INHERITANCE OF THE DIETARY RIBONUCLEIC ACID REQUIREMENT OF *DROSOPHILA MELANOGASTER*¹

B. W. GEER

*Department of Genetics, University of California, Davis*²

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D*ROSOPHILA melanogaster* larvae grow at a near maximal rate on a synthetic diet when yeast ribonucleic acid (RNA) is supplied. Investigations (HINTON 1956; SANG 1957; GEER 1963) have shown that RNA can be replaced in the diet by its nucleotide derivatives. Interstrain variability in the dietary RNA requirement is significant (SCHULTZ and SERVICE 1951); as a consequence the impact of dietary-inclusions and omissions of nucleotides varies markedly, depending upon the strain examined.

SANG (1957) has found that the viability of the Oregon-S strain is nearly normal when RNA is omitted from the diet. Adenylic acid increases the larval growth rate but guanylic acid is only slightly effective as a growth promoting substance. The inclusion of the pyrimidines with adenylic acid strengthens the adenylic acid effect, but in the absence of adenylic acid the pyrimidines exert no apparent growth effects. The penetrance of the *tu*^K allele of Oregon-K is dependent upon the nucleotide content of the medium (SANG and BURNET 1963). HINTON's RNA derivative analysis using the Oregon-R strain (HINTON 1956) coupled with the discoveries of a number of adenine requiring strains by his laboratory (HINTON, ELLIS, and NOYES 1951; HINTON and ROBERTS 1952; HINTON 1955, 1959) point to the importance of adenine in *Drosophila* nutrition.

GEER (1963) has shown that the Canton-S wild-type strain has a near absolute requirement for yeast RNA when casein is employed as the nitrogen source. However, the requirement becomes only a partial one when the nitrogen source is altered by adding various amino acids, changing the concentration of casein, or using proteins other than casein. Further examination of the Canton-S RNA requirement revealed that the pyrimidine derivatives increase larval viability, whereas the purine components accelerate the larval developmental rate. Addition of the four nucleotide derivatives to the diet in the proper proportions improves both viability and growth rate, thus completely replacing whole RNA. The present study is concerned with the examination of the genetic mechanism which controls the dietary RNA requirement of the Canton-S strain.

MATERIALS AND METHODS

In addition to Canton-S, the studies used the Oregon-R, Riverside, and Bikini wild-type strains. Each has been characterized by its ability to utilize dietary RNA (GEER 1963). Strain charac-

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² Present address: Department of Biology, Knox College, Galesburg, Illinois.

teristics are summarized in Table 1. The requirement was assessed by the percentage of larvae that survived on unsupplemented medium, whereas the response was evaluated by two criteria. One, viability, was the increase in larval survival when 1.0 mg RNA/ml was added to the medium, the second, developmental time was the reduction of the developmental period from egg to pupation by RNA supplementation. Each strain was unique in its utilization of RNA, being quantitatively different from every other strain for at least one of the three measureable traits.

For growth comparisons eggs were sterilized, and hatched larvae were grown axenically on synthetic medium at 25°C. The composition of the basal medium and techniques were as described previously by GEER (1963). Unless otherwise indicated, RNA deficient medium was the basal medium without RNA; complete medium was the basal medium supplemented with 1.0 mg RNA/ml. Yeast RNA and nucleoside-3'(2')-phosphoric acids were obtained from the California Corporation for Biochemical Research. The minimal levels of nitrogen and phosphorus in the yeast RNA were 15 and 9 percent respectively, while the moisture content was less than 7 percent. The RNA gave a negative biuret test.

To avoid variability in preparation, media were prepared and autoclaved together before each experiment. Where long periods of time elapsed before use, culture vials were sealed to avoid desiccation and stored at 4°C.

Hybridization, backcrossing, selection, and chromosome substitution were the genetic methods employed in the experimental work.

EXPERIMENTS

Hybridization: Reciprocal crosses between the RNA dependent Canton-S strain and the nonRNA requiring Oregon-R strain (Table 2) yielded dissimilar results. Viability of larvae from the cross of Oregon-R females to Canton-S males was

TABLE 1

The requirement for and response to dietary RNA of wild-type strains

Strain	Requirement	Response	
		Viability	Development time
Canton-S	++	++	++
Riverside	+	++	++
Oregon-R	—	—	—
Bikini	—	+	+
Oakland	—	—	+

Strong, ++; measureable, +; negligible, —.

TABLE 2

Growth of hybrid larvae on RNA deficient medium

Females × Males	Number of experiments	Number of larvae	Percent larvae to pupation	Percent larvae to adulthood	Development time ± s.d.
OR × CS	5	206	32.5	28.2	7.7 ± 0.9
CS × OR	6	243	13.9	13.6	8.0 ± 0.9
CS × CS	7	266	3.7	1.8	9.0
OR × OR	5	146	71.9	56.8	8.8 ± 1.4

almost twice as great as that of the offspring of the reciprocal cross. The difference appeared to lie in sex-linked genetic factors, there being more viable male offspring than female offspring when Oregon-R females were used in the cross. The sex ratio was normal when Canton-S females were the maternal parents, but the viability of the offspring was only half that of the offspring of the reciprocal cross.

Larvae from the cross of Oregon-R females to Canton-S males were cultured on four dietary levels of RNA; 0, 0.1, 0.5, and 1.0 mg RNA/ml (Figure 1). The performance of the hybrid larvae was approximately what would be expected on the basis of an additive genetic scheme, the only exception being the growth response at 0.5 mg RNA/ml. The optimal response at this level of RNA suggests the dominance of certain Canton-S alleles under these particular dietary conditions.

Backcrossing: Oregon-R/Canton-S females were backcrossed to Canton-S and Oregon-R males and the growth of the progeny was examined at four dietary RNA levels (Figure 2). If epistatic relationships of gene loci are prominent in the determination of the requirement, one would expect backcross progeny to closely resemble the parent strains, more so than if additive relationships predominate. Results were indicative of epistatic relationships. Canton-S backcross larvae were very similar to the Canton-S parental line in their utilization of RNA, surviving somewhat better than Canton-S on unsupplemented medium and slightly less at 1.0 mg/ml. Oregon-R backcross larvae also resembled the parent strain, surviving quite well without dietary RNA, but the data in gen-

FIGURE 1.—The frequency of larvae which pupate when cultured on 5 percent casein media supplemented with 0, 0.1, 0.5, and 1.0 mg RNA/ml. Canton-S is indicated by a solid line, Oregon-R by a segmented line, and the hybrid larval response by a segment-dot line.

FIGURE 2.—The frequency of larvae which pupate when cultured on 5 percent casein media supplemented with 0, 0.1, 0.5, and 1.0 mg RNA/ml. The Canton-S larval response is indicated by a solid line, Oregon-R by a long segment line, progeny of hybrid females crossed to Canton-S males by a short-segment line, and progeny of hybrid females crossed to Oregon-R males by a segment-dot line.

eral were more suggestive of additive relationships between Oregon-R genetic elements.

An extensive backcross experiment was performed with the Canton-S and Riverside strains. Canton-S was marked with a scarlet eye color mutant (st^{cs} , 3-44) which arose spontaneously in the Canton-S stock and which did not greatly alter the nutritional characteristics of the strain. By using this marker both backcross lines could be grown in the same cultures, thus minimizing environmental influences. After the initial cross and backcross, females were chosen by eye color, for backcrossing to the parental lines. Flies were grown on ordinary cornmeal medium; in each generation egg samples were collected and larvae were cultured on RNA deficient medium. This was continued for three backcross generations. Thus, the proportion of foreign genome was diluted at known rates in each backcross line. When Riverside males were mated to hybrid females, the percent of the genome contributed by the Riverside strain increased from 50 to 75 at the first backcross, from 75 to 87.5 at the second backcross, etc. Employment of Canton-S males had an inverse effect upon the hybrid genome.

The heritability of the RNA requirement was evidenced by the backcross results (Figure 3). Viability of the hybrid larvae was somewhat less than the midpoint between the parental lines. However, this value was, necessarily, the mean of the reciprocal crosses of the Canton-S and Riverside strains, since X-chromosome factors are important in the determination of the RNA requirement. Genetic factors which determined the difference in the RNA requirement between the two strains appeared to be additive, as evidenced by the relatively linear pattern produced by successive reductions of the foreign genetic material in the two backcross lines. Each of the three backcrosses markedly increased or decreased the frequency of genes contributing to the larval viability. Results were similar whether viability was measured by survival to pupation, or by emergence.

Selection: Selection was practiced on RNA deficient medium for two traits: increased larval viability, and more rapid larval development. The selection line was established by choosing RNA deficient cultures containing viable larvae and mating the emerging males to unselected females. Each generation, males in RNA deficient cultures were selected on the first day of imago eclosion and were mated to female progeny of the previous selection generation, which had been multiplied in mass culture on cornmeal medium.

The response to selection was immediate for increased larval survival on RNA deficient medium (Figure 4). Within three generations of selection, survival to pupation had increased from 23 to 72 percent, but it leveled off at this point. Following the fourth generation of selection, selection was relaxed for one generation. No loss in survival resulted from mass mating, but the percentage of larvae reaching adulthood decreased slightly. Variability of the developmental time was strikingly increased by the relaxation of selection. The succeeding generations of selection resulted in viability increases, and reduced developmental time variability to its former level. Throughout the experiment, selection for more rapid development was unsuccessful.

Chromosome substitution: The distribution of the genes determining the

FIGURE 3.—The viability of backcross larvae as measured by the percent of larvae which survive to pupation (open circles) and to adulthood (open triangles) when cultured on 5 percent RNA deficient casein medium. The solid lines represent backcross generations to the Riverside strain, whereas the segmented lines represent backcross generations to the *stCS* strain.

FIGURE 4.—Selection for early emergence and improved larval viability on a 3.5 percent RNA deficient casein diet. Developmental time (lower graph, solid line), percentage of pupating larvae (upper graph, broken line), and percentage of larvae to emerge as adults (upper graph, solid line) are indicated. Each generation except 1 and 5 represents a selection generation. The standard deviation of the developmental time is shown by a vertical line at each generation.

Canton-S RNA requirement was investigated using $\gamma^{51} sc^8 In-S \gamma^{3P}; al^2 Cy lt^3 cn^2 sp^2/dp b Pm; ru h DcxF ca/ Sb In(3R)Mo$, an X, second, and third chromosome marker strain permitting no crossing over in these chromosomes. For convenience the genotype of the marker strain is abbreviated to $\gamma; Cy/Pm; D/Sb$.

F₁ progeny from the cross between Canton-S and the marker strain were backcrossed to the Canton-S strain, and F₂ females heterozygous for a single marker were collected. These females in turn were mated to Canton-S males, and the progeny were cultured on RNA deficient and on complete media. The experiment was repeated with females heterozygous for the γ , *Pm*, and *Sb* marker chromosomes, so that the influence upon the RNA requirement of one foreign chromosome at a time was observed.

Development of progeny from the marker/Canton-S matings (Table 3) provided the following results. All matings produced a greater percentage of surviving offspring on RNA deficient medium than the parental Canton-S strain, with the mating involving the X-chromosome marker strain exhibiting the greatest effect. On RNA deficient medium marked surviving offspring significantly outnumbered unmarked survivors in the progeny of two of the three crosses; female

larvae heterozygous for the *Pm* chromosome were relatively inviable, reducing the marker class to the level of the wild-type progeny. The sex ratio of the surviving offspring of all matings was distorted owing to the low viability of marked females. Chi-square analysis showed that the sex ratio of the wild-type offspring of the crosses was normal, whereas the sex ratio of the marked offspring deviated significantly from normal ($P < 0.005$). Inclusion of RNA in the diet improved the viability of the progeny of all crosses and returned the sex ratio to normal.

Chromosome substitution strains of the Canton-S and Bikini strains differing in one and two pairs of chromosomes were derived by a series of pair-matings. γ ; *Cy/Pm*; *Sb/D* males were mated to Canton-S and Bikini females. From these crosses heterozygotes were selected to be mated, so as to maximize the frequency of the desired F_2 genotypes. For example, $\gamma/+$; *Cy/+*; *D/+* $\times$ $+$; $+$; *Cy/+*; *Sb/+* was used to obtain $\gamma/+$; $+/+$; *Sb/D* females and $+$; $+/+$; *Sb/D* males which were then mated. Cultures showing no yellow males were used to propagate the stock.

Stocks of the following genotypes were derived from both the Canton-S and Bikini strains: (1) $+/+$; $+/+$; *Sb/D* (2) $+/+$; *Cy/Pm*; $+/+$ (3) γ/γ ; $+/+$; $+/+$ (4) $+/+$; *Cy/Pm*; *Sb/D* (5) γ/γ ; $+/+$; *Sb/D* (6) γ/γ ; *Cy/Pm*; $+/+$.

The Y chromosome came from the marker stock, and the fourth chromosome was randomized in all stocks.

Each Canton-S derived stock was crossed to a Bikini derived stock, and F_1 progeny were selectively mated to maximize the chances of acquiring the unmarked genotype. Stocks 1, 2, and 3 derived from Canton-S were crossed to Stocks 4, 5, and 6 derived from Bikini, and similarly Stocks 1, 2, and 3 derived from Bikini were crossed to Stocks 4, 5, and 6 derived from Canton-S. The genomes given in Table 4 were established from the F_3 offspring and compared nutritionally. The control genomes were Canton-S and Bikini genomes which had been taken apart and resynthesized in the same manner as the substitution genomes.

Growth comparisons consider the response of various chromosome combina-

TABLE 3

Growth with (+) and without (—) dietary RNA, of Canton-S larvae which are heterozygous for single marked chromosomes

Cross	Supplement	Number of larvae	Percent larvae to pupation	Percent larvae to adulthood	Development time	Mutant/+	Sex ratio
<i>Sb/CS</i> $\times$ CS	—	287	35.8	21.6	8.2	1.8	1.5
	+	147	72.1	66.6	8.4	1.0	1.1
<i>Pm/CS</i> $\times$ CS	—	164	32.9	25.6	7.7	1.0	3.3
	+	89	79.7	67.4	7.6	1.5	1.1
γ/CS $\times$ CS	—	188	62.2	43.6	8.0	1.6	1.8
	+	110	97.2	90.0	7.6	1.2	1.2
Canton-S	—	296	3.5	1.6	8.8	..	..

TABLE 4

Growth of the larvae of Canton-S (CS) and Bikini (B) chromosome substitution stocks on 3.5% RNA deficient casein medium and deficient medium supplemented with 0.51 mg/ml uridylic acid and 0.51 mg/ml cytidylic acid. The increase in frequency of emerging adults on supplemented medium is the nucleotide response

Stock	Number of experiments	Number of larvae	Percent larvae to pupation	Percent larvae to adulthood	Nucleotide response
<i>Supplemented</i>					
X, 2, 3-B	6	372	70.6	58.0	— 0.8
X, 2 B 3-CS	5	304	81.9	65.4	17.4
X, 3-B 2-CS	10	484	73.3	56.4	35.0
2, 3-B X-CS	5	242	69.8	46.2	16.3
X, 2, 3-CS	4	202	91.0	78.7	62.7
X, 2 CS 3-B	6	327	79.8	44.6	5.4
X, 3-CS 2-B	5	329	68.9	60.7	25.9
2, 3-CS X-B	3	109	88.9	78.8	47.6
<i>Unsupplemented</i>					
X, 2, 3-B	5	236	75.4	58.8	...
X, 2 B 3-CS	6	329	57.1	48.0	...
X, 3-B 2-CS	6	316	28.1	21.4	...
2, 3-B X-CS	4	157	49.6	29.9	...
X, 2, 3-CS	4	137	22.6	16.0	...
X, 2 CS 3-B	5	292	70.2	39.2	...
X, 3-CS 2-B	4	192	38.0	34.8	...
2, 3-CS X-B	3	141	37.5	31.2	...

tions to supplemented nucleotides, and the abilities of the different genomes to cope with an RNA deficient diet. The nucleotide response was the increase in percentage of larvae to survive to adulthood when uridylic and cytidylic acids were added to the diet. These nucleotides improve larval viability but have little effect upon developmental time.

A striking nucleotide response was exhibited by the Canton-S strain, whereas the response of the Bikini strain was slight. In contrast to their development on unsupplemented medium, Canton-S larvae not only survived better when fed pyrimidine nucleotides but they survived better than Bikini larvae. Substitution of the Bikini third chromosomes for their Canton-S counterparts increased viability almost to the level of the Bikini strain, but the nucleotide response was quite low. Replacement of the Canton-S second chromosomes with the Bikini counterparts strongly influenced survival and the nucleotide response. A weak suppression of the nucleotide response was the only effect of the Bikini X chromosome pair when placed in the Canton-S genome. Thus, the Bikini third chromosomes were the most effective in altering the nutritional traits of the Canton-S genome.

A second comparison was made between the alterations of the nutritional characteristics of the Bikini genome by the presence of a single pair of Canton-S

chromosomes. The Canton-S second chromosomes reduced viability to the greatest extent on RNA deficient medium and produced the greatest increase in the nucleotide response. Only a moderate response to the nucleotides was induced by two Canton-S X chromosomes, but larval viability on deficient medium was halved. Larval viability was suppressed only slightly by the Canton-S third chromosomes, while the nucleotide response was measureably increased. The Bikini genome thus was most sensitive to the introduction of the Canton-S second chromosomes and somewhat less sensitive to the X chromosomes. Greater differences between the influences of the individual chromosome pairs indicate that the genes determining the Bikini nutritional characteristics are less uniformly distributed throughout the genome than the genes determining the Canton-S traits.

DISCUSSION

The polygenic mode of inheritance of the RNA requirement is indicated by each of the experimental approaches. Substitution of chromosome pairs between the Canton-S and Bikini genomes indicates the nonrandom linkage of gene loci to the chromosome pairs of the RNA requiring and nonrequiring genomes. All of the major chromosomes contain influential gene loci however. Loci with prominent effects are linked to chromosome 3 of Bikini and to the X and second chromosomes of Canton-S. Reciprocal matings of Canton-S to Oregon-R also suggest the presence of important factors on the sex chromosomes.

Heterozygosity for single foreign chromosomes shows that single chromosomes may markedly affect nutritional characteristics and again reveals the polygenic nature of the RNA requirement. The Riverside-Canton-S backcross experiment furnishes evidence for a broader relationship of nutritional influence to the proportion of foreign chromosome material.

Under certain dietary conditions, gene interaction appears to be prominent in determining strain nutritional traits. The optimal growth response of Oregon-R/Canton-S hybrid larvae at 0.5 mg RNA/ml may be due to dominance of certain Canton-S alleles, but this is of minor impact in comparison with the apparent epistatic relationships revealed by the growth of larvae of the cross of hybrid females to Canton-S males. Offspring of the Canton-S backcross closely resembled the strain at all RNA levels. This is consistent with expectations based upon the epistatic action of a few gene loci.

Selection for improved larval viability on an RNA deficient diet as performed in the present investigation establishes the heritability of the nutritional requirement and minimizes the possibility of the cytoplasm being the major hereditary vehicle. The rapid response to selection again suggests that a limited number of gene loci exert major influences, whereas the 'plateau' reached after three selection generations implies that maximal growth is unlikely on an RNA deficient diet.

Lack of response to selection for more rapid larval development is of interest because it indicates the complexity of the relationships of the traits usually chosen to gauge growth response, i.e., larval viability, larval developmental

time, and adult body size. In this selection experiment larval viability and developmental time remained autonomous. SANG (1962) selected successfully for all three growth traits on both pyridoxine deficient and low casein media. ROBERTSON (1960) found that strains selected for large body size on low protein and diluted synthetic diets also developed more rapidly, but a strain selected on a live yeast diet did not exceed the control. It would thus seem that all diets do not expose the growth traits for simultaneous selection.

In conclusion, the polygenic inheritance of the Canton-S RNA requirement is very evident. The nonrandom distribution of loci on all major chromosomes and apparent dominance and epistatic relationships are indicative of a complex controlling mechanism. That a rigid nutritional requirement, such as exhibited by the Canton-S strain, may be polygenically inherited suggests the possibility that many of the nutritional requirement differences within species may have evolved by natural selection for polygenic systems.

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SUMMARY

The RNA requirement of Canton-S is under polygenic control. Chromosome substitution experiments with the Canton-S and Bikini strains established linkage of the genes controlling the RNA requirement with all major chromosomes. In Canton-S genes linked to the X and second chromosomes were predominant, while in the Bikini genome genetic factors of the third chromosome were most influential. Depending upon the strain to which it was crossed and the culture conditions, the Canton-S genome exhibited varied dominance and epistatic relationships with other genomes.

Selection for increased larval viability on an RNA deficient diet was practiced with success. After three generations of selection, viability was increased to approximately the same level exhibited by non-RNA-requiring strains, but subsequent selection produced no appreciable increase.

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