE CHROMOSOME ANALYSIS[1]

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ARTIFICIAL selection often produces changes in quantitative characters other than the character under direct selection. This phenomenon of correlated response has been attributed in varying degrees to sampling error (CLAYTON *et al.* 1957c), linkage (WIGAN and MATHER 1942; MATHER and HARRISON 1949) and pleitropy (LATTER and ROBERTSON 1962). We have attempted to determine the relative importance of linkage and pleiotropy by locating the genes important in the direct response to selection in one character and in a correlated response in another character. We studied the relationship of genes affecting sternopleural and abdominal bristle number in directional selection lines of *Drosophila melanogaster* in which correlated responses occurred, and also in lines in which both characters were under directional selection. These lines and the analysis at the whole chromosome level of the distribution of effects on the two bristle number characters are described in this paper. In the accompanying paper (DAVIES 1971) THODAY's (1961) method is used to assign effects on the characters to small regions of the genome.

MATERIALS AND METHODS

Origin of selection lines: About ten adult male and female *Drosophila melanogaster* were captured in Athens, Greece in 1964. Non-virgin females from the progeny of these flies were placed singly in tubes. Random samples of their progeny were used to set up a number of mass cultures. After one generation of mass transfer selection lines were started. Flies were raised on oatmeal-agar medium in 1/3 pint milk bottles at 25 ± 1°C.

Selection procedure: The characters chosen for selection were the sum of the number of sternopleural bristles on the two sides of the fly, and the number of bristles on the third abdominal (sternital) segment of the male and the fourth of the female. Selection was carried out up to and including the 34th generation. The four single directional selection lines were named as follows:

STH selection for higher sternopleural bristle number
STL selection for lower sternopleural bristle number
ABH selection for higher abdominal bristle number
ABL selection for lower abdominal bristle number

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and the four dual selection lines as follows:

HH selection for higher sternopleural, higher abdominal bristle number
LL selection for lower sternopleural, lower abdominal bristle number
HL selection for higher sternopleural, lower abdominal bristle number
LH selection for lower sternopleural, higher abdominal bristle number

The S_0 generation was made up of 8 groups of 20 flies of each sex taken at random from the Athens base population and randomly assigned a line designation. In each generation the bristle numbers of 20 virgin flies of each sex were counted, and four pairs of flies were selected from these as parents of the next generation. In HL and LH flies with the greatest difference (in the desired direction) between the two bristle numbers were chosen. In HH and LL the sum of the two bristle numbers was used as a selection criterion. When sufficient eggs had been laid the parents were removed to prevent the culture from becoming overcrowded. A reserve culture was always set up with a further four flies of each sex. STH died out at generation 25, and selection was continued on an isolate from a relaxed line which had been taken from generation 21. The main culture of ABL died out at generation 5 and was reconstituted from reserves.

Chromosome extraction: The balancer chromosomes used were: *FM6* for the *X* chromosome, *SM5Cy* and Px^4 carrying bw^D-like (bw^D-like dominant brown eye color isolated in Cambridge from Px^4 by Drs. J. R. S. WHITTLE and J. B. GIBSON) for chromosome II, and *TM3 Sb Ser* and Ubx^{130} for chromosome III. These were readily distinguishable in all combinations. They were tested for crossover suppression in the presence of one another, and produced very low levels of recombinants. All of these stocks were put onto a standard background, Oregon WD, which is a completely homozygous derivative of the inbred Oregon stock of SPICKETT and THODAY (1966).

Random samples of virgin females were taken from generation 27 for HH, LL, ABH and ABL, and from generation 33 for all the lines. The bristle numbers of the lines at these generations are given in Table 1. Each female was mated separately with males of the appropriate balancer chromosome constitution. The breeding programmes used were completely standard and were constructed so that the *Y* chromosomes always derived from Oregon WD. The only uncontrolled variable is the small fourth chromosome. Control programs with Oregon WD chromosomes showed no evidence of any recombination between the balancers and other chromosomes. It was found that the original *FM6* stock was showing secondary *X*-chromosome nondisjunction (CHARLESWORTH and DAVIES 1968). This was guarded against in the second chromosome extraction, and chromosomes from the first extraction were not used in further experiments. The multiple line chromosome combinations used in the investigation of interactions were then made using single chromosome stocks from the groups marked in Table 2. Stocks were assayed by counting both bristle number characters of 20 flies of each sex from each of two replicate cultures. Salivary gland chromosomes were examined using a method based on that given by NICOLETTI (1959).

RESULTS AND DISCUSSION

Responses to selection: The responses to selection of the eight selected lines are shown in Figures 1 and 2. All the features of these responses are commonly observed in selection experiments (CLAYTON, MORRIS and ROBERTSON 1957a; CLAYTON and ROBERTSON 1957b; FRANKHAM, JONES and BARKER 1968a,b; JONES, FRANKHAM and BARKER 1968). Excessive discussion of irregularities in selection responses is given by FRANKHAM *et al.* (1968a,b) and by JONES *et al.* (1968).

Correlated responses occurred in all four single directional selection lines, the correlation always being positive. The correlated responses in STL and ABH were maintained; those in STH and ABL were lost by generation 6. Correlation coefficients were calculated for each generation of each line. In every line there was considerable fluctuation in the value of the correlation coefficient from

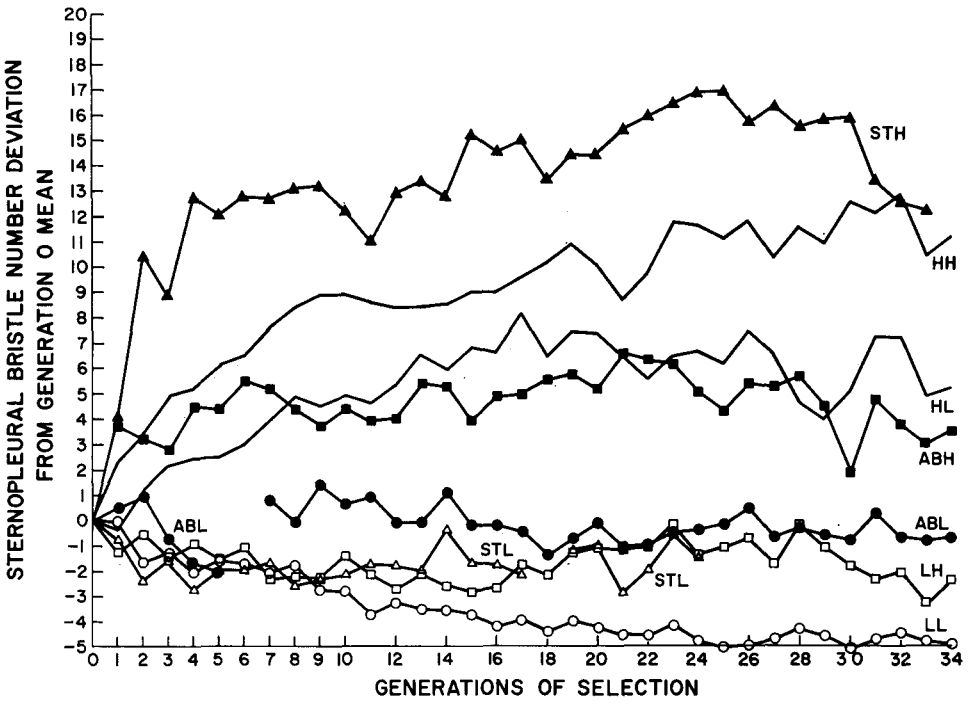

FIGURE 1.—Responses to selection: sternopleural bristle number.

generation to generation and no obvious trend with time.

In LL there was progress in sternopleural bristle number during a plateau in abdominal bristle number. In several cases the characters altered to different extents after relaxation of selection (DAVIES, 1969). This suggests that the sets of genes affecting the two characters are to an extent different.

Accelerated responses to selection were clearly seen in ABL abdominal and LL abdominal bristle number. JONES *et al.* (1968) found that rapid responses were often associated with a large increase in variance. This is supported by the striking increases in the coefficients of variation of LL and ABL abdominal bristle number at the time of accelerated response. In other lines the coefficient of variation shows no significant trend after a slight fall in the first four to ten generations. LATTER (1965a) has shown that large increases in additive genetic variance occur when a gene of large effect at low initial frequency increases in frequency. DAVIES (1971) has shown that the ABL and LL abdominal bristle number responses are largely attributable to a single effective region of very large effect.

Effects of single chromosomes: A number of each of the three main chromosomes were extracted from each selection line as described in the section on MATERIALS AND METHODS. Lethals occurred at low frequency in the ABL and LL second chromosomes and the ABL, HH and LL third chromosomes. All samples of HH second and STH third chromosomes were lethal, the lethals falling into two complementation groups in both cases. None of these lethals were colethal

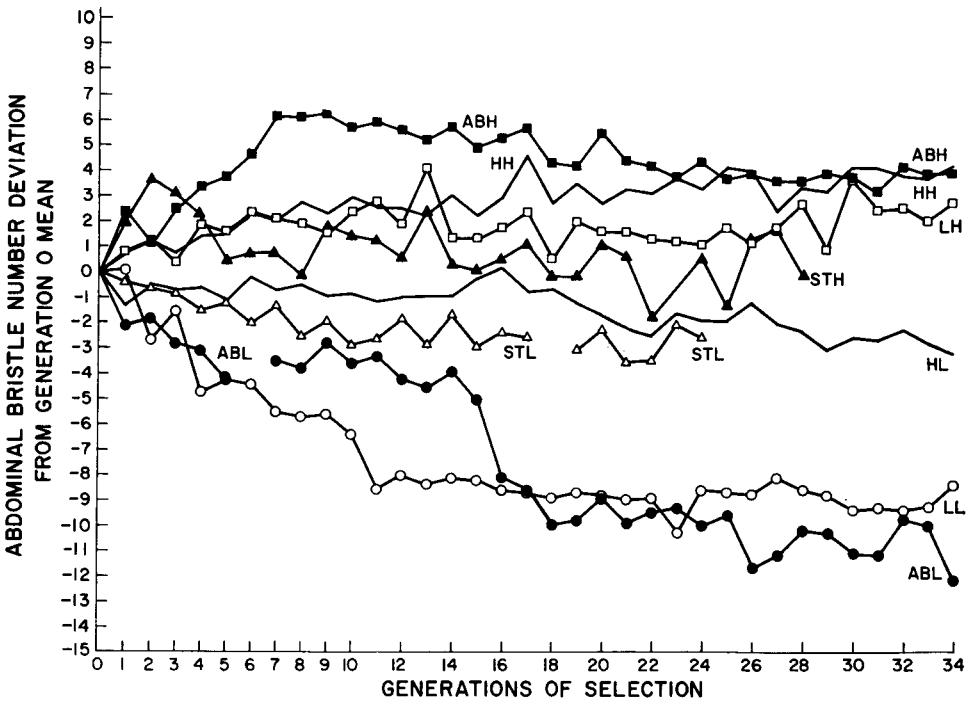

FIGURE 2.—Responses to selection: abdominal bristle number.

with any balancer chromosomes. *In(2L)t* (LINDSLEY and GRELL, 1968) occurred in all samples of one HH II lethal class. *In(3R)Mo* occurred in all samples of one class of STH III lethals, and in all HH, HL, LH, LL and ABL third chromosomes.

TABLE 1

Mean abdominal and sternopleural bristle numbers of the selected lines at the time of chromosome extraction

Line, generation	Sternopleural			Abdominal		
	Mean	Difference from Oregon (WD)	Difference from "Athens base"	Mean	Difference from Oregon (WD)	Difference from "Athens base"
ABH, g 27	23.8	+ 4.0	+ 4.9	23.9	+ 3.8	+ 3.6
ABL, g 27	17.9	— 1.9	— 1.0	9.2	—10.9	—11.1
HH, g 27	29.6	+ 9.8	+10.7	23.3	+ 3.2	+ 3.0
LL, g 27	13.3	— 6.5	— 5.6	11.0	— 9.1	— 9.3
STH, g 33	31.2	+11.5	+12.4	21.0	+ 0.9	+ 0.7
ABH, g 33	21.6	+ 1.8	+ 2.7	24.2	+ 4.1	+ 3.9
ABL, g 33	17.8	— 2.0	— 1.1	10.2	— 9.9	—10.1
HH, g 33	29.7	+ 9.9	+10.8	24.6	+ 4.5	+ 4.3
LL, g 33	13.2	— 6.6	— 5.7	9.9	—10.2	—10.4
HL, g 33	25.5	+ 5.8	+ 6.6	18.7	— 1.4	— 1.6
LH, g 33	16.0	— 3.7	— 2.9	22.3	+ 2.2	+ 2.0

TABLE 2

Individual chromosome effects expressed as deviations from the mean of Oregon WD (sexes pooled)*

Selected line	X chromosome ST	X chromosome AB	Chromosome II ST	Chromosome II AB	Chromosome III ST	Chromosome III AB
STH	+0.9 (7)†§	+2.0 (10)	+0.7 (6)	+1.7 (3)§	+8.5 (3)§	+2.4 (7)
	+4.0 (3)§			0.0 (3)	+6.5 (4)§	
ABH	[−0.1 (3)]‡	+1.4 (3)	+1.8 (6)¶	+0.1 (6)¶	+6.9 (3)	+0.3 (4)
					+2.7 (7)§	+3.3 (6)§
ABL	+0.1 (7)	+1.3 (7)	−2.2 (8)	−2.8 (4)§	[+4.4 (5)]	−10.2 (9)
				−4.8 (4)	[+0.4 (3)]§	
					−2.1 (1)	
HH	+2.9 (5)	+3.5 (6)	+2.1 (5)	+1.4 (5)	+6.4 (4)§	+2.3 (13)¶
	+1.3 (2)§	+2.1 (2)§			+4.2 (6)	
	[−0.6 (3)]	+0.8 (2)			+1.6 (1)	
					+1.0 (2)	
LL	−1.1 (9)	−0.1 (9)¶	−2.1 (3)§	−2.2 (3)§	−3.0 (7)	−4.9 (7)
			−0.4 (2)	−3.7 (2)		
HL	0.0 (4)	−0.2 (4)	[−1.0 (4)]	−2.7 (4)	+7.2 (4)§	[+0.4 (5)]
					+1.9 (1)	
LH	−0.4 (3)	+2.1 (3)	−0.4 (5)	+0.1 (5)	−2.2 (4)	+0.4 (4)

* Oregon WD mean: ST, 19.8; AB, 20.1.

† The number in round brackets is the number of chromosomes definitely assigned to the group.

‡ Numbers in square brackets indicate that the effect of this group of chromosomes is in the opposite direction to the direction of selection response.

§ Where chromosome samples from a line show multiple levels of effect on a character, only chromosomes from certain groups were used in making multiple chromosome genotypes, and these groups are marked.

¶ There are small differences between samples of these chromosome types.

The bristle numbers of the selected lines at the time of chromosome extraction are given in Table 1. The presence or absence of significant heterogeneity among chromosomes of any particular type was ascertained by analysis of variance of the replicated assays. Where there was heterogeneity, chromosome samples were divided into subgroups by t-testing, giving the groups shown in Table 2. The details of the decision process are given by DAVIES (1969). All the lines except LH (from which only small numbers of extracted chromosomes were obtained) showed heterogeneity for one or other character among the samples of at least one chromosome. A large amount of genetic variance clearly remains after long continued selection. This was also found by CLAYTON and ROBERTSON (1957b), while JONES *et al.* (1969) found their lines capable of responding to reverse selection after forty generations of directional selection.

The overall mean bristle numbers of the various extracted chromosomes and their subgroups are given in Table 2. The significance levels of chromosome effects were determined in the factorial analyses of variance used to analyze interchromosomal interactions (DAVIES, 1969). All samples of HH second and STH third chromosomes were lethal when homozygous, so that the means given represent mean bristle numbers of various heterozygotes for chromosomes from the two lethal classes in each case.

The distribution of effects on sternopleural and abdominal bristle number among the subgroups of those chromosome types that are heterogeneous provides evidence concerning the relationships of the genes affecting these characters. Whenever among samples of a particular chromosome type from a line there are several levels of effect of one character, but only one level of effect for the other character, it is reasonable to suppose that only the lowest level of expression of the heterogeneous character can have any relation to pleiotropic effects of genes affecting the two characters. This situation occurs in the *X* chromosome of STH, the second chromosome of STH and ABL, and the third chromosome of STH, ABL, HH and HL. Also in some cases individual chromosomes fall into a high group for one character and a low group for the other (e.g., HH *X*, HH III, ABH III). Therefore, the sets of loci affecting these two characters are to an extent non-overlapping.

Table 2 shows that for each character-direction combination the same chromosome or chromosomes tend to be associated with large effects in all the relevant lines. This implies a non-random distribution of genes affecting the character, which is more likely to occur if the number of important segregating loci in the base population were small.

It is also clear that the distributions of effects on sternopleural bristle number are different from the distributions of effects on abdominal bristle number. This distributional difference means that many of the loci affecting these characters which were segregating in the base population are different. In HL and LH effects on the two characters are almost completely on separate chromosomes, but this could reflect difficulty in applying the selection criterion rigorously.

Chromosome effects and correlated responses: Most of the correlated responses are due to effects of the chromosomes which are most important in the direct responses, showing correlated response to be largely a directed process with a minor chance component. The only exception is the second chromosome contribution to STH abdominal bristle number.

If linkage is a major mechanism of correlated response, then there will be a greater association of chromosome effects on the two characters in the lines in which only one character was selected than in lines where both are selected. This is because when one selects for both characters in the same direction genes which are not closely linked may be utilized. Comparison of HH with ABH, and ABL with LL (noting that the third chromosome may be atypical [DAVIES, 1971]) shows this effect, providing further evidence against pleiotropy and for linkage. For all three chromosomes there is usually a positive correlation between abdominal and sternopleural effects if both occur.

It seemed from the responses to selection that the reconstituted ABL line showed very little correlated response in sternopleural bristle number. The chromosome analysis shows that all second and some third chromosomes have low sternopleural bristle numbers compared with Oregon WD and the ABL line. The majority of third chromosomes have high sternopleural bristle numbers, and their maintenance may explain the loss of correlated response. Genes increasing sternopleural bristle number were probably associated with the third chromo-

TABLE 3

Sternopleural and abdominal bristle number means of reconstructed "+++" genotypes and of possible combinations of extracted chromosomes, expressed as a percentage of the difference between the selection line and Oregon WD

Selection line	Possible summations of effects of extracted chromosomes† ST	AB	Mean of +++ genotype‡ ST	AB
STH§	115*[a], 97, 87, 70*[b]	711*, 511	106[a] 83[b]	208
ABH	481, 248*	43, 118*	128	111
ABL	[115]¶, 89* 215	118*, 139	96	130
HH	114, 98*, 80, 92, 76, 58, 66, 50, 32, 60, 44, 25	160, 128*, 84	100	73
LL	94*, 70	70*, 85	98	82
HL	107*, 15	180*	135	72
LH	79*	120*	77	124

* These are the particular summations which involve the group means of the particular chromosomes used in making the "+++" genotype.

† The numbers in this column are obtained by expressing the deviations from Oregon WD of groups of extracted chromosomes as percentages of the deviation of the selection line from Oregon WD, and then summing X, II and III effects in all possible ways.

‡ These are the bristle numbers of reconstructed genotypes homozygous for particular samples of the three main chromosomes from a line, expressed in percentages as in the previous column.

§ Two STH +++ genotypes were made, one (a) with both the higher *X* and higher third chromosome, the other (b) with both the lower *X* and the lower third chromosome.

¶ Numbers in square brackets indicate that the effect is in the opposite direction to the direction of selection response.

some abdominal gene that became very important in the abdominal response around generation 15.

Recovery of selection line phenotype: For each selected line flies homozygous for all three major chromosomes were constructed. The extracted chromosomes used in these reconstructions were chosen from the most frequent class wherever there was heterogeneity. The mean sternopleural and abdominal bristle numbers of these reconstructed genotypes are compared with possible summations of effects of individual chromosome types in Table 3.

The recovery of the bristle number phenotype of the selected lines at the time of sampling is very good. Discrepancies are probably due to the lack of homogeneity in the lines. The lines cannot be reconstructed exactly because the frequencies of the various segregating alleles are unknown, and some may not have been extracted. The only large disagreement is in STH abdominal bristle number, which is discussed separately.

It seems likely that a large proportion of the important genes affecting the bristle number characters has been obtained for each line in this set of extracted chromosomes. It was not considered worthwhile to extract samples of the small fourth chromosome.

Interactions between chromosomes: Interactions between chromosomes were

investigated by making all possible crosses between genotypes comprising the eight possible combinations of single and multiple homozygous chromosomes. This gives a total of 27 female and 18 male genotypes. Two replicates of each type were assayed. The significance of interactions was determined by a factorial analysis of variance in each case, treating males and females separately. Therefore the effect of a chromosome is considered over all levels of other chromosomes. Since representatives of particular groups of extracted chromosomes were used, all possible interactions have not been investigated. In table 3 a comparison of the summed effects with the mean of the "+++" genotype gives some idea of where interactions might *a priori* have been expected to be important.

The percentage contributions of interchromosomal interactions and main effects of chromosomes to the total variance are summarized in Table 4. Since a fixed-effects model was used, these percentages should not be regarded as real components of variance, but merely as some measure of the importance of the different effects. The particular interaction components and their significance levels are given by DAVIES (1969).

Interactions are relatively least important in those line-character combinations where there was a large and efficient response to selection. Within each line the interactions which are significant for the two characters are frequently between different chromosomes, which implies a certain amount of non-identity between the genes which affect the characters.

Interactions were more important in unselected than selected characters. In STH abdominal and ABH sternopleural bristle number there are predominantly negative interactions between chromosomes. The contribution of interactions to

TABLE 4

Percentage contributions of chromosome main effects and interactions to the total variance

Line	Sex	Sternopleural			Abdominal		
		Amount of variance relative to STH ♀ low	Main effects (percent of total)	Interactions (percent of total)	Amount of variance relative to ABH ♀	Main effects (percent of total)	Interactions (percent of total)
STH	♀	100	98.8	1.2	12	28.5	71.5
low	♂	97	99.4	0.6	12	28.3	71.7
STH	♀	120	94	6	12	28.5	71.5
high	♂	144	93.1	6.9	12	28.3	71.7
ABH	♀	5	78.4	21.6	100	96.3	3.7
	♂	7	89.6	10.4	33	92.9	7.1
ABL	♀	6	89.9	10.1	503	98.5	1.5
	♂	7	71.7	28.3	420	99.6	0.4
HH	♀	68	94.2	5.8	33	85.3	14.7
	♂	69	93	7	15	77.5	12.5
LL	♀	33	95.5	4.5	318	95.4	4.6
	♂	26	94	6	198	91.7	8.3
HL	♀	87	96.9	3.1	56	64.1	35.9
	♂	83	98	2	48	88.9	11.1
LH	♀	9	88.4	11.6	56	92.7	7.3
	♂	16	90.3	9	7	72.7	27.3

variance in the unselected character is similar and fairly small in ABH and ABL, but in STH abdominal interactions contribute more than main effects of chromosomes. Summation of the effects of individual STH chromosomes on abdominal bristle number gives a value 7 times that of the selected line (Table 3), whereas the reconstructed genotype, containing all three of these same chromosomes, had a value only twice that of the selected line. It is as if the alleles responsible for the correlated response had not been lost by segregation or recombination but their effects had been covered up by negative interaction effects. This could be due to chance, but it could reflect the action of strong natural stabilizing selection.

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SUMMARY

A set of lines selected for sternopleural and/or abdominal (sternital) bristle number was produced. The effects of individual chromosomes and their interactions were assayed. A considerable amount of variability remained in some of these lines despite thirty generations of selection. This was directly shown to be due to continued segregation at loci involved in the responses to selection. Correlated responses occurred in all four single directional selection lines, and in all cases were in the same direction as the direct response to selection in the other character. Whenever a chromosome had effects on both characters, the effects were positively correlated. The distribution of effects among chromosomes suggested that sampling error was not an important cause of the correlated responses. Interactions between chromosomes were not important in direct responses, but contributed significantly to the variance in unselected characters. The loss of one correlated response was not due to the breaking up of linked gene complexes but apparently to the accumulation of negative interaction effects. A number of considerations lead to the conclusion that loci which were involved in the responses to selection for sternopleural bristle number were to a significant extent separable from loci involved in the responses to selection for abdominal bristle number. This suggests that linkage played an important role in the correlated responses.

LITERATURE CITED

A joint literature citation is given at the end of the second paper.