

Nutritional requirements of inbred lines and crosses of *Drosophila melanogaster*

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1. INTRODUCTION

It is commonly assumed that hybrids are more efficient and less variable than their parents, and this is sometimes taken to imply that they have less stringent nutritional needs (Lints, 1962). Recent results of crossing *Drosophila* strains selected on diets deficient in protein or pyridoxine (Sang, 1962*a*) suggest that any such generalizations concerning the characteristics of hybrids may require qualification, since these particular F₁'s did not need less pyridoxine or casein than their parents, nor were they necessarily less variable in development rate when tested on the deficient diets used for selection. There were also indications of genotype-environment interactions of such an order as would preclude any simple, general relationship between the variability of parents and their crosses, which might be anticipated from measurements made in only one environment. This paper examines this problem in greater detail, using inbred lines.

The problem of the 'efficiency' of hybrids is of economic importance. In commercial practice, complex crosses (up to 4-way) of poultry and other animals are exploited, and these are taken to be more efficient than the lines from which they are derived. There are few data to confirm this (Anon, 1961) or to suggest how the performance of the 4-way relates to its 2-way parents. This latter presents a more complex problem, which it has been possible to examine only cursorily here. Its interest in the present context is to see how far, if at all, the merits of a particular 2-way cross are also found in the 4-way cross.

These are essentially practical problems, since there is no reason to believe that the kind of result found with *Drosophila* would not also be found for domesticated animals if they could be studied on the same scale. It is therefore important to note another issue; namely, the extent to which environmental improvement can permit the full expression of a particular genotype: and, conversely, how far a poor environment may restrict the expression of a 'good' genotype compared with a 'poor' one. It is easy to envisage, for instance, that a particular genotype (or even allele) may restrict the phenotypic expression of some character under optimal conditions, whereas this might be of no significance under sub-optimal conditions. That is, either genotype or environment may be limiting; and the relevance of this to hybrid performance does not appear to have been examined.

These problems can all be studied by finding the dose-response of lines and their crosses to different nutrients. Such nutritional measures might also be expected to throw some light on the biochemical basis of hybrid vigour, even if only in general terms. Preliminary experiments suggested that varying the major nutrients (casein, choline, ribosenucleic acid) would provide the data most relevant to our topic and these were examined first. Four vitamin responses were also determined for the information they might be expected to provide about some of the metabolic systems possibly involved in hybrid vigour.

2. MATERIALS AND METHODS

Larvae were cultured axenically using the technique previously described (Sang, 1956). The synthetic medium used was Sang's (l.c.) medium C, which contains a more or less optimal provision of the major nutrients (casein, fructose, ribosenucleic acid and lecithin) and a more than sufficient supply of vitamins and salts. Dose-responses were found for each genotype as indicated below: three cultures each containing 40 newly hatched larvae were set up for each point on the response graphs. Two pure lines and their F_1 were inoculated at the same time and into the same batch of media, since this represented the maximum which could be handled by one person. No distinction was made between reciprocals. The 4-way cross was set up from the progeny of the F_1 's and usually on separately prepared media. The pure lines were Ore-S, Ore-R, Nettlebed (collected in Edinburgh) and Crianlarich (collected in West Scotland) and all had been brother-sister mated for over 125 generations before the experiments started. Development rate was measured in log days, for reasons already given (Sang, 1962*a*), and the size of flies was measured by finding the weight of a sample of 25 or more day-old males hatching from the cultures.

3. EXPERIMENTAL

(i) *General*

The parent lines have different characteristics of survival, weight and development time under the conditions chosen as standard (Table 1). Of these three 'characters', larval survival is the one most commonly taken to be heterotic; larval development time is usually found to be faster in crosses of inbred lines, but not always in crosses of selected lines, whereas size is not found to behave regularly, except that selected small strains frequently show heterosis on crossing (see Sang (1962*a*) for references). Past work suggests, therefore, that the three 'characters' may behave differently in crosses, and it is convenient to look at this first with respect to the standard conditions.

The four lines have characteristic mortalities: S survives best and C worst, with the other two lines lying together between them (Table 1). The S/C cross is not superior to the S line, but it is some 18% better than its mid-parent. The R/N cross is superior to both parents, and about 20% better than its mid-parent. The 4-way cross is indistinguishable from its better parent or from the average of the two F_1 's.

The variances of the F_1 's are not lower than those of the parent lines and the 4-way cross is nor more variable than the F_1 's. The data lend support to the view that pre-adult survival shows heterosis, but not to any assumption that this is invariably accompanied by a reduction of variability in a cross of inbred lines. On the basis of survival, the genotypes rank: S, SC/RN, R/N, S/C, R, N, C.

Table 1. *Characteristics of the lines and crosses*

Genotype	Per cent survival		Adult ♂ weight (100 × mg.)		Development time (100 × log days)	
	Mean	Variance	Mean	Variance	Mean	Variance
S	88.7	335	86.7	1.3	68.6	24.0
C	40.1	104	83.5	6.4	69.6	49.5
S/C	75.8	186	87.8	8.3	63.1	10.5
R	73.0	165	79.9	1.9	71.8	35.5
N	66.8	125	97.4	5.9	71.2	35.6
R/N	84.4	87	93.2	12.6	61.2	24.0
SC/RN	84.4	89	92.2	6.7	58.9	27.5

Survival was assessed from the casein experiment, since there was no change of mortality throughout the range of diets, giving 24 cultures per genotype on which to measure between-culture variances. Weight was averaged from the six or seven highest concentrations of pyridoxine tested. Development time was taken from the riboflavine experiment since the cultures containing eight or more μg riboflavine all gave optimal conditions and allowed an estimate to be made of the within-culture variance on samples ranging from 120–320 according to survival.

Weight also displays heterosis (Table 1), if this is judged by superiority over the mid-parent; but the R/N cross is better than the S/C by this criterion, and the 4-way cross is somewhat superior to the average of the F_1 's. In this case, the variances of the 2-way crosses tend to be greater than those of the inbred lines, and the 4-way cross is less variable than the 2-way crosses. Larval development time behaves similarly: both simple hybrids grow faster than either parent, and the 4-way cross grows fastest of all. Both hybrids tend to be less variable than their parents, but the 4-way cross is no more variable than its parents. The rank order for both weight and development time differs from that already listed; but considering all three characters together, the SC/RN and R/N crosses tend to be similar and superior to S/C, and all three crosses are superior to the parent lines.

(ii) *Casein*

Although *Drosophila* larvae show a notable ability to maintain size at the expense of development time under some circumstances (see below and Robertson, 1960), their response to different casein levels is by change of size and development rate, not only as a consequence of deficiency but also when fed an excess of this protein. As already noted, there were no significant alterations of survival within the range of casein supplies tested (Table 1).

Development time changes with the amount of casein in the diet to give dose response curves with distinct optima (Sang, 1956); more, as well as less, than this amount of casein slows growth. These optima can be determined only approximately from dose-response graphs, but they range from about 5.0 to about 5.5% casein for the genotypes tested. The 4-way hybrid appears to require the smallest amount, and the S line most, for optimal growth. It follows, by this criterion, that there is no one standard optimum medium for *Drosophila*, but that each genotype has its particular requirement which is determined by its reactions to a deficiency, and to an excess, of casein (see also Sang, 1962*a*). These two reactions are not correlated and have to be considered separately (Table 2). Reactions to low casein can be measured, with sufficient accuracy for our purpose, by finding the amount by which development time is slowed when casein is reduced from 5 to 2.5%. The 4-way cross is least affected by this decrease and the F₁'s are remarkably like their more resistant parent. The parent lines rank C, R, S and N in their ability to withstand a decrease of dietary protein. When the effects of excess protein are measured in the same way (Table 2)

Table 2. *Reactions of the genotypes to suboptimal, and to excess, casein provision*

Genotypes	S	C	S/C	R	N	R/N	SC/RN
Casein reduced from 5 to 2.5%:							
Development time delay (100 × log days)	20	16	16	19	23	18	14
Weight loss (100 × mg.)	12	17	15	5	1	13	7
Casein increased from 6 to 10%:							
Development time delay	1	2	3	2	8	0	3
Weight loss	6	4	7	10	11	9	9

the situation is found to be quite different. The R/N genotype is least affected by excess and the 4-way is indistinguishable from its S/C parent. The parent lines rank S, C, R and N, indicating that the N line is the one least able to cope with departures from its optimal supply. Otherwise the reactions of the different genotypes to too little or too much casein are complex, and for this reason it seems improbable that the optimal supplies (inflexions of responses) described above indicate the relative efficiencies of the genotypes, except in a narrow sense. Since the weights of the flies are also different (Table 1), measurement of the efficiency of the genotypes from only development rate optima are misleading for this reason, too.

When selected for, size is positively correlated with development time (Robertson, 1960). This relationship is not found to hold for the inbred lines even under optimal conditions: the trend is for the largest lines to develop fastest. Line N is exceptional in this respect (Table 1), being the heaviest and most slowly growing line. As would be expected, maximum size is attained with about the optimal provision of casein, but the various genotypes respond differently to reduced (or excess) casein (Table 2). Line N is particularly notable in that its size is scarcely altered at the lowest casein level and this presumably explains its unique behaviour, previously noted.

When the regressions of weight on development time are examined it is found that they fall into three groups; the exceptional N line, the S, R/N and SC/RN genotypes which lose size slightly as development is delayed, and C, R and S/C which lose size more rapidly. Further, each genotype had its own particular size-development time relationship, and, as far as this could be determined, a somewhat different response to excess protein. These interrelationships demand that the data be brought together in a simpler and more meaningful way.

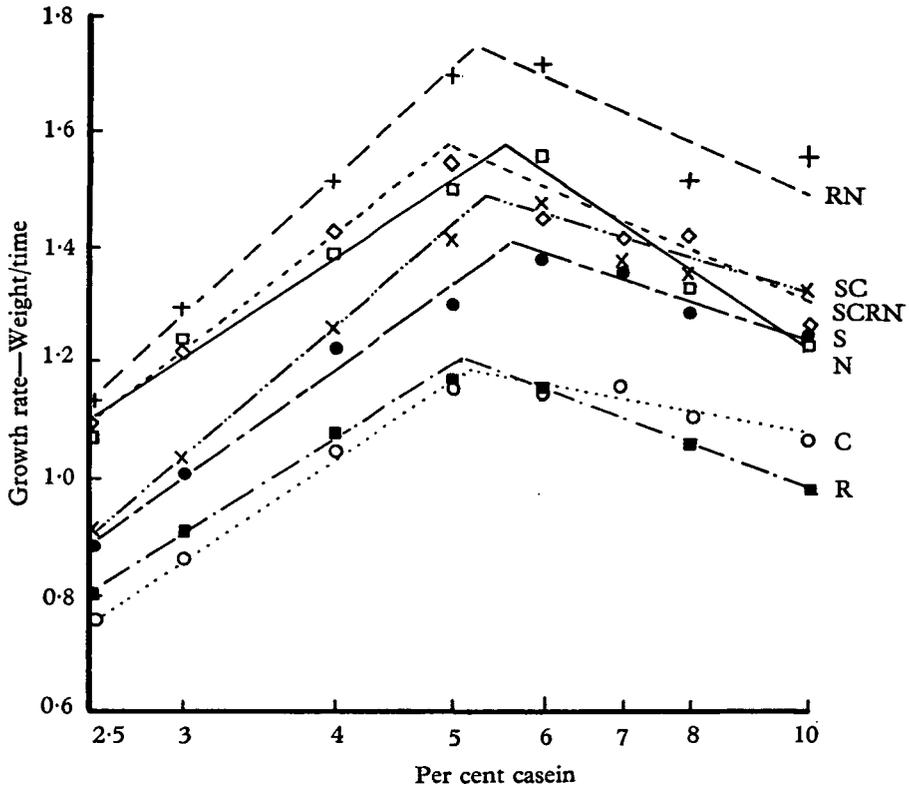


Fig. 1. Growth of the lines and their crosses when provided with different amounts of casein in the diet. The responses in this, and in subsequent figures, are fitted by eye. Line S solid circle, C open circle, R solid square, N open square, SC cross, RN plus and SCR.N diamond.

The simplest measure of the performance of the genotypes is their growth rate (weight divided by development time) which, in effect, measures their efficiency in converting food materials per unit time. Efficiency (as defined) is then found to be proportional to the amount of casein in the diet, up to an optimum beyond which it declines in proportion to the excess. The lines have different efficiencies, ranking N, S, R and C; with R and C being similar except in their reactions to an overdose. The S/C cross is superior to its better parent, and the R/N cross is the best genotype. It is particularly notable that both crosses, and the 4-way cross, show an improved superiority over their parent lines as the casein supply is raised to the optimum.

They do not show any greater resistance to the effects of excess casein. At low casein levels the 4-way cross is similar to the R/N and only slightly inferior to it, but the improvement of efficiency which it achieves with higher supplies of casein is similar to that of the S/C genotype. The 2-way hybrids tend to be like, but somewhat superior to, their better parent at low protein levels, but they use higher protein supplies more effectively than the pure lines. The 4-way cross behaves similarly, but it is not as efficient as its better F₁ parent at the higher casein levels. The C and N lines are notable for their different reactions to excess protein. These results may be stated conversely, since it is reasonable to assume that optimal nutritional environments permit the most complete expression of the genotypes: the efficiencies of the hybrid genotypes are then seen to be more depressed by casein shortage than are those of the parent lines. The 4-way cross loses less than the average of its parents. Reactions to excess are different for all three hybrids.

Perhaps the most important conclusion indicated by Fig. 1 is that departures from the optimal protein provision for each genotype must necessarily lead to a difference in any measures of the interrelationships of the genotypes to each other. It also follows that the standard (5.5% casein) medium is not optimal for all the genotypes, and the experiments to be described do not take account of this.

(iii) Ribose nucleic acid

Most *Drosophila* strains can synthesise the ribose nucleic acid (RNA) required for their development, although this ability depends on other dietary conditions (Ellis, 1959) and is never adequate for normal growth requirements (Sang, 1957; Robertson, 1960). As with casein, an excess supply is detrimental to all three characters. Omission of RNA from the diet, which is a measure of ability to synthesize RNA, affects the lines very differently (Table 3). Mortality of the S line is virtually unaffected whereas only a rare pupa of the R line is viable. The S/C cross is like its more viable parent and the R/N cross is like its less viable parent, under these conditions. The 4-way hybrid is almost as inviable as the R/N cross. Excess RNA affects the 2-way crosses in the converse fashion but has no effect on the survival of the 4-way cross, which is the best genotype in this respect.

Table 3. Responses of the genotypes to removal of RNA from the diet and to provision of 3.2% RNA, as measured by difference from the control (0.4% RNA) medium

Genotypes	S	C	S/C	R	N	R/N	SC/RN
Per cent survival loss							
RNA zero	0	12	0	75	22	75	59
RNA 3.2%	44	33	61	16	38	13	0
Development time delay (100 × Log days):							
RNA zero	19	19	16	(30)	24	21	25
RNA 3.2%	14	11	12	7	13	8	6

Datum in brackets obtained by extrapolation, since insufficient flies hatched for accurate measurement.

As judged by their development rates, the optimal provision of RNA is very similar for all genotypes, but they are all affected differently by departures from this amount (Table 3). When deprived of RNA, the 2-way hybrids tend to be less delayed than their parent lines, whereas the 4-way cross is the second most severely retarded genotype. Delays due to excess RNA again follow a different pattern, the 4-way being least affected and the two-way crosses similar to their least delayed

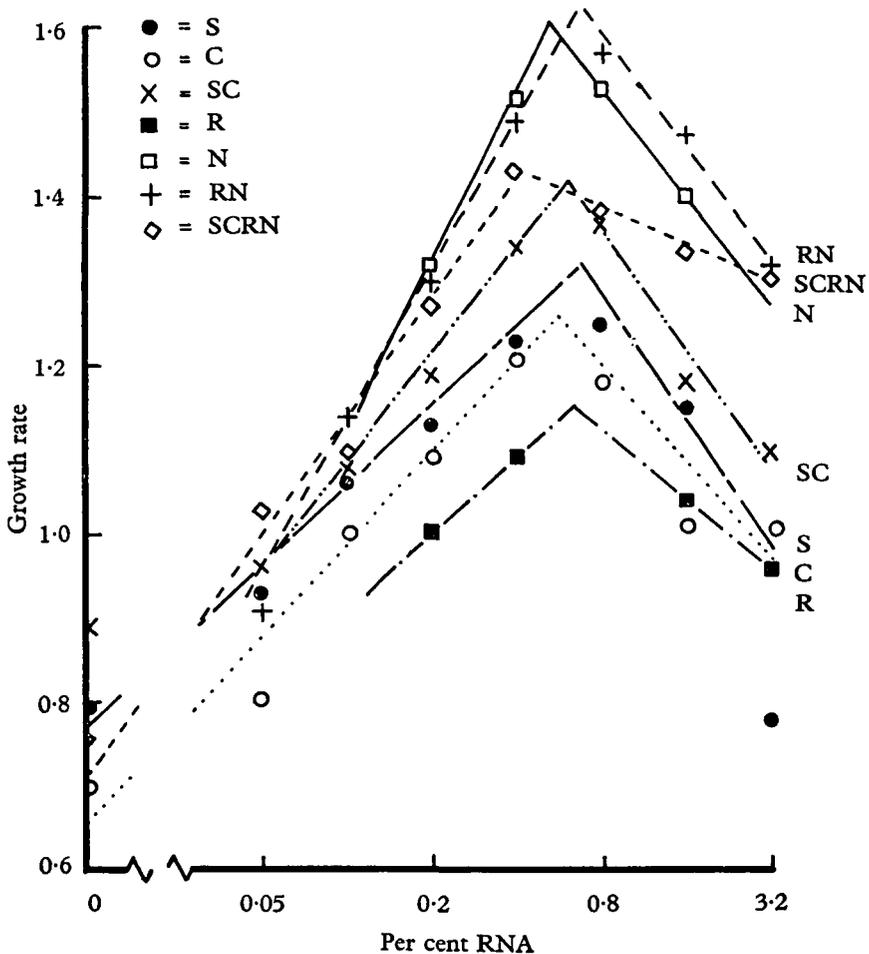


Fig. 2. Growth rates of the lines and their crosses on different levels of dietary RNA.

parent. No useful comparisons of weight changes could be made due to the high mortalities of some of the lines, but it was noted that there was a regular negative correlation between weight and development time, as reported by Robertson (1960).

The influence of RNA supply on the interrelations of the lines and crosses is most readily seen in the alterations produced in their growth rates (Fig. 2). As before the S/C hybrid is always superior to its parents, but the R/N hybrid is not superior to the N line when the supply of RNA is low. The SC/RN hybrid is notable for its reaction

to excess RNA. The optimal provisions of RNA by this criterion are higher than those estimated from development rates (which are close to 0.4% RNA for all genotypes) and this reflects the fact that the optimum supply for weight tends to be higher, at between 0.6 and 0.8% RNA. There are considerable differences in the requirements of the inbreds for their most efficient growth, S needing most and N least. The hybrids tend to be intermediate, but the 4-way cross is the lowest requirer. As with casein, however, the most interesting point illustrated by Fig. 2 is the notable change of ranking of the genotypes caused by departures from the optimal dietary RNA provisions. Comparable results have been recorded by Robertson (1961).

(iv) *Choline*

Tests of the effects of choline levels have to be made on media without lecithin and, since choline is an incomplete substitute for lecithin, this affects development rate, size and survival (Table 4). The C line survives better on the choline medium by about 20% and the N line by nearly 10%; whereas the R line is about 20% less viable. Survival of the S line and the three hybrids is little affected, so the other genotypes must show specific reactions to the removal of lecithin from the diet; that is in their abilities to synthesise the phosphatidic portion of the molecule or to utilize dietary lecithin. Similarly, all the lines and crosses, except R, are reduced in size; and all, except N, are significantly slowed in development. The 4-way hybrid is most affected in this respect (Table 4). By substituting choline for lecithin in the diet, the relationships of the crosses to their parent lines are altered in all the aspects which we have considered.

Table 4. *Characteristics of the genotypes when reared on the complete choline medium, and their reactions to a reduction of the choline supply to 20 µg. per culture*

Genotype	S	C	S/C	R	N	R/N	SC/RN
Survival per cent:							
Standard medium	89	40	76	73	67	84	84
Complete choline	83	64	85	47	71	84	84
Reduced choline	7	15	60	16	0	15	64
Development time (100 × log days):							
Standard medium	69	70	63	72	72	61	59
Complete choline	74	75	64	80	71	70	68
Reduced choline	90	95	79	95	(108+)	101	88
Weight (♂ in 100 × mg.):							
Standard medium	87	84	88	80	97	93	92
Complete choline	73	62	74	76	93	89	77
Reduced choline	66	(47)	58	72	(60)	(74)	71

Removal of choline from this medium results in larval death, and a sub-optimal supply lowers survival and weight, and prolongs development. These reactions are different from those just described, since they reflect the function of choline as the main dietary source of labile methyl groups. For instance, the N line does not survive on low choline, whereas replacement of lecithin by choline improves its survival (Table 4). Only two of the genotypes (S/C and SC/RN) have their survival reduced by a fifth or less when the choline is reduced to 20 μg . per culture; about half or more of possible survivors die in the other cases. Similarly, the R/N cross is delayed in development almost as much as the N line would appear to be, whereas the S/C cross is not as affected as its parent lines. The 4-way hybrid is about the

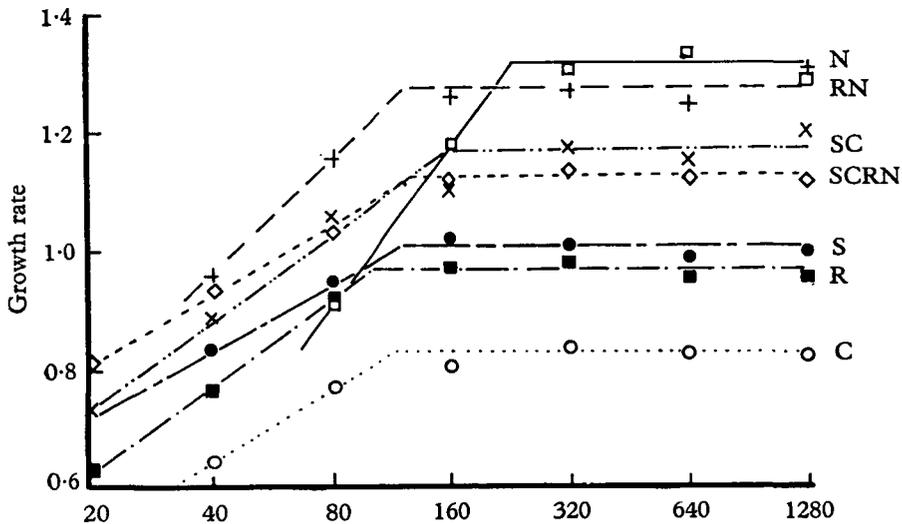


Fig. 3. The influence of choline supplies on the growth rates of the genotypes.

average of its F_1 parents in this respect, but not as regards weight loss. The other genotypes seem to have particular individual weight responses to low choline (Table 4).

As might be expected, the 'efficiencies' of the genotypes are all lowered on the choline diet (Fig. 3), to the greatest extent for the SC/RN hybrid and the C line, and least for the R line. The lines have about the same choline requirement for full growth (*ca.* 100/ μg . per culture), except for N which requires over twice this amount. The hybrids require somewhat more choline (*ca.* 120 μg .) than the lines. Given adequate choline, the N line is the most efficient genotype, but its efficiency falls most rapidly when the choline is reduced below this amount. The R/N cross responds in an intermediate fashion to sub-optimal choline: the 4-way cross is not as affected as either of its parents by choline reduction. Since there is no overdose complication when extra choline is provided (Fig. 3) the minimal amount of choline needed to produce one unit of each genotype can be calculated (Table 5). This shows that the N line requires most and the R line least. Both 2-way hybrids require less than

their parental average (the R/N needing relatively less than the S/C) and the 4-way cross requires more than its mid-parent. Put in other terms, the data of Table 5 imply that the R line uses choline most effectively, and that the R/N hybrid behaves similarly although its other parent is the least efficient genotype. The S/C hybrid is more efficient than either of its parents, but the SC/RN hybrid is intermediate in performance between its parents and grandparents.

Table 5. *Minimal requirements for unit growth of the genotypes*

Genotype	Casein %	Choline μg.	RNA %	Riboflavine μg.	Niacin μg.	Pyridoxine μg.	Folic acid μg.
S	3.95	110	0.455	3.57	5.85	1.72	0.44
C	4.40	120	0.395	6.00	10.00	1.89	1.13
S/C	3.75	100	0.420	5.40	6.27	1.52	0.55
R	4.15	97	0.580	4.05	11.75	0.80	< 3.00
N	3.40	173	0.315	2.85	10.50	1.16	0.43
R/N	3.00	97	0.400	3.36	7.90	1.28	< 2.00
SC/RN	3.25	110	0.340	4.12	8.75	1.32	0.81

The optimal supply, or the minimum giving optimal growth, has been corrected proportionally to unit weight and unit development time, in order to adjust for the genetic differences in these characteristics among the genotypes.

(v) *Riboflavine*

Survival and weight of all the genotypes were little altered by reduction of the dietary riboflavine to the lowest levels tested (1.5 and 2 μg. per culture) and no useful assessment could be made of these small changes. Rate of development was slowed by lesser reductions, and the genotypes were then found to have different minimal requirements. Figure 4 suggests that the crosses need more dietary riboflavine than their parent lines, but adjustment for weight differences and development times shows this is not so (Table 5). Indeed, both line hybrids are intermediate in minimal requirements for normal growth when adjusted to this unit scale (Table 5), and the 4-way cross also approximates to its parental average. Thus, although there is a considerable range of minimal requirements among the genotypes, this characteristic appears to behave additively, and in so far as these riboflavine needs may be taken to indicate the level of the energy transfer mechanisms of each genotype, they would then rank: N, R/N, S, R, SC/RN, S/C and C. This is different from their performance ranking with respect to the other characters which we have considered, and suggests that the superiority of the hybrids is not due to the greater efficiency of their riboflavine-dependent oxidation systems.

Figure 4 also shows that most of the genotypes react in the same way to sub-optimal riboflavine. The exceptions are that the development time of the S line is slowed more than the others, and those of the R line and of the R/N hybrid by less than the average. The S/C hybrid is like its C parent, the R/N hybrid like its N

parent, and the 4-way cross like the average of the four grandparental lines, indicating considerable interactions between genotypes and sub-optimal riboflavine supplies, different from those found when optimal riboflavine is provided.

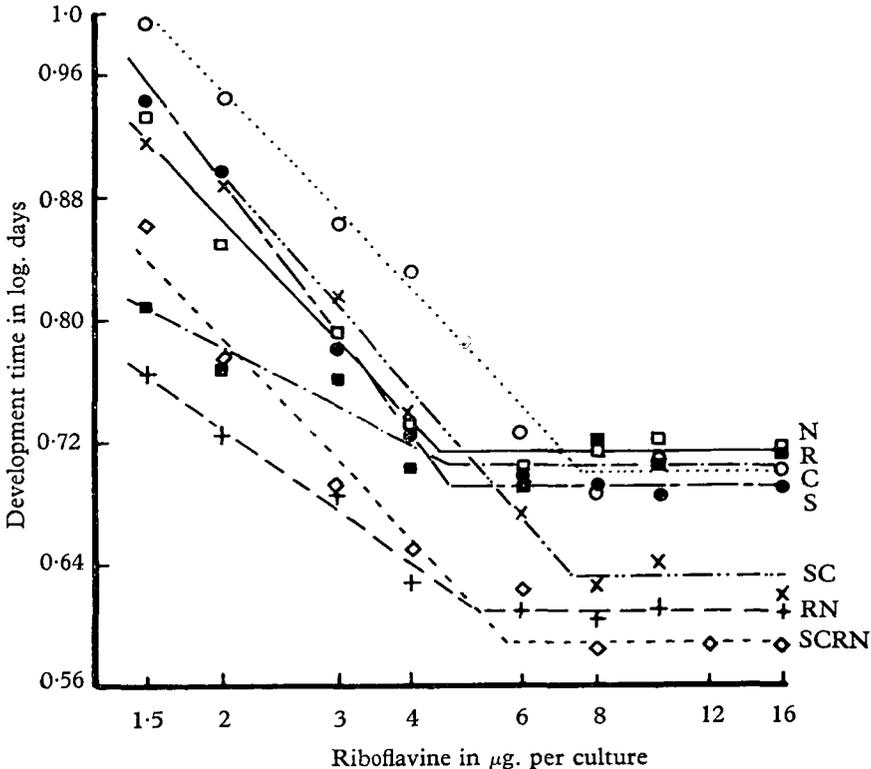


Fig. 4. The relationships between development rate and riboflavine supply for the seven genotypes.

(vi) Nicotinic acid

Both survival and development rate are affected by sub-optimal supplies of nicotinic acid: adult weight is reduced only at the lowest levels tested (2.5 and 3 µg. per culture).

Survival of the R line is affected least and that of the N line most, by reduction of the nicotinic acid supply to 3 µg. per tube (Table 6). Both 2-way crosses are intermediate, but the 4-way cross approximates to its R/N parent. As would be expected from these reactions, the N line has the highest minimum requirement for normal growth, but the S line, not R, has the lowest need per culture. Correction for weight and development time (Table 5) demonstrates that the R line has the highest requirement per unit of tissue formed. On this scale, both 2-way hybrids require less nicotinic acid than the mean of their parents, but the 4-way cross requires more than the average of its parents. It follows that the genotypes react differently in

development rate to sub-optimal supplies of nicotinic acid (Table 6). The S and S/C genotypes are least affected, while the others, except N, are very similar.

Nicotinic acid is not synthesized from tryptophan by *Drosophila* (Schultz, St. Laurence & Newmeyer, 1946) and the dietary requirement depends on its involvement as a cofactor in energy transfer mechanisms, and in the metabolism of glutamic acid, tryptophan and, possibly, other amino-acids (Sang, 1962*b*). It might therefore be anticipated that there would be a correlation between nicotinic acid requirements and riboflavine requirements, but none is found (data of Table 5). Nor is there a correlation with casein needs, although increased dietary casein raises the nicotinic requirement within a strain (Sang, 1962*b*). However, the lower casein requirements of the hybrids, compared with their parents, are paralleled by similar lower requirements for nicotinic acid. These two needs may be causally related, and the lower nicotinic acid requirements of the hybrids may depend on their higher efficiency in utilizing dietary casein.

Table 6. *Requirements of the genotypes for nicotinic acid, and the effects of reducing its supply to 3 μ g. per culture*

Genotypes	S	C	S/C	R	N	R/N	SC/RO
Minimal requirements in μ g.	7.5	11.5	8.8	14.0	16.0	12.5	12.0
Per cent survival loss	44	12	24	7	65	49	45
Development time delay (100 \times log days)	13	17	14	17	—	16	20

(vii) *Pyridoxine*

Since the responses to casein suggest that hybrids make better use of near-optimal supplies of casein than their parent lines (pp. 52–55) it is of particular interest to see if the relationship is also reflected in requirements for pyridoxine, the vitamin most involved in protein metabolism. Reduction of pyridoxine from the standard 50 μ g. to 0.8 μ g. per culture scarcely affects survival or weight, although both decline with smaller amounts. As with riboflavine, the response to deficiencies of this vitamin is first by prolongation of development time, as indicated in Fig. 5. The minimal requirements of pyridoxine for normal growth are similar for each genotype, except for line R which seems to require less than the others. Corrected for weight and development rate differences (Table 5) one 2-way cross is found to require less (S/C), and the other (R/N) more, pyridoxine than its parent lines. The 4-way requires somewhat more than the R/N cross. If line R is excluded as clearly exceptional, there is a high correlation ($r = 0.92$, $p < 0.01$) between the minimal pyridoxine requirement and the optimal casein provision for the genotypes (data of Table 5). Thus the pyridoxine requirement seems directly determined by the quantity of casein needed by each genotype. The R line needs much less pyridoxine than its casein requirement would imply, but this is not reflected in its crosses.

Figure 5 shows that the majority of genotypes respond in much the same way to suboptimal supplies of pyridoxine. The most obvious exception to this general trend

is line N, which declines in efficiency more rapidly than the other genotypes (*cf.* its reactions to departures from an optimal casein supply). There is no evidence that the two-way crosses are less affected than the pure lines, but the 4-way cross does seem to be most resistant to the effects of sub-optimal pyridoxine.

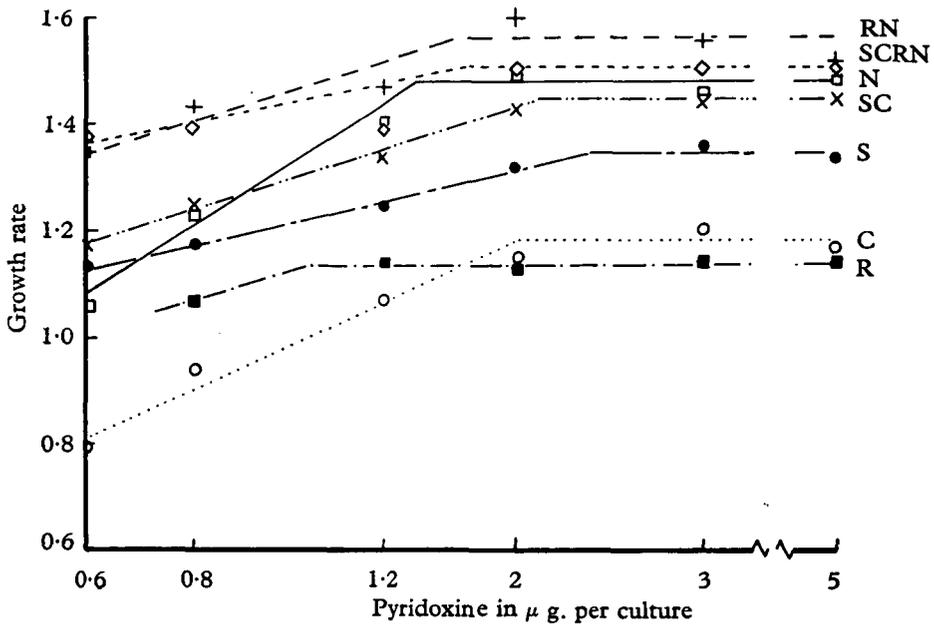


Fig. 5. Growth rates of the lines and crosses on different levels of pyridoxine supply.

(viii) *Folic acid*

Omission of folic acid from the standard diet has little effect on the weight of such flies as may hatch, but it slows their growth and, generally, causes a high proportion of pupal mortality. The S line is only marginally affected in this latter respect (Table 7) and the R line most. The strain crosses are superior to the mean of their parents in their abilities to survive on zero folic acid, but the 4-way cross is inferior to its parents.

The requirements of the genotypes for full growth also differ as do their reactions to reduced supplies of folic acid (Fig. 6). Line R and its cross with line N appear to require very much more folic acid than the other genotypes. These high requiring genotypes (Table 5) are the two most affected by omission of dietary RNA (Table 3) suggesting a relationship between the two requirements. However, line R is not the one most affected in development rate by withdrawal of dietary folic acid (Table 7), nor is its efficiency greatly affected.

Comparison with the effects of withdrawal of RNA (Table 3) shows that the two do not act in the same way; development of line N, for instance, being slowed by more than the average on zero RNA, but by considerably less than average on zero folic

acid. All three hybrids respond in very much the same way to omission of folic acid (Table 7): the parent lines have diverse reactions.

There is evidence (Sang, 1956) that *Drosophila* larvae can synthesize folic acid for themselves, but at a limited rate inadequate for normal growth. Omission of folic

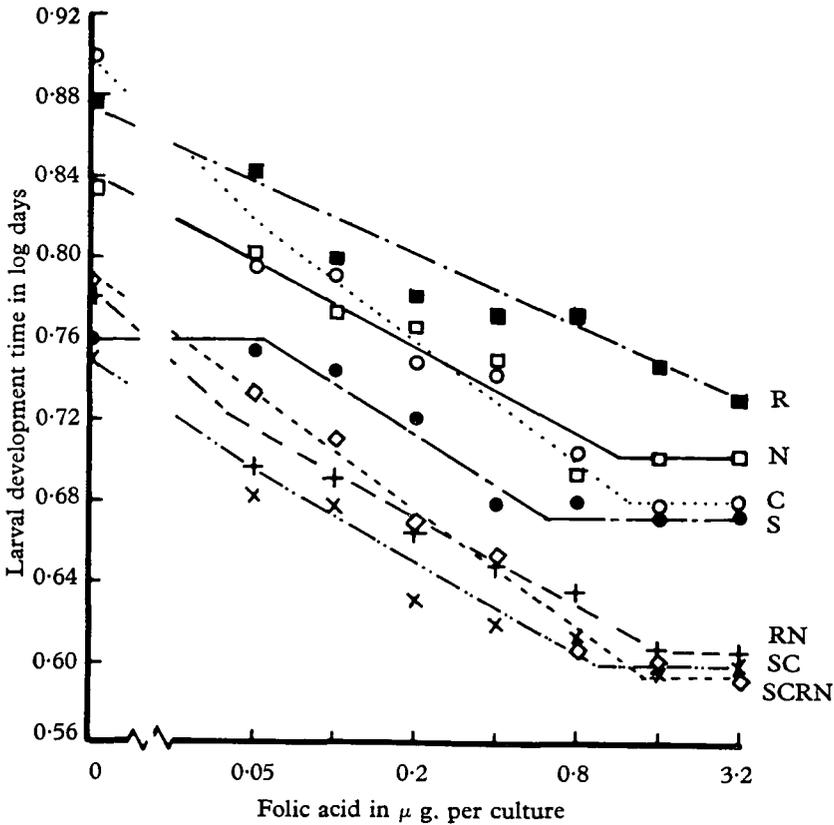


Fig. 6. Larval development times of the genotypes on different levels of dietary folic acid.

acid raises their dietary requirement for RNA, and *vice versa* (unpublished observations), indicating that even when optimal RNA is provided the vitamin is being used extensively for RNA synthesis. Consequently, it is not surprising that there is a correlation between folic acid and RNA requirements for optimal growth ($r = 0.703$, significant at $p = 0.05$: data of Table 5). The S line is exceptional in that its folic requirement is considerably less than would be anticipated from its RNA

Table 7. Effects of reducing folic acid from 0.4 μ g. to zero per culture

Genotypes	S	C	S/C	R	N	R/N	SC/RN
Per cent survival loss	0	37	12	50	30	31	57
Development time loss (100 \times log days)	8	15	13	10	9	13	14

requirement. However, as folic acid is also needed for at least the metabolism of glycine-serine by *Drosophila* (Sang, 1962*b*), a high correlation with RNA needs is not to be expected.

4. DISCUSSION AND CONCLUSIONS

While the present study was planned to provide some information about the biochemical characteristics of hybrids, it was always recognized that nutritional methods could indicate only those aspects of the metabolism of the organism which might be worth examining in greater detail, using more refined procedures. The limitations of nutritional manipulation will now be obvious, but it is interesting that the technique has shown how each genotype has different requirements and, consequently, that there is no 'standard food medium' optimal for all genotypes. It follows that the experiments have been performed under conditions which are sub-optimal for each genotype, in one way or another (cf. Tantawy, 1961). No doubt this is always so, but it is worth emphasizing that the genotypes examined may have not only different optimal (or minimal) requirements for the nutrients tested but also different responses to sub-optimal supplies. There is no evidence either that crosses are less stringent in their nutritional needs or that they are less affected by departures from optimal dietary provisions.

There is little to be gained from summarizing the detailed nutritional characteristics of the lines since another sample of inbreds would provide, presumably, a different spectrum of needs. It is worth noting that these characteristics are frequently subtle; for instance, protein deficiency has little effect on the growth rate of the C line but greatly reduces its weight, whereas the N line reacts in the converse fashion. Nor are there any evident relationships between the requirements of the lines and those of their simple crosses. The S/C cross, for example, requires less nicotinic acid, but considerably more riboflavine, than the mean of its parents. And so on. The only obvious generalization is that these hybrids make better use of casein (i.e. of a particular amino acid balance) than their parent lines (Table 5); consequently they tend also to need less pyridoxine, though the R line is anomalous in this respect, indicating the danger of generalizing from information obtained for a restricted range of parents. Similar anomalies in requirements of strains have been found for other species (Williams, 1956; Hutt, 1961).

Except for casein, heterosis is not found among the optima determined for nutrient requirements (Table 5). The casein requirement of the S/C hybrid is about 11% less than its mid-parent, of the R/N hybrid about 20% less and of the SC/RN about 4% below its mid-parent level. This at once suggests that ability to utilize dietary protein effectively is the main distinguishing characteristic of the hybrids. There is not a significant correlation between development time and casein requirement, but there is one between adult male weight and RNA requirement. Rather surprisingly, this is negative ($r = -0.84$), indicating that the heavier genotypes make more efficient use of the nucleotides available to them. Deficiencies of the casein supply affect the crossbred populations relatively more than they do the

inbreds (Fig. 1), and this also tends to be true of their reactions to reduced RNA (Fig. 2). There are clear differential effects of departures from optimal nutritional conditions on the performance of the various genotypes, and the crosses are not better 'buffered' in this respect. None the less, hybrid efficiency in growth rate is superior, and this seems to be reflected, if only approximately, in the superiority of their protein metabolism. The data do not exclude the possibility that other metabolic systems may also be involved; but it seems unlikely that nucleic acid synthesis, transmethylation or energy transfer systems play a part.

Table 8. *Within-culture variance of development time, when the genotypes are reared on sub-optimal diets*

Sub-optimal nutrient	S	C	S/C	R	N	R/N	SC/RN
Casein 2.5%	18	27	37	33	35	45	58
RNA zero	84	436	108	—	16	—	64
Choline 40 μ g.	16	22	17	18	—	32	15
Riboflavin 1.5 μ g.	24	15	41	116	40	59	27
Nicotinic acid 2.5 μ g.	31	20	16	17	—	36	47
Pyridoxine 0.6 μ g.	28	—	26	31	28	22	23
Folic acid zero	30	73	34	—	17	31	41
Optimal diet	24	50	11	36	36	24	27

The samples range between 30 and 100, depending on survival as indicated in preceding Tables: dashes indicate survival below 5%.

The variability of the genotypes when reared on sub-optimal diets has not been stressed in the text since previous observations (Sang, 1962*a*) have shown that all genotypes may change their relationships under these treatments, just as they show different average reactions to deficiencies (see also Prabhu & Robertson, 1961). Table 8 summarizes the data for development time, and it indicates only too clearly the sensitivity of some genotypes to deficiency states. These gene-environment interactions illustrate the difficulty in the way of estimating the environmental component of phenotypic variance from inbred lines and F_1 's, and emphasizes the fallacy inherent in the assumption that all inbreds and F_1 's are equal in this respect.

The importance of genetic constitution in relation to variability might be expected to be evident in the 4-way cross population, which is equivalent to a random mated, full-sib family (Falconer, 1953); but this population is not found to be more variable than the single-crosses (Tables 1 and 8). Two explanations seem possible. First, the 4-way will be homozygous only for such alleles as are common to all parent lines, whereas the 2-way crosses will be homozygous for alleles common to two parent lines. In so far as hybrid vigour depends on dominance (or on overdominance) the 4-way cross would then be at an advantage. Alternatively, since hybrid mothers of a 3-way cross lay eggs which have a higher hatchability than eggs laid by inbred females mated to hybrid males (unpublished observations), it seems probable that the eggs formed by hybrids are qualitatively superior to those laid by inbreds, and

that the larvae which hatch from them are then initially more viable and less responsive to early environmental stresses. Such a maternal effect could therefore also account for the unexpectedly low variability of the 4-way cross.

When judged by the more complex measures of survival, development time and size, the general superiority and lower variability of hybrids has suggested that they also have 'a reduced sensitivity to environmental fluctuations' (Robertson & Reeve, 1952). The data tend to confirm this generalization (Table 1), when the fluctuations represent uncontrolled, minor departures from a near-optimal environment. On the other hand, major divergences due to specific nutritional deficiencies may affect the hybrids as greatly as they do their parent inbreds, and some inbreds may be more resistant to particular changes than hybrids (Table 8). The general conclusion suggested by the data is, then, that the particular genetic constitutions of the inbred lines, and of their crosses, are of paramount importance in specifying their reactions to different nutrient supplies.

SUMMARY

1. The problem of whether or not hybrids are more efficient, less variable and have less stringent nutritional needs than their parents is examined by finding the dose-responses of four inbred *Drosophila* lines and their crosses to casein, choline, RNA, riboflavine, nicotinic acid, pyridoxine and folic acid, under germ-free conditions.

2. Under more or less optimal conditions, survival, development rate and weight of the hybrids are generally superior to those of the inbreds; the 4-way cross is not inferior to the 2-way crosses. Variability of the crosses is not necessarily lower than that of the inbreds, and the 4-way cross is no more variable than the 2-way crosses.

3. As measured by growth rate, the hybrids use casein more effectively than the pure lines, but their relative efficiency declines as the casein supply is decreased. There is a positive correlation between casein requirements for optimal growth and minimum requirements of pyridoxine. Hybrids also tend to be more efficient in their use of choline, but not of the other nutrients examined.

4. Deficiencies of particular nutrients (and also of excess provision of the non-vitamins) affect the lines and crosses differently, so that their relationships to one another are altered. The hybrids show no special advantage in resisting departures from the optimum. Variability is also changed significantly under sub-optimal conditions and, in some situations, the hybrids may then be more variable than the inbreds.

5. Each line and each cross is found to have its own optimal nutritional environment, and its particular reactions to departure from this. The full potential of the genotypes cannot be manifest, therefore, by tests in a single, standard environment.

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