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# Isolation and characterization of deficiencies exposing the *rudimentary* locus of *Drosophila melanogaster*

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SUMMARY

The *rudimentary* locus of *Drosophila melanogaster* contains the structural information for the first three enzymes of the *de novo* pyrimidine pathway. Two new *rudimentary* (*r*) mutants have been isolated following mutagenesis with ICR-170. From complementation analysis and cytogenetical observation both were shown to be deficiencies which expose the *r* locus. $Df(1)r^{l9}$ is deleted from band 14D1 to 15D1 and $Df(1)r^{lD}$ from band 14B6 to 15A2. These deficiencies were combined with several characterized *r* alleles each giving a single enzymatic defect in one of the first three enzymes of the pyrimidine pathway. An unusual semilethal *trans* effect was observed in some but not all the combinations. The effect was not observed with a third smaller deficiency which also exposes the *rudimentary* locus.

## 1. INTRODUCTION

*Rudimentary* (*r*; 1–55·3) is a sex-linked recessive pleiotropic mutation affecting both wing phenotype and female sterility (Morgan, 1915). More recently, *rudimentary* mutants have been shown to be defective in any single one or all of the first three enzymes of *de novo* pyrimidine biosynthesis, namely, carbamyl phosphate synthetase (CPSase), aspartate transcarbamylase (ATCase) and dihydroorotase (DHOase) (Norby, 1973; Jarry & Falk, 1974; Rawls and Fristrom, 1975). Moreover Rawls and Fristom (1975) and Jarry (1979) have shown that the *rudimentary* locus encodes the protein concerned with the activities of these enzymes.

Pyrimidine auxotrophy is lethal in bacteria and lower eucaryotes in the sense that the organisms are unable to grow in the absence of pyrimidines. The situation is of course more complex in animals: the various tissues may have different needs in pyrimidine which can or cannot be met by *de novo* synthesis. In *Drosophila* the developing egg and the pupa are closed environments and as such could provide restrictive conditions. We reasoned, therefore, that it might be possible to isolate lethal *rudimentary* mutations. In the course of a large screening designed to test

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this hypothesis, two chromosomes were isolated which exhibited the expected phenotype. This paper reports the isolation and characterization of these lethals, which proved to be deficiencies of the *rudimentary* locus.

## 2. MATERIALS AND METHODS

### (i) *Genetic markers and strains*

The $v^{24}f$ stock used for the mutagenesis screening was obtained from M. Gans. The flies used in this paper have all been generated from a single male crossed to an attached-*Xvf* virgin. $Df(1)r^{D17}$ was isolated by M. Green; it is a small X-ray-induced deficiency cytologically deficient for chromosome region 15A1-5 (M. Green & G. Lefevre, personal communication). This chromosome is deleted for the *r* locus (Jarry, 1979) and one or more adjacent recessive lethal loci. It carries also the *v* and *f* markers. $Dp(1:4)r^+f^+$ is a small segment of the *X* chromosome, from band 13F to 15F, that is attached to a chromosome 4 (Green, 1962). Genetical tests show that the presence of two doses of this duplication is generally lethal. Mutants $r^1$, $r^{11}$, $r^{12}$, $r^{29}$, $r^{38}$ and $r^{45}$ were described by Carlson (1971) and biochemically characterized by Jarry & Falk (1974) and Rawls & Fristrom (1975). Markers are described in Lindsley & Grell (1968).

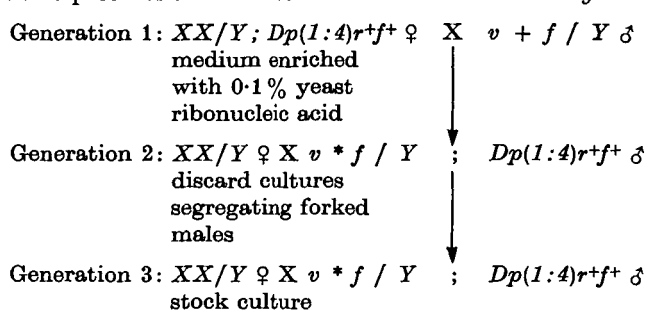
### (ii) *Growth medium*

The medium used is yeast cornmeal-dextrose. Flies were grown at 22 °C.

### (iii) *Mutant selection*

The protocol used in the selection of presumptive lethal-*rudimentary* mutations is presented in Fig. 1. $v^{24}$ *f* males were mutagenized by injection with 0·1% ICR-170. Eight lethals were isolated amongst approximately 1000 chromosomes. Among these, two, *l9* and *lD*, proved to be non-complementing with *r*.

Fig. 1. The protocol used for isolation of lethal *rudimentary* mutations




Vermilion forked males in generation 1 were injected with 0·1% ICR-170. Chromosomes bearing a lethal mutation, marked with an asterisk, in the chromosomal region covered by the duplication $Dp(1:4)r^+f^+$, will be recovered at generation 2 in phenotypically $f^+$ males. The male progeny of generation 3 will be exclusively $f^+$ if a lethal mutation has been induced on the *vf* chromosome, but both $f^+$ and $f^-$ in the other case.

### (iv) *Genetic characterization*

Genetic characterization is essentially based on the analysis of lethality and developmental delay exhibited by the isolated lethals and their interaction with other selected genetic markers. Since mutant selection was initially aimed at isolating lethal-*rudimentary* alleles, whenever possible *rudimentary* wing phenotype and female-sterility were also scored.

In general, progeny tests were carried out using at least ten culture bottles. In each bottle, crosses involved ten females and ten males. Female parents were allowed five days of oviposition and segregant female progeny were scored daily. Whenever possible, five culture vials, each containing ten females and ten males, were set to test the female sterility of the particular combination. Statistical handling of the data is as described by Naguib (1976).

### (v) *Biochemical characterization*

Wild-type and rudimentary-winged female progeny were harvested at daily intervals and frozen at $-80$ °C, until they could be assayed for CPSase, ATCase and DHOase activities. Methods of enzyme assay were those reported by Jarry (1979).

### (vi) *Cytology*

G. Lefevre established all the cytology described in this paper.

## 3. RESULTS

### (i) *Genetic mapping*

The isolated lethals were mapped by measuring recombination among male progeny of $v^{24}lxf/+r^{12}+$ ; $Dp(1:4)r^{+}f^{+}$ mothers, where *lx* stands for *l9* or *lD* (Table 1). Results for a third lethal (*l7*), isolated from the same mutagenesis screen yet rejected as an allele of *rudimentary* are also included. Clearly, a strong or perhaps complete linkage exists between *r* and both *l9* and *lD*, as evidenced by the absence of forked male progeny. *l7*, on the other hand, generates forked males

Table 1. *Results of crossing* vlx f / + $r^{12}$ + ; Dp(1:4)$r^{+}f^{+}$ *females with* v + f / *Y males*

| | *lx* | | |
|---|---|---|---|
| Phenotype of male progeny | *l9* | *lD* | *l7* |
| *r* (n.c.o.) | 177 | 171 | 198 |
| *vr* (s.c.o.) | 57 | 28 | 60 |
| *f* (s.c.o.) | 0 | 0 | 4 |
| *rf* (s.c.o.) | 2 | 2 | 5 |
| *vf* (d.c.o.) | 0 | 0 | 0 |
| *v* (t.c.o.) (n.c.o.); *Dp(1:4)r⁺f⁺* | 125 | 21 | 108 |
| *r* (d.c.o.) (n.c.o.); *Dp(1:4)r⁺f⁺* | 997 | 903 | 784 |
| Total | 1358 | 1125 | 1159 |

n.c.o, non-crossover; s.c.o, single crossover; d.c.o, double crossover; t.c.o, triple crossover.

and would, therefore, seem to map between *v* and *r* at approximately 1·34 centimorgans from the latter.

(ii) *Test of allelism of* l9, lD *and* l7 *to the* Df(1)r$^{D17}$ *region*

Complementation tests were performed between the lethals and *Df(1)r$^{D17}$* because the latter has been shown to be deleted for the *r* locus. For these tests, *Df(1)r$^{D17}$/FM7*; *Dp(1:4)r$^{+}$f$^{+}$* females were crossed to *lx*; *Dp(1:4)r$^{+}$f$^{+}$* males,

Table 2. *Results of crossing* v Df(1)r$^{D17}$f/FM7; Dp(1:4)r$^{+}$f$^{+}$ *females to* v lx f ; Dp(1:4)r$^{+}$f$^{+}$ *males*

| *lx* | Phenotype of ♀ progeny | Number of ♀ progeny | Mean* developmental time (days) ± S.D. |
|---|---|---|---|
| *l9* | *vf* | 0 | — |
| | *v* | 478 | 19·3 ± 2·7 |
| | *B* | 781 | 20·3 ± 3·3 |
| *lD* | *vf* | 0 | — |
| | *v* | 329 | 18·1 ± 2·5 |
| | *B* | 557 | 19·8 ± 3·6 |
| *l7* | *vf* | 341 | 19·1 ± 2·9 |
| | *v* | 481 | 19·8 ± 3·1 |
| | *B* | 776 | 19·3 ± 3·0 |

* Length of development from egg laying to adult in days ± standard deviation.

Table 3. *Results of crossing* vlxf; Dp(1:4)r$^{+}$f$^{+}$ *males with* vl(x)f/ClB; Dp(1:4)r$^{+}$f$^{+}$ *females*

| *lx* | Phenotype of ♀ progeny | Number of ♀ progeny | % relative* viability | Mean developmental time (days) ± S.D. |
|---|---|---|---|---|
| *l9* | *vf* | 0 | — | — |
| | *v* | 265 | 29·5 | 20·3 ± 3·1 |
| | *B* | 897 | — | 17·2 ± 3·0 |
| *lD* | *vf* | 0 | — | — |
| | *v* | 239 | 35·2 | 19·1 ± 3·9 |
| | *B* | 679 | — | 17·5 ± 3·7 |
| *l7* | *vf* | 0 | — | — |
| | *v* | 811 | 62·1 | 19·1 ± 3·9 |
| | *B* | 1291 | — | 18·2 ± 3·6 |

* % relative viability is the ratio of the *v* ♀ progeny to the *B* ♀ progeny multiplied by 100.

were *lx* stands for *l9*, *lD* or *l7*, and the results are presented in Table 2. The combination *lx/Df(1)r$^{D17}$* will have the phenotype vf, and the table shows that, while *l9/Df(1)r$^{D17}$* and *lD/Df(1)r$^{D17}$* were completely lethal, about 70% of *l7/Df(1)r$^{D17}$* survived. These results fit in with those of Table 1, in that *l7* can recombine with *r* to give *r$^{+}$* flies and is not lethal opposite the *Df(1)r$^{D17}$* deficiency, while *l9* and *lD* are lethal opposite this deficiency and do not recombine with *r*. The combinations *l7/l9*, *l7/lD* and *l9/lD* were completely lethal.

The three mutations are phenotypically different. Table 3 presents the results of the crosses between *lx*; *Dp(1:4)r⁺f⁺* males and *lx/ClB*; *Dp(1:4)r⁺f⁺* females. It appears clearly that, despite the presence of *Dp(1:4)r⁺f⁺*, the survival of both *l9/l9*; *Dp(1:4)r⁺f⁺* and *lD/lD*; *Dp(1:4)r⁺f⁺*, scored as v, is much lower than of *l7/l7*; *Dp(1:4)r⁺f⁺*, when compared to the heterozygous combinations scored as Bar-eyed. Moreover, these females show large delays in their development ranging from nearly a day in the case of *l7* to three days in the case of *l9*. Neither the low viability nor the developmental delay had been observed in the non-complementing combination *lx/Df(1)r$^{D17}$*; *Dp(1:4)r⁺f⁺* presented on Table 2.

Both *l9* and *lD* strongly enhanced the forked phenotype. In addition, *lD/lD*; *Dp(1:4)r⁺f⁺* females exhibited a strong minute phenotype and a scalloped-like wing phenotype in at least 70% of the adults. This phenotype ranged from a single nick in the medial edge of the wing to strong scalloping.

### (iii) *Cytogenetical analysis*

Salivary gland chromosome squashes from heterozygous females carrying each of the three mutations were prepared and analyzed by Dr G. Lefevre. He could see no obvious change in the banding pattern of the chromosome carrying *l7*, but the *l9* chromosome was deleted from band 14D1 to 15D1 and the *lD* chromosome from band 14B6 to 15A2.

### (iv) *Comparison with* Df(1)r$^{D17}$

In an attempt to draw a parallel between the already characterized deficiency *Df(1)r$^{D17}$* and the newly induced deficiencies, each lethal chromosome was combined with various *rudimentary* alleles chosen to represent the three complementation units described by Jarry & Falk (1974). Previous results had shown that each of these complementation units corresponded to one of the first three enzyme activities of the pyrimidine biosynthetic pathway, CPSase, ATCase and DHOase.

Table 4. *Results of crossing* Df(1)r$^{D17}$/Df(1)r$^{D17}$; Dp(1:4)r⁺f⁺ *Females with* r$^{x}$/*Y males*

| *r$^{x}$* | Enzyme defect | Phenotype of ♀ progeny | Number of ♀ progeny | % relative viability | Mean developmental time (days) ± S.D. |
|---|---|---|---|---|---|
| *r$^{45}$* | CPSase, ATCase, DHOase | *r* | 240 | 87·0 | 13·4 ± 2·3 |
| | | + | 276 | | 13·5 ± 2·4 |
| *r$^{12}$* | CPSase | *r* | 404 | 97·6 | 14·5 ± 2·3 |
| | | + | 414 | | 14·6 ± 2·4 |
| *r$^{11}$* | CPSase | *r* | 247 | 84·3 | 14·3 ± 1·8 |
| | | + | 293 | | 14·5 ± 1·9 |
| *r$^{29}$* | ATCase | *r* | 384 | 97·0 | 14·7 ± 2·3 |
| | | + | 396 | | 15·2 ± 2·4 |
| *r$^{38}$* | ATCase | *r* | 166 | 79·1 | 14·8 ± 2·3 |
| | | + | 210 | | 15·5 ± 2·4 |
| *r$^{1}$* | DHOase | *r* | 286 | 91·4 | 14·5 ± 2·2 |
| | | + | 313 | | 14·8 ± 2·4 |

Table 5. *Results of crossing* lx; Dp(1:4)r+f+ *males with* $r^v$/ClB *females*

| *lx* | $r^v$ | Phenotype of ♀ progeny | Number of ♀ progeny | % relative viability | Mean developmental time (days) ± S.D. |
|---|---|---|---|---|---|
| *l9* | $r^{45}$ | *r* | 116 | 11·5 | 15·8 ± 2·6 |
| | | + | 1005 | | 13·6 ± 2·3 |
| | | *B* | 1796 | | 14·6 ± 2·6 |
| | $r^{12}$ | *r* | 418 | 77·7 | 15·4 ± 1·9 |
| | | + | 538 | | 12·8 ± 2·3 |
| | | *B* | 1075 | | 13·8 ± 2·5 |
| | $r^{11}$ | *r* | 140 | 80·9 | 21·0 ± 1·5 |
| | | + | 173 | | 17·7 ± 2·1 |
| | | *B* | 303 | | 19·3 ± 2·4 |
| | $r^{29}$ | *r* | 19 | 2·0 | 18·1 ± 2·8 |
| | | + | 960 | | 16·4 ± 3·1 |
| | | *B* | 1681 | | 17·8 ± 3·2 |
| | $r^{38}$ | *r* | 61 | 15·0 | 21·2 ± 2·0 |
| | | + | 407 | | 17·4 ± 2·4 |
| | | *B* | 636 | | 19·2 ± 2·4 |
| | $r^{1}$ | *r* | 1 | 0·0 | — |
| | | + | 897 | | 19·7 ± 4·4 |
| | | *B* | 1691 | | 20·5 ± 4·3 |
| *lD* | $r^{45}$ | *r* | 234 | 35·9 | 16·0 ± 2·3 |
| | | + | 651 | | 13·7 ± 2·2 |
| | | *B* | 989 | | 14·6 ± 2·8 |
| | $r^{12}$ | *r* | 330 | 52·9 | 15·9 ± 1·8 |
| | | + | 624 | | 13·9 ± 2·7 |
| | | *B* | 1042 | | 14·1 ± 2·8 |
| | $r^{11}$ | *r* | 331 | 74·8 | 23·2 ± 2·1 |
| | | + | 448 | | 18·1 ± 2·2 |
| | | *B* | 830 | | 20·8 ± 3·5 |
| | $r^{29}$ | *r* | 75 | 9·6 | 19·8 ± 2·5 |
| | | + | 779 | | 16·1 ± 2·5 |
| | | *B* | 1284 | | 18·5 ± 3·5 |
| | $r^{38}$ | *r* | 67 | 19·7 | 2·2 ± 1·9 |
| | | + | 341 | | 17·1 ± 2·4 |
| | | *B* | 545 | | 19·4 ± 3·9 |
| | $r^{1}$ | *r* | 0 | 0 | — |
| | | + | 956 | | 18·5 ± 1·0 |
| | | *B* | 1805 | | 21·0 ± 4·6 |

* A delay of one day is significative at the 1% level.

Table 4 gives the results of a complementation test between $Df(1)r^{D17}$ and six different *rudimentary* alleles. None of these mutations can complement positively the deficiency. However, the mean developmental times for flies of genotypes $Df(1)r^{D17}/r$ and $Df(1)r^{D17}/r$; $Dp(1:4)r^+f^+$ are very similar, indicating that no deleterious effect is caused by any of the *rudimentary* alleles. On the other hand, when a similar experiment was performed with *l9* and *lD* chromosomes, it was observed that the relative viability of *lx*/*r* and *lx*/*r*; $Dp(1:4)r^+f^+$ progeny depen-

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ded upon the complementation unit to which the particular *r* allele belonged (Table 5). Flies heterozygous for both *l9* and *lD* chromosomes associated with CPSase⁻ *rudimentary* alleles ($r^{11}$ and $r^{12}$) exhibited a more or less normal relative viability (53–81 %, Table 5, column, 5), but showed a delay in development ranging from 3–5 days (column 7). By contrast, when associated with ATCase⁻ ($r^{23}$, $r^{38}$) or DHOase⁻ ($r^{1}$) alleles, both chromosomes strongly reduced the viability of heterozygous flies (compare Table 5, column 5 and Table 4, column 5). The *l7* chromosome had no effect either on survival or on developmental duration when tested under the same conditions (results not shown). The combination $lD/r^{1}$; $Dp(1:4)r^{+}f^{+}$ gave rise to 0·4 % mosaic adults, all showing a *rudimentary* wing with either a missing eye or a deformed leg. Similar mosaics have not been detected with any of the other combinations investigated.

### (v) *Pyrimidine biosynthetic enzyme activities*

Direct confirmation of the complementation data was obtained by measuring the activities of the three enzymes of the pyrimidine pathway coded for by the *rudimentary* locus (Jarry, 1979). These enzymes exist *in vivo* as a high molecular weight complex containing multiples of a single trifunctional polypeptide (Jarry, 1978).

Enzyme assays were carried out on females heterozygous for the deficiencies and $r^{45}$, a non-complementing *rudimentary* allele which exhibits low activity for the three enzymes simultaneously. The results of Table 6 indicate that although females heterozygous for a wild type chromosome and $r^{45}$ have, as expected, about half the activity of wild type flies, females heterozygous for the deficiencies and $r^{45}$ do not exhibit increased activity compared with homozygous $r^{45}$ females. This is direct evidence that both *l9* and *lD*, are defective in their *rudimentary* enzyme activities. Moreover, low activity for the three enzymes simultaneously is in perfect agreement with the observed *rudimentary* wing phenotype associated with survivors from the various genotypic combinations between the lethals and the various *rudimentary* alleles (see Table 5).

Table 6. *CPSase, ATCase and DHOase levels in females carrying* rudimentary *deficiencies*

| Genotype | Enzyme activities | | | N* |
|---|---|---|---|---|
| | CPSase† | ATCase‡ | DHOase‡ | |
| $r^{+}/r^{+}$ | 72·6 ± 3·2 | 47·5 ± 2·1 | 115·0 ± 5·0 | 5 |
| $r^{45}/r^{45}$ | 8·1 ± 1·0 | 12·2 ± 1·2 | 1·3 ± 1·0 | 5 |
| $r^{45}/r^{+}$ | 35·0 ± 2·4 | 28·2 ± 1·2 | 56·0 ± 4·8 | 5 |
| $r^{45}/Df(1)r^{D17}$ | 3·0 ± 1·2 | 5·0 ± 2·1 | 2·1 ± 1·2 | 5 |
| $r^{45}/l9$ | 2·6 ± 1·5 | 9·8 ± 2·4 | 2·1 ± 1·9 | 3 |
| $r^{45}/lD$ | 11·0 ± 3·8 | 8·5 ± 2·4 | 1·6 ± 1·0 | 3 |

* N is the number of different experiments.
† Picomoles citrulline produced in 30 minutes per mg of protein.
‡ Nanomoles carbamylaspartate produced in 30 minutes per mg of protein.

## 4. DISCUSSION

In this paper, we report the isolation and characterization of two mutations induced by ICR-170 and selected as lethal *rudimentary* alleles. Complementation analysis and cytological examination of the chromosomes indicate that both mutations are in fact deficiencies which expose the 15A1 band of the salivary gland chromosome, already shown to contain the *rudimentary* locus (Jarry, 1979). They should, therefore, be renamed $Df(1)r^{l9}$ and $Df(1)r^{lD}$.

ICR-170 is believed to produce mostly frameshift mutations in bacteria and fungi (Auerbach, 1976). Strong support for the view that this mutagen has similar effects in Drosophila is provided by the results of Carlson *et al.* (1967) and Rawls & Porter (1979). However Woodruff & Gander (1974) isolated temperature-sensitive lethal mutations in Drosophila after mutagenesis with ICR-170 and concluded that this chemical could also induce missense mutations. Our results demonstrate that ICR-170 can also induce chromosome rearrangements. The specificity of ICR-170 in Drosophila remains therefore to be established with a larger sample of mutations suitable for analysis at the molecular level.

The complementation patterns of the two deficiencies when associated with several *r* alleles characterized genetically (Carlson, 1971) and biochemically (Jarry & Falk, 1974; Rawls & Fristrom, 1975) exhibit some interesting characteristics which have not been described before. Females heterozygous for either $Df(1)r^{l9}$ or $Df(1)r^{lD}$ and a CPSase$^-$ *r* allele ($r^{11}$, $r^{12}$) have the same viability as siblings with an additional wild type gene copy. However, heterozygous combinations with either an ATCase$^-$ *r* allele ($r^{29}$, $r^{38}$) or to a greater extent a DHOase$^-$ allele ($r^1$) exhibit a large decrease of relative viability. These effects are not observed with another deficiency which also exposes the *rudimentary* locus, $Df(1)r^{D17}$, under the same experimental conditions. One must conclude that there is a *trans* effect associated with the newly isolated deficiencies but not with $Df(1)r^{D17}$. Comparison of the break points of the three deficiencies indicates that the only chromosomal region deleted in both $Df(1)r^{l9}$ and $Df(1)r^{lD}$ but not in $Df(1)r^{D17}$ is the interval 14D1 to 14F1. Experiments are in progress to isolate point mutations in this region in order to test whether they produce a similar phenotype in association with ATCase$^-$ and DHOase$^-$ *rudimentary* alleles.

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