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Genetics of the screwworm fly

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Characterization of three eye mutants

Abstract: **Three spontaneous eye mutants of *Cochliomyia hominivorax*, the screwworm fly are described and characterized. The mutants, yellow eye (*ye*), orange eye (*or*), and white eye (*wh*), segregate independently and are recessive. Yellow eye is recessively epistatic to orange eye and dominantly epistatic to white eye. All three mutants have complete penetrance, are autosomal, and have little effect on fly viability under laboratory conditions.**

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DESPITE the screwworm eradication program being cited as one of the success stories of genetic control, genetic studies of *Cochliomyia hominivorax*, the screwworm fly, have been limited to the description of karyotypes and characterization of morphological mutants. LaChance and coworkers described and characterized 24 morphological mutants[7,8]. Unfortunately all of their lines were lost in the late 1960s. Evidence of reduced competitiveness of released flies due to genetic selection under factory rearing conditions[1] and reports of reproductively isolated "gamodemes" of screwworms in Mexico[13] rekindled interest in screwworm genetics. The existence of population substructuring was never substantiated[6,10–12] and all of the subject populations were eradicated during 1983–1984.

The potential benefits of implementing genetic technologies in the screwworm eradication effort are clear. Areas of special interest are genetic sexing mechanisms, production of genetically superior mass release strains, and genetic characterization of the parameters governing the effectiveness of a mass produced strain in the field. An adequate supply of well characterized genetic markers is needed in order that these and other program needs may be effectively addressed. A program to isolate and characterize genetic markers of screwworm flies has been initiated to supply the biological material required for further research on the genetics of this species. The present paper describes and characterizes three eye mutants of the screwworm fly. In addition, linkage and epistatic relationships between the three loci are discussed.

Materials and Methods

The yellow eye and orange eye phenotypes were first observed in the F_2 generation of colonies originating from isofemale field collections. Yellow eye was isolated from a line designated LH-31 from the state of Oaxaca on the Pacific Coast of Mexico. This line was out-crossed to increase vigor. The orange-eye phenotype was isolated from the line designated PA-34 from the state of Chiapas, Mexico. Both of these phenotypes have been maintained in pure breeding colonies. The white eye phenotype was observed in the F_3 generation of a cross between a single male of the LH-31 strain (yellow eye) and several females from the CIH-34 strain, a line with normal colored eyes from the state of Michoacan, Mexico. This phenotype was isolated and also has been maintained as a pure breeding colony.

Screwworm larvae were reared in a medium of either fresh horse meat supplemented with dried blood[2] or a gel medium consisting of a starch graft Polymer gelling agent, dried blood, dried milk, dried egg, and water[4]. Bacterial growth was controlled in both media by the addition of 0.12 percent formalin. Larvae were maintained at approximately 37° C. Mature larvae were allowed to pupate in sawdust. Adults were maintained in screen cages at approximately 25° C with honey provided as a carbohydrate source.

Crosses were made in 30 × 30 × 50 cm

cages with approximately 500 females and 250 males per cage. A small amount of fresh warm horse meat was provided as an oviposition substrate. Oviposition was induced by placing the females in a dark room with elevated temperature, 35° C. Females were allowed to oviposit collectively for F_1 and F_2 crosses. However, eggs were collected from individual females for the backcrosses. The progenies of the individual females were reared separately allowing each family to be scored separately and treated as a replicate. All flies were scored for eye color and sex within 24 hours of emergence.

Results

Yellow eye (ye)

Flies with the yellow-eye phenotype exhibited mustard yellow eyes as opposed to the dark brick red to brown eye color of normal screwworm flies. The dorsal ocelli appeared normal in color. The F_1 progeny of reciprocal crosses between yellow-eye flies and normal-eye flies had normal colored eyes. The results of the backcrosses between these F_1 flies and yellow-eye flies are presented in Table I. The ratio of normal-eye flies to yellow-eye flies did not differ from the 1:1 ratio expected if the yellow-eye phenotype were the result of a single recessive gene ($P > 0.05$). The *ye* gene segregated independently of sex in crosses with heterozygous males (crosses 1 and 2) ($\chi^2 = 1.13$ and 1.10, 1 *df*). The yellow-eye allele had complete penetrance and flies carrying this gene had good viability, 0.982 (observed mutants/expected mutants).

White eye (wh)

White-eye flies exhibited clear white eyes with normal colored dorsal ocelli. The F_1 progeny of reciprocal crosses between white-eye flies and normal wild-type flies had normal eyes. Three different eye colors were observed among the F_2 progeny of these crosses: normal—1498 flies; yellow—331 flies; and white—99 flies. These results approximate, but differ significantly from, the 12:3:1 proportion expected if *ye* was dominantly epistatic to *wh* (the dominant allele of the *ye* locus, normal-colored eyes, is epistatic to the *wh* gene; *ye wh*/*ye wh* = white eye, *ye wh*/+ *wh* = normal eye) ($\chi^2 = 8.28$, 2 *df*). Backcross results are presented in Table II, crosses 1–4. Again, three phenotypes were observed. The observed ratios of normal, yellow, and white-eye flies did not differ significantly from the 2:1:1 ratio expected if *ye* were dominantly epistatic to *wh* and the two genes were segregating independently ($P >$ 0.05). The ratio of male to female progeny in each of the phenotypic classes of the two backcrosses with heterozygous males (crosses 1 and 2) did not differ from the expected ratio of 1:1 ($\chi^2 = 1.49$ and 4.07, 2 *df*). In addition, white-eye flies were crossed reciprocally to yellow-eye flies of the LH-31 strain and then backcrossed to white eye (Table II, crosses 5–8). The F_1 progeny of these crosses had yellow eyes. The observed ratio in crosses 6, 7, and 8 did not differ significantly from the expected ratio of 1 yellow eye:1 white eye. The observed ratio of male to female progeny within the phenotypic classes differed significantly from the expected 1:1 ratio in the crosses with heterozygous males (crosses 5 and 6) ($\chi^2 = 4.65$ and 8.50, 1 *df*). However, the observed pattern was not that expected if the *wh* gene was sex linked. The *wh* gene had complete penetrance and flies carrying this gene had good viability, 0.965.

Orange eye (or)

Flies with the orange-eye phenotype exhibited reddish orange eyes and dorsal ocelli. The F_1 progeny of both reciprocal crosses between orange-eye flies and normal-eye flies had normal eyes. Results of backcrosses between these F_1 flies and orange-eye flies are presented in Table III. The observed ratios in three of the four crosses did not differ from the 1:1 ratio expected if orange eye was controlled by a single recessive gene, $P >$ 0.05. In the other cross an excess of orange-eye flies was observed. However, 25 of the 31 families in this cross did not differ significantly from the expected 1:1 ratio ($P >$ 0.05). The observed ratio of males to females within each phenotype in cross 1 did not differ significantly from the 1:1 ratio expected ($\chi^2 = 1.99$, 1 *df*). In cross 2, the second backcross with heterozygous males, a signifi-

Table I. Results of backcrosses for the mode of inheritance of yellow eye

Cross	Parental genotypes ♀♀ × ♂♂	No. fam.	Normal ♀♀	Normal ♂♂	Yellow eye ♀♀	Yellow eye ♂♂	χ^{2*} eye color
1	*ye*/*ye* × *ye*/+	18	615	606	635	584	0.00
2	*ye*/*ye* × +/*ye*	15	725	688	665	679	1.73
3	*ye*/+ × *ye*/*ye*	12	358	357	334	351	0.64
4	+/*ye* × *ye*/*ye*	11	391	408	382	373	1.25

* The expected ratio is 1 normal:1 yellow; the chi-square statistics have 1 degree of freedom

Table II. Results of backcrosses for the determination of the mode of inheritance of white eye

Cross†	Parental genotypes ♀♀ × ♂♂	No. fam.	Normal ♀♀	Normal ♂♂	Yellow eye ♀♀	Yellow eye ♂♂	White eye ♀♀	White eye ♂♂	$\chi^{2\ddagger}$ eye color
1	$\frac{ye\ wh}{ye\ wh} \times \frac{ye\ wh}{+\ +}$	3	61	56	28	21	19	23	3.72
2	$\frac{ye\ wh}{ye\ wh} \times \frac{+\ +}{ye\ wh}$	3	35	35	14	26	19	15	0.61
3	$\frac{ye\ wh}{+\ +} \times \frac{ye\ wh}{ye\ wh}$	38	1220	1151	597	617	589	580	0.88
4	$\frac{+\ +}{ye\ wh} \times \frac{ye\ wh}{ye\ wh}$	24	668	590	301	328	289	307	1.32
5	$\frac{ye\ wh}{ye\ wh} \times \frac{ye\ wh}{ye\ +}$	11	0	0	152	133	98	127	7.06*
6	$\frac{ye\ wh}{ye\ wh} \times \frac{ye\ +}{ye\ wh}$	4	0	0	12	29	26	18	0.11
7	$\frac{ye\ wh}{ye\ +} \times \frac{ye\ wh}{ye\ wh}$	36	0	0	1363	1317	1314	1274	1.61
8	$\frac{ye\ +}{ye\ wh} \times \frac{ye\ wh}{ye\ wh}$	20	0	0	593	581	566	593	0.10

† White eye flies were crossed to normal flies in crosses 1–4 and to yellow eye flies for crosses 5–8

‡ The expected ratio for crosses 1–4 is 2 normal:1 yellow:1 white. The expected ratio for crosses 5–8 is 1 yellow:1 white; the chi-square statistics have 2 degrees of freedom for crosses 1–4 and 1 degree of freedom for crosses 5–8

* $P < 0.05$

cant deficiency of normal males was observed ($\chi^2 = 4.93$, 1 *df*). The orange-eye gene had complete penetrance and flies carrying this gene had good viability, 1.009.

Interactions between *or* and *ye* were examined by crossing orange-eye females with yellow-eye males and scoring the F_2 progeny. The results of this cross were 1411 normal, 626 yellow, and 434 orange-eye flies. These results did not differ significantly ($\chi^2 = 2.28$, 2 *df*) from the 9:4:3 ratio expected if *ye* was recessively epistatic to *or* (the recessive allele of *ye* locus, yellow eye, was epistatic to *or*) and the two genes assorted independently.

Segregation of or *and* wh

Due to the recessive epistatic interaction of *ye* to *or*, and the dominant epistatic interaction of *ye* to *wh*, the segregation of *or* with respect to *wh* could not be observed in F_2 or backcrosses. The status of the *or* gene cannot be established in *ye/ye* flies and the status of the *wh* gene cannot be determined in *ye/+* or *+/+* flies. Therefore, the linkage relationship between *or* and *wh* was determined by using a third genetic marker, the isozyme phosphoglucomutase (PGM), which could be scored in each of the eye color phenotypes. The PGM locus was linked to *wh* ($\chi^2 = 0.30$, 1 *df*) and segregated independently of *or* ($\chi^2 = 4.61$, 3 *df*).

Discussion

LaChance and coworkers[7] described four eye color mutants of *C. hominivorax*, yellow eye (*y*), white eye (*w*), ruby eye (*ru*), and rough eye (*ro*). Their yellow-eye mutant (*y*) did not differ phenotypically from the yellow-eye mutant (*ye*) described in this paper. Unfortunately, no representative material of the *y* mutant is available to confirm the genetic relationship between *y* and *ye*. The white-eye mutation (*w*) of LaChance differed both phenotypically and genetically from that described in this paper (*wh*). The ocelli of *w* flies were white whereas they appeared normal in *wh* flies. Genetically, LaChance's white-eye phenotype was controlled by a single recessive gene, *w*. Two independently assorting recessive genes *ye* and *wh* are required for the expression of the *wh* white-eye phenotype. Also, flies carrying *w* had low viability, 0.682, while those carrying the *wh* gene described herein had high viability, 0.965.

Crossing over does not occur in male screwworm flies[7,8]. The lack of crossing over facilitates the placement of loci onto genetic linkage groups. The independent segregation of *ye*, *or*, and *wh* in the dihybrid F_2, and backcrosses with heterozygous males, indicates that each of these loci is on a different chromosome. The screwworm fly has six pairs of chromosomes, five autosomes, X and Y[5]. The characterization of these three loci tentatively places a genetic marker on three of the five screwworm autosomes. Work is currently in progress to correlate these genetic markers with their respective chromosomes.

The color of the eye of an insect is primarily determined by ommochrome and pteridine screening pigments found in the primary and secondary pigment cells. The biosynthetic pathways leading to pteridines and ommochromes are distinct, pteridines are derived from purines while ommochromes are derived from the amino acid tryptophan[9]. The epistatic interactions observed between genes affecting eye pigments usually reflect the biosynthetic pathways leading to the production of the affected pigments. The recessive epistasis of *ye* to *or* indicates that *ye* inhibits the synthesis of the precursors to the orange pigment observed in orange eye flies. The dominant epistasis of *ye* to *wh* indicates that the *wh* allele inhibits the expression of the yellow pigment observed in yellow-eye flies but does not affect the expression of those pigments inhibited by the *ye* allele. These interactions indicate that the biosynthetic pathway leading to the yellow pigment is independent from that of the other pigments.

Only one ommochrome, xanthommatin, has been isolated from the eyes of cyclorraphan Diptera. Xanthommatin is brownish-yellow in its oxidized state[9]. A mechanism by which the *wh* mutation inhibits ommochrome expression, xanthommatin, but does not affect pteridine synthesis and the *ye* mutation inhibits pteridine expression with no affect on ommochrome synthesis can be proposed. This would explain the colorless eyes of the *ye wh/ye wh* flies, both pteridines and ommochromes inhibited, as well as the normal colored eyes of the *ye wh/+ wh* flies, with the full complement of pteridines present and only xanthommatin absent. Continuing with this mechanism, the orange-eye mutation either inhibits or modifies one of the pteridine pigments. This would explain the yellow-eye color observed in *or ye/or ye* flies, the precursor of the orange pigment is inhibited by the yellow-eye mutation. This mechanism awaits experimental confirmation.

Table III. Results of backcrosses for determining the mode of inheritance of orange eye

Cross	Parental genotypes ♀♀ × ♂♂	No. fam.	Normal ♀♀	Normal ♂♂	Orange eye ♀♀	Orange eye ♂♂	χ^2† eye color
1	*na/na* × *na/+*	23	898	945	892	930	0.12
2	*na/na* × *+/na*	31	1015	930	1094	1043	9.03*
3	*na/+* × *na/na*	33	1132	1151	1106	1153	0.13
4	*+/na* × *na/na*	30	1135	1076	1131	1091	0.03

† The expected ratio is 1 normal:1 orange; the chi-square statistics have 1 degree of freedom
* $P < 0.05$

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