Two New Eye Color Mutants in the Mediterranean Fruit Fly, *Ceratitis capitata*[1,2]

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ABSTRACT

Two new eye color mutants are described in the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). Light eye *(lt)* is an autosomal recessive gene linked to the previously described double chaetae *(dc)* locus. Purple eye *(Pr)* is an autosomal dominant and segregates independently from *dc* and *lt*. *Pr* is the first mutant described in this species which is dominant in expression.

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) is a major agricultural pest in many tropical and sub-tropical areas around the world. Interest in genetic studies of this species has begun to increase recently with the realization that such work is of potential importance in programs of population control (Rössler and Koltin 1976). The acquiring of a library of mutant genes is a basic first step in the genetic analysis of a species. In the Mediterranean fruit fly the complex eye color pattern has proven to be the most variable trait. This is not surprising, considering the example of *Drosophila melanogaster.* In *D. melanogaster,* eye variants (followed by bristle or hair alterations and wing shape or venation changes) predominate in one list of easily scored mutations (Strickberger 1962). In *C. capitata,* a series of eye color variants have been described (Sharp and Chambers 1973; Rössler and Koltin 1976; Carante 1981, 1982; Rössler 1982; Saul 1982a,b). To date all the mutant genes described have been autosomal and recessive in expression. In this paper two new eye color variants are described. One is an autosomal recessive, the other is the first autosomal dominant gene reported in this species.

MATERIALS AND METHODS

The flies used in this study were derived from a laboratory stock which had been kept in culture for 7–9 generations in the Entomology Department, University of Hawaii. The stock was originally founded with approximately 150 flies reared from figs collected by T. Wong (U.S. Dept. of Agriculture/Agricultural Research Service) at Kula, Maui, Hawaii. Larval and adult rearing methods are similar to those described in Rössler and Koltin (1976). Parental crosses involving mutant strains were made by sorting male and female flies into separate containers within 24 hours of eclosion. After 3–4 days the adults were placed together into a single container and 7 days later eggs were collected. Under the conditions of our laboratory (temperature range 23–27°C, relative humidity (RH) 65–75%) the egg to egg generation time is approximately 28 days. Flies were immobilized by etherization and examined under a fiber optic light of color temperature 3350°K.

RESULTS

Description of the light eye trait

The light eye trait first was observed in 2 male flies in the F_3 of a line derived from larvae treated with a 0.8% concentration of formaldehyde (volume of 37% formalin per volume of liquid used in larval medium). The normal eye color pattern

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of *C. capitata* is complex. There are red, blue, green, and yellow components in both fixed and reflective patterns (Carante 1981). This pattern varies with the intensity, color temperature, and angle of incidence of the light (Saul 1982b). In the pure breeding light eye stock the eye colors are all greatly reduced in intensity. The predominant color is a light pink-red. In the light eye stock, as in all eye color variants, wild-type and mutants, so far described in this species, the male eye has a more intense blue-violet central spot than the female eye. The light eye trait is easily distinguished from wild-type at all adult stages. Crosses to determine the genetic basis of light eye are shown in Table 1. The *lt/lt* homozygote is 100% penetrant and fully expressed.

Description of the purple eye trait

The purple eye trait first appeared in a female fly in the F_1 of laboratory colony flies exposed as mid pupae to 5000R of radiation from a cobalt-60 source. For several hours after eclosion the eye of the wild-type fly appears relatively dark in color in comparison to the translucent white body. There is also a distinct dark green reflective background color. Over a period of several hours the body darkens and assumes its adult appearance. The eye appears to lighten in color and the green background takes on blue, yellow and green reflective colors. From eclosion in purple eye flies the blue coloration of the eye is distinctly deeper and more intense, rather purple-violet than blue. Also, the green reflective background is more intense than wild-type and remains so throughout the adult stage. Purple is fully penetrant, but, somewhat variable in expression with the color ranging from a deep purple to purple-violet. The phenotype is always clearly distinct from wild-type. The deeper green background color of purple is the easiest difference to use for rapid sorting of flies. When flies from a true breeding purple line are crossed reciprocally to wild-type flies, the F_1 are all purple eye (Table 2).

Preliminary mapping of the lt and Pr genes

Reciprocal crosses were made of true breeding purple and light lines to the *dc/dc* (double chaetae) stock. The F_1 were intercrossed and the F_2 phenotypes scored. Since normal karyotype *C. capitata* males exhibit only 0.4–1.2% recombination (Rössler 1982) those intercrosses involving recessive genes like *lt* and *dc* can only distinguish loci on the same chromosome (with expected 1:2:1 F_2 ratio) from loci on different chromosomes (with expected 9:3:3:1 ratio). In crosses involving the dominant *Pr*, recombinant chromosomes from the female parent can be scored in the F_2, and a wide range of phenotypic ratios is possible depending on the crossover frequency. The results of crossing *lt* with *dc* and *Pr*, and *Pr* with *dc* are in Table 3.

DISCUSSION

Mode of inheritance of light (lt)

From the data in Table 1 it appears that *lt* behaves as an autosomal recessive gene. In genetic crosses with all known eye mutants except reflectionless and ruber (which were not available for testing) the F_1 phenotypes are wild-type. From the published descriptions it appears that *lt* differs phenotypically from ruber and reflectionless since it is not fluorescent or matte, respectively (Carante 1981, 1982). These observations indicate that *lt* is a previously undescribed mutant. It is given the genetic symbol *lt*.

Mode of inheritance of Purple (Pr)

The data in Table 2 indicate that *Pr* is an autosomal, dominant gene. This is the first dominant gene described in this species. It is given the genetic symbol *Pr*.

TABLE 1. Crosses to determine the genetic basis of the light eye trait in the Mediterranean fruit fly.

		No. of progeny			
		wild-type		light eye	
Type of mating	No. pairs	males	females	males	females
1) wild-type ♂♂ X light eye ♀♀	12	90	74	0	0
2) wild-type ♀♀ X light ♂♂	12	92	82	0	0
F_1 from cross 1*	mass mated	94	121	35	37
F_1 from cross 2*	mass mated	150	159	42	59

*The 4 offspring ratios (wild-type:light) in the F_2 do not differ significantly from a 3:1 ratio by chi-square analysis. (P = 0.05)

TABLE 2. Crosses to determine the genetic basis of the purple eye trait in the Mediterranean fruit fly.

		No. of progeny			
		wild-type		purple eye	
Type of mating	No. pairs	males	females	males	females
1) wild-type ♂♂ X purple eye ♀♀	12	0	0	104	109
2) wild-type ♀♀ X purple eye ♂♂	12	0	0	177	183
F_1 from cross 1*	mass mated	32	39	108	124
F_1 from cross 2*	mass mated	50	38	175	162

*The 4 offspring ratios (purple:wild-type) in the F_2 do not differ significantly from a 3:1 ratio by chi-square analysis. (P = 0.05)

Preliminary linkage analysis for light and Purple

The data from the first 2 crosses in Table 3 indicate that *lt* is linked to the *dc* locus of Rössler and Koltin (1976). The *dc/dc* genotype is incompletely penetrant and highly variable in expression (especially in females) and therefore difficult to score in genetic crosses. It has been shown that a pleiotropic eye facet alteration is associated with the *dc* locus and this trait is fully penetrant and constant in expression in both sexes (Saul and Rössler, unpublished data). In scoring the crosses involving *dc* we retained the nomenclature for the gene, but scored the phenotypes on the basis of the pleiotropic eye trait. No recombinants have yet been found between *lt* and *dc* which might suggest allelism. However the wild-type phenotypes of the F_1 flies would indicate that the genes are closely linked rather than allelic.

Pr appears to segregate independently from *dc* (Table 3). The purple eye/double chaetae flies when mated yield a population of F_3 flies which are all double chaetae

TABLE 3. Linkage analysis of the *lt, Pr,* and *dc* genes in the Mediterranean fruit fly.

Parental phenotypes	F_1 phenotype	Phenotypes of the F_2 progeny [a]					
		wild-type	light	purple	dc	dc/purple	X^2 (ratio)
lt/lt ♂♂ X *dc/dc* ♀♀	wild-type	205	112	n.a.	91	n.a.	(2:1:1) 2.17[n.s.]
lt/lt ♀♀ X *dc/dc* ♂♂	wild-type	195	98	n.a.	76	n.a.	(2:1:1) 3.82[n.s.]
Pr/Pr ♂♂ X *dc/dc* ♀♀	purple	29	n.a.	79	8	28	(3:9:1:3) 0.35[n.s.]
Pr/Pr ♀♀ X *dc/dc* ♂♂	purple	36	n.a.	69	7	24	(3:9:1:3) 5.41[n.s.]
Pr/Pr ♂♂ X *lt/lt* ♀♀	purple	114	148	368	n.a.	n.a.	(3:4:9) 1.24[n.s.]
Pr/Pr ♀♀ X *lt/lt* ♂♂	purple	110	134	371	n.a.	n.a.	(3:4:9) 4.60[n.s.]

[a]Offspring ratios in the F_2 did not differ significantly between male and female progeny and the numbers are pooled in this table. (P = 0.05)
n.a. = not applicable to this particular cross.
n.s. = not significant.

and approximately 90% purple and 10% wild-type. The F_2 purple/double chaetae flies are thus presumably of either *Pr/Pr;dc/dc,* or *Pr/+;dc/dc* genotype. An intercross of F_2 double chaetae flies yielded a true breeding *dc/dc* line.

The third group of crosses in Table 3 present some difficulty in interpreting. As mentioned in the Results section, intercrosses involving a dominant allele like *Pr* can produce scorable recombinant genotypes in the F_2 when crossed with a gene in the same linkage group. If *lt* and *Pr* are linked, the new genotypes in the F_2 would be *Pr/Pr;lt/+, Pr/+;lt/lt, Pr/+;+/+;* and *+/+;lt/+*. The phenotypic ratio would depend on the recombination frequency in females, r, with Purple, light, wild-type, and purple-light flies in expected frequencies of 0.75–0.25r, .25–.25r, .25r, and .25r, respectively. If *lt/lt* is linked to, and epistatic to *Pr* the expected frequencies would be .25 for light with no Purple-light flies. I believe that *lt/lt* is epistatic to *Pr/Pr* since light flies from the F_2 are true breeding while purple flies produce about 12% (also true breeding) light flies. This epistatic relationship means that the expected numbers of light flies is the same (25%) under both the linkage and independence models. The observed frequency of wild flies in the F_2 (18%) compares with a maximum possible frequency of 12.5% (when r = 0.5) under the linkage model.

If *Pr* and *lt* are on separate chromosomes, there are 9 possible genotypes in the F_2: *Pr/Pr;+/+, Pr/Pr;lt/+, Pr/Pr;lt/lt, Pr/+;+/+, Pr/+;lt/+, Pr/+;lt/lt, +/+;+/+,+/+;lt/+,* and *+/+;lt/lt* in a ratio of 1:2:1:2:4:2:1:2:1. Since *lt/lt* is epistatic to *Pr* the phenotypic ratio should be (9)Purple:(3)wild:(4)light. On the basis of the first 4 crosses in Table 3, *Pr* should segregate independently from *lt* since *Pr* is independent from *dc* and *lt* is linked to *dc.* The ratios observed in the last 2 crosses in Table 3, the high frequency of wild flies in the F_2, and the results of the crosses between *Pr* and *dc,* and *lt* and *dc* all support the conclusion that *Pr* and *lt* are unlinked.

One immediate goal for genetic research in the Mediterranean fruit fly is to construct a linkage map with at least one marker locus on each of the 5 autosomes and to find sex linked markers. To date we have not found a sex linked locus and there appear to be markers for only 3 of the 5 linkage groups (Rössler 1982). The addition of light eye and especially purple are steps along the process which has as its ultimate goal the development of genetically based autocidal techniques for this important agricultural pest species.

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