SCIENTIFIC NOTE

Field Discovery of a Pearly Eye Melon Fly, *Bactrocera cucurbitae* (Hendel) (Diptera: Tephritidae), Mutant

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Abstract. A single female pearly eye melon fly *Bactrocera cucurbitae* was reared from field collected ivy gourd, *Coccinia grandis* L. Allele analysis of pearly eye revealed that it is a mutation that was determined to be autosomal recessive and a true breeding strain. We examined further genetic crosses of normal and pearly eye to evaluate its potential for use as a genetic marker in SIT programs .

The melon fly**,** *Bactrocera cucurbitae* (Coquillett), was first discovered in Hawaii in 1895 and since it became a serious pest of cucurbits, beans, peppers and tomatoes (Back and Pemberton 1918). Among the four species of fruit flies known to occur in Hawaii, the melon fly is the most serious pest of vegetables (Harris 1977). The principal tools available for control of the melon fly are protein bait sprays, male annihilation, and the sterile insect technique (SIT) (Cunningham and Steiner 1972, Kakinohana et al. 1990, Prokopy et al. 2003). However, among these tools, SIT was demonstrated to be the most effective in eradicating the melon fly from the Okinawa Islands once the native melon fly population was reduced by 5% by means of male annihilation (removal of the males from a native population with an attractant, cue-lure, plus a toxicant) (Koyama et al. 1984). It is necessary to reduce the population of wild melon by5% or more in order to obtain a minimum 10:1 or higher overflooding ratio of sterile melon flies to wild melon flies for SIT to be successful in eradicating melon fly. In contrast, both protein bait sprays and male annihilation (Cunningham and Steiner 1972) have not been demonstrated to be effective tools for eradication of melon fly.

At present, fruit fly pupae are marked with flourescent dyes to identify and to separate the lab reared sterile flies from the native flies. As the marked adults eclose, their extruded ptilinial sacs and bodies are usually marked with the dye. After the ptinilial sacs are retracted and the integument hardens, the dye is sealed in the inner fold of the ptilinial sacs. Even though the dye may be removed from the rest of released fruit fly bodies, crushing the heads of marked flies in acetone releases the dye for positive identification. However, McInnis and Cunningham (1986) reported that two classes of errors occur when dyed release flies are used, namely, that some flies, although rarely, may not become marked and that marked flies may accidently mark native flies. To avoid this problem, genetically marked flies with easily distinguishable phenotypic characteristics can potentially be substituted for external marking with dyes (Saul and McCombs 1992). The use of genetic markers, which have the potential for use in sterile insect technique (SIT) programs and release-recapture studies, have two advantages: (1) the markings are permanent, easily distinguishable from wild markings in both living and dead flies, and distinctive; and (2) the labor and cost required to mark flies externally with a dye and dye powder mixture (Schroeder and Mitchell 1981) are eliminated (Saul and McCombs 1992).

Kobayashi et al. (1973) were the first to report on a naturally occurring "yellow-eyed" mutant melon fly. Subsequently, chemically induced light eye (*le*) color melon fly mutants bearing light yellow eyes and white thoracic and scutellar vittae, which were genetically distinct but phenotypically similar, were produced by Saul and McCombs (1992). Finally, a white eye (*we*) was reported by McCombs et al. (1996).

In this note, we describe a field collected pearly eye mutant melon fly, and discuss the genotype of the mutant and its link with an autosomal chromatid.

A single pearly eye mutant (PEM) female melon fly was reared from fruit of ivy gourd, *Coccinia grandis* (L.), which was collected from Laie, Oahu Island on 12 March 2003. In experiment 1 (Table 1), a single field collected pearly female (P_1) was mated with six normal or dark reddish blue eye (NE) (Saul and McCombs 1992) melon fly males, which were collected along with the pearly female. At ten days old, the mated pearly female was allowed to oviposit in field collected ivy gourd fruit for a period of 48 hours in a 25 x 25 x 25 cm screen cage. For the adult flies, water was provided in a 200 ml plastic cup with a 9 cm diam lid (Highland Plastics, Pasadena, CA, CS300-04) fitted with a cotton wick (Pearsons Dental Supply, Sylmar, CA, dental cotton roll, 15.2 x 0.9 cm diam), and yeast hydrolysate combined with granulated sugar was given as food. Egg infested fruit was placed on melon fly diet inside a 200 ml plastic cup, which was in turn placed in a 1700 ml plastic bucket (LT512-64) with vermiculite. After mature larvae left the fruit and diet and pupated in the vermiculite, pupae were sifted from vermiculite, transferred to a 200 ml plastic cup, and placed in clean 25x 25 x 25 cm cage. The resulting F_1 progeny were inbred to the F_2 generation. In experiment 2 (Table 2), 1 PEM male and 1 NE female, and 1 PEM female and 1 NE male were outcrossed. Also, 1 NE male and 1 NE female, and 1 NE female and 1 male NE were intercrossed. Each of the four cohorts were inbred to the F_2 generation. In experiment 3, 100 PEM and 100 NE living adult flies were placed in a 1700 ml plastic container and placed outside of a laboratory exposed to the ambient environment and the eye colors were noted. Subsequently, the flies were transferred to petri dishes and held in a laboratory and propagated for a period of 12 months and the PEM and NE eye colors were recorded.

In experiment 1 (Table 1), an outcross of the PEM female with NE males yielded NE progeny. Inbreeding of the F_1 progeny yielded NE and PEM progeny (3:1 ratio) indicating that the PEM allele is recessive as compared with the dominant NE allele. In experiment 2 (Table 2), reciprocal outcrosses of cohort 2 and 3 both yielded a 3:1 phenotypic ratios indicating that the PEM allele is not sex linked, but rather, it is linked to an autosomal chromosome. Additionally, reciprocal intercrosses of cohorts 1 and 4 indicated that both phenotypes are true-breeding strains. In previous reports, the alleles for the "yellow eyed" (Kobayashi et al. 1973), "light eye-white scutellum" (Saul and McCombs 1992) and "white eye" (McCombs et al. 1996) mutants were determined to be autosomal recessive. In regards to eye color, Kobayashi et al. (1973) reported no change in eye color after death in the "yellow eyed' mutant. Saul and McCombs (1992) reported that the eyes of a "light eye-white scutellum" mutant (*le*) darkens after death, but is distinguishable from the wild melon fly. In contrast, the PEM adult eye color changed from pearly white to tan after death and can be easily distinguished from living and dead NE flies. At the time of this writing, a comparison with the white eye melon fly (*we*) reported by McCombs et al. (1996) and the PEM was not made and, therefore, the differences in the two strains was not ascertained. According to Tables 1 and 2, the sex ratio remained near 1:1 indicating that survival of the PEM adults was not affected by the PEM allele (Rossler 1979).

In the context of the area-wide pest management program (AWPM) of fruit flies in Hawaii, which was funded in 1999, the use of PEM flies would have two important advantages over using externally marked NE flies for SIT and release-capture studies. According to Saul and McCombs (1992): (1) genetically marked flies do not incur additional costs and

Table 1. Summary of progeny production and phenotypic ratios from crosses of normal eye and pearly eye melon fly through three generations (P1 – F2).

Generation	Phenotype cross	No. of pupae	No. of adults				Phenotype ratio (NE: PEM)	Goodness of fit F2 (3:1)
			NE	NE	PEM	PEM		
P1	NE x PEM	-	6	0	0	1	-	-
F1	NE x NE	37	17	20	0	0	37:0	-
F2	-	1885	759	687	230	209	1446:4	2.21

NE = normal eye phenotype; PEM = pearly eye phenotype.

Table 2. Summary of progeny production and phenotypic ratios from crosses of cohorts of normal eye and pearly eye melon fly. Normal eyed melon fly crossed with F_4 PEM.

Cohort	Generation	Phenotype cross	No. pupae	NE	NE	PEM	PEM	Phenotype ratio (NE: PEM)	Goodness of fit F2 (3:1)
1	P1	PEM x PEM	0	0	0	1	1	-	-
	F1		45	0	0	22	23	0:45	-
	F2		222	0	0	74	63	0:137	-
2	P1	PEM x NE	0	1	0	0	1	-	-
	F1		32	13	12	0	0	25:0	-
	F2		69	21	25	8	5	46:13	0.21
3	P1	NE x PEM	1	0	1	1	-	-	-
	F1		42	16	21	0	0	37:0	-
	F2		57	24	20	6	5	44:11	0.55
4	P1	NE x NE	0	1	1	0	0	-	-
	F1		20	6	7	0	0	348:0	-
	F2		402	188	160	0	0	0:348	-

NE = normal eye phenotype; PEM = pearly eye phenotype.

labor for dyeing and scoring as NE flies; and (2) genetic markers are permanent, unambiguous, and dead and living wild flies are easily scored. the disadvantage of using the PEM is due to the absence of a genetic sexing system to separate out the females, which can cause sting damage to crops and interfere with sterile male matings with wild females, prior to release (Saul 1990, McCombs and Saul 1992). In addition, sterilization of release PEM flies would be necessary step to prevent the mass introduction of the PEM allele into the wild population. From the mass production standpoint, the recessive nature of the PEM allele makes these flies easy to rear in genetically uniform colonies and recovery of colonies contaminated with NE melon flies is a relatively simple matter. Finally, further studies are necessary to determine the relative fitness of the PEM reproductive potential and mating success as compared with the NE phenotype.

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