CHARACTERIZATION OF A 'YELLOW-EYE' MUTANT OF TOBACCO BUDWORM

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Abstract

Seven 'yellow-eyed' tobacco budworm, *Heliothis virescens* (F.), adults (3 females:4 males) were found in a laboratory culture at the State University of West Georgia. The moths were mated in a single breeding chamber, and eggs were collected to establish a true-breeding, 'yellow-eye' strain. It was noted that crosses between 'yellow-eye' moths produced 'yellow-eye' progeny only. Crosses between 'yellow-eye' and 'wild-type' phenotypes were made to characterize the inheritance of the 'yellow-eye' trait. Sex ratios of the progeny generated from all crosses were approximately 1:1 (female:male). Reciprocal crosses of 'yellow-eye' and 'wild-type' moths produced F_1 progeny. Crosses of the F_1 'wild-type' heterozygotes with 'yellow-eye' moths produced 'wild-type' and 'yellow-eye' progeny in a 1:1 ratio. Crosses of F_1 'wild-type' heterozygotes produced F_2 progeny in a 3:1 ('wild-type' : 'yellow-eye') ratio. Furthermore, 'yellow-eye' males were produced from this cross indicating that the 'yellow-eye' trait was not X-linked. Based on the results generated by the crosses performed, the 'yellow-eye' trait was inherited in accordance with simple Mendelian genetics as an autosomal, homozygous recessive trait. Crosses are now in progress to generate a true-breeding, homozygous dominant 'wild-type' strain. Once this objective has been accomplished, further and more refined characterizations of the inheritance of the 'yellow-eye' trait will be possible.

Introduction

Variations in eye color/pigmentation have been reported in a variety of insects. However, outside of certain Dipteran species very little is known concerning the genetics and the expression of the gene(s) that code for those variations. In *Drosophila melanogaster* (Birchler et al. 1994; Ewart et al. 1994; Sun et al. 1995; Kirby and Stephan 1996; and Croop et al. 1997), the Mediterranean fruit fly, *Ceratitis capitata* (Zwiebel et al. 1995) and the tsetse fly, *Glossina* morsitans (Challoner and Gooding 1997), recent reports describe the isolation and characterization of genes correlated with spontaneous mutations resulting in pigment-deficient compound eyes. This gene, designated 'white gene', is a member of a superfamily of genes known as ATP-Binding Cassette (ABC) transporters that code for the synthesis of ATPase transmembrane proteins involved in the cellular uptake of guanine and tryptophan, precursors of red and brown eye pigments including xanthommatin. In addition, this gene superfamily also includes the subfamily of multidrug resistance genes (Allekmets et al. 1993; Rubio and Cowman 1996), and the Drosophila white gene has shown significant homology to a human gene located on chromosome 21. In addition to the possibility of contributing to an understanding of the pathophysiology of Downs syndrome (Chen et al. 1996), it is suggested that human homologs of the Drosophila white gene may contribute to the understanding of a variety of neurological disorders since tryptophan is also a precursor required for the synthesis of the neurotransmitter seratonin (Croop et al. 1997).

To date, very little is known about the evolutionary significance/importance of eye and body color variations in insects and the factors that may mediate or maintain these color variants within a population. In some insects, eye color mutations have been linked to insecticide resistance (Scott et al. 1984). However, in Lepidoptera, correlations between insecticide resistance and eye color, larval melanization and wing pigmentation have not been observed (Dittrich and Leutkemeier 1980; Brown, personal comm.). Although eye color mutants in tobacco budworm moths have been noted, reports in literature are lacking. However, a characterization of the inheritance of a 'yellow-wing' trait in a mutant tobacco budworm strain has been reported (Mitchell and Leach 1994). Brown et al. (unpublished data) recently demonstrated that the *Aceln* locus, which controls acetylcholinesterase sensitivity in an organophosphate resistant strain of tobacco budworm (Heckel and Brown, unpublished data), was not linked to the 'yellow-wing' trait characterized by Mitchell and Leach (1994). Nevertheless, the previously described literature suggest that the potential use of these color mutants as genetic markers and molecular probes for a variety of genes, including multidrug-resistance genes, is enormous and a worth pursuit. In addition, these mutant strains with visible markers may be useful in population field studies (Saul and McCombs 1992). This study attempts to characterize the inheritance of one eye color variation, the 'yellow-eye' trait, in an agriculturally important pest insect, the tobacco budworm.

Materials and Methods

Genetic Analysis

The original 'yellow-eye' moths (3 female:4 male) were mated in a single, 3.8 L paper chamber with a muslin top for oviposition to establish a true-breeding 'yellow-eye' strain. The 'yellow-eye' strain and the 'wild-type' strains were maintained separately following a standard method of rearing lepidopterous insects (Young et al. 1976). Adults from each of the parental strains were reciprocally crossed as single pairs to produce hybrids that could be reared to adults for eye color determinations. F_1 'wild-type' heterozygote adults were reciprocally backcrossed to adults from each of the parental strains and intercrossed to produce F_2 progeny as single pairs. Single pair matings (at least 5

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replications of each cross) were made in 0.4 L paper chambers with a muslin top for oviposition. The adults were fed a honey:molasses:water (1:1:20) solution. Eggs were collected from the breeding chambers of each cross and washed in a 0.0125% hypochlorite solution (2.5% Clorox®) to minimize fungal and viral contamination. The eggs were then brushed onto an artificial diet in 30 mL clear plastic cups capped with a pressed paper lid and labeled appropriately. Larvae from each cross were then sorted and reared individually on the artificial diet in 30 mL clear plastic cups until pupation. Pupae were removed, sorted by sex, and held individually for adult emergence. All stages were maintained under controlled environmental conditions at 27± 1°C, 40-70% relative humidity, and a 14:10 (L:D) photoperiod.

Biological Data

In general, the overall fitness of the parental 'wild-type' and 'yellow-eye' strains was evaluated by comparing developmental times, fecundity, and egg hatch data between the two strains. At least 25 neonate 'wild-type'and 'yellow-eye' larvae were maintained individually on artificial diet to determine their mean developmental times. Observations of molt, pupation, emergence and death were made every 24 h, and mean developmental times were calculated. For fecundity comparisons, at least 25 single pairs of newly-emerged female and male moths were held in individual 0.4 L containers. The moths were fed a honey:molasses:water (1:1:20) solution, and the containers were covered with a muslin top for oviposition. Eggs were collected and counted every 24 h for the entire egg production period, and the mean number of eggs/female was calculated for each strain. Percent egg hatch was determined form at least two replicates of 25 eggs from each parental strain ('wild-type' or 'yellow-eye'). Eggs were placed individually in 30 mL clear plastic cups. Observations were made every 24h for a maximum five day period.

Inheritance of the 'yellow-eye' trait was analyzed by calculating Chi square values (Steel and Torrie, 1960) for the observed phenotypic ratios for the F_1 backcrosses and F_1 intercrosses as compared to the expected phenotypic ratios assuming a simple Mendelian inheritance model. Biological data were analyzed by calculating and comparing the respective means ± standard errors.

Results and Discussion

A 'yellow-eye' mutant strain of tobacco budworm, *Heliothis virescens* (F.), was isolated from a laboratory culture. The adults of the mutant strain appeared normal with the exception of the uniform pale green-yellow compound eye color and the absence of the darker pigmentation pattern of the central compound eye. In contrast, the compound eyes of 'wild-type' tobacco budworm adults are typically a darker green color with characteristic black-brown pigmented, centrally located ommatidia of the compound eye. In addition to differences in eye color between the 'wild-type' and 'yellow-eye' strains, it was noted that the larvae resulting from the crosses between heterozygous, 'wild-type' adults and 'yellow-eye' mutant adults could be sorted based on larval body pigmentation. 'Yellow-eye' adults arose from pale green larvae exclusively. The coloration of larvae that developed into 'wild-type' moths was more variable. Although fourth and fifth instar tobacco budworm larvae exhibit a wide range of color from pale green to dark brown, earlier instar larval coloration was more uniform. The pale green coloration of early instar larvae could be used to identify those larvae that would eventually developed into 'yellow-eye' adults. Of the 241 pale green larvae, selected from three separate crosses between 'wild-type' heterozygote moths and 'yellow-eye' moths, that emerged as adults, 238 (98.8%) of the moths that developed from those larvae exhibited the 'yellow-eye' trait. The three 'wild-type' moths originated from pale green larvae selected as fourth instars when larval coloration was a less discriminating character for identification. Otherwise, the identification of 'yellow-eye' moths based on larval color would have been 100%.

Segregation for eye color in crosses of the 'wild-type' and 'yellow-eye' strains indicated that the 'yellow-eye' phenotype was an autosomal, homozygous recessive trait (Table 1). When 'yellow-eye' moths were crossed with 'yellow-eye' moths, all of the progeny produced that developed to adulthood possessed the 'yellow-eye' trait. When 'yellow-eye' moths were mated with homozygous, 'wild-type' moths, all of the progeny produced that developed to adulthood possessed the 'wild-type' trait. Reciprocal crosses of the F_1 'wild-type' heterozygotes with 'yellow-eye' moths produced 'wild-type' and 'yellow-eye' progeny in a 1:1 ratio. Intercrosses of F_1 'wild-type' heterozygotes produced F_2 progeny in a 3:1 ('wild-type':'yellow-eye') ratio. The observed phenotypic ratios resulting from the F_1 backcrosses and F_1 intercrosses were not significantly different from the expected phenotypic ratios assuming a simple Mendelian inheritance model. Furthermore, the 'yellow-eye' trait was not X-linked. Since male tobacco budworm are homogametic (XX) and females are heterogametic (XY), all F_2 male moths resulting from the F_1 intercross should exhibit the 'wild-type' phenotype. However, the F_1 intercross generated 'yellow-eye' F_2 male moths. In addition, the sex ratios of the progeny resulting from each cross were approximately 1:1 (female:male).

Comparisons of biological data (i.e., mean developmental time, frcundity, and % egg hatch) suggested no significant developmental or reproductive differences between the parental 'wild-type' and 'yellow-eye' strains (Table 2). Mean developmental times (days ± s.e.) for each growth stage of 'wild-type' tobacco budworms were as follows: egg, 2.0 ± 0.08; 1st instar, 2.1 ± 0.78; 2nd instar, 1.4 ± 0.19; 3rd instar, 1.8 ± 0.24; 4th instar, 2.5 ± 0.16; 5th instar, 6.4 ± 0.27; pupal, 7.5 ± 0.45; and adult, 11.2 ± 2.24. Mean developmental times (days ± s.e.) for each growth stage of 'yellow-eye' tobacco budworms were as follows: egg, 2.0

± 0.07; 1st instar, 2.0 ± 0.05; 2nd instar, 1.4 ± 0.23; 3rd instar, 1.8 ± 0.21; 4th instar, 2.1 ± 0.12; 5th instar, 7.2 ± 0.18; pupal, 6.0 ± 0.11; and adult, 11.9 ± 0.62.

Conclusions

In summary, the 'yellow-eye' trait was inheroited as an autosomal, homozygous recessive trait. Bioogical data suggest that he 'yellow-eye' trait does not compromise the reproductive fitness of the 'yellow-eye' strain. The 'yellow-eye' mutation may be similar to the 'white gene' of *Drosophila* which belongs to a superfamily of genes known as ABC transporters. The products of some of these genes are required for the cellular uptake of precursor molecules required for the synthesis of pigments and some neurotransmitters. Furthermore, this superfamily includes the 'multidrug-resistance' gene subfamily. Future studies will focus on the isolation and characterization of the 'yellow-eye' gene to determine its similarity to the *Drosophila* 'white gene', its potential use as a genetic marker or molecular probe, and the significance of this gene in the study of tobacco budworm populations.

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Table 1. Segregation for eye color in crosses of 'wild-type' (WT) and 'yellow-eye' (YE) tobacco budworm adults.

Cross	Number of Adults				Ratio	
Male x Female	Male		Female		Actual	Expected
Actual Expected	WT	YE	WT	YE	WT:YE	WT:YE
Parental Crosses						
YE x YE	0	404	0	357	0:761	0:761
WT x YE*	41	0	33	0	74:0	74:0
YE x WT*	35	0	36	0	71:0	71:0
Backcrosses						
F_1 x YE	114	88	121	96	235:184	209:209
YE x F_1	70	68	89	53	159:121	140:140
F_1 x WT*	13	0	19	0	32:0	32:0
WT x F_1*	27	0	18	0	45:0	45:0
F_1 x F_1	476	117	398	135	874:252	844:282

*Homozygosity of the wild-type parents were based on the results from the single pair matings reported. The genotypes of the "wild-type' adults used in the single pair matings were unknown. "Wild-type' adults could be homozygous or heterozygous. The results reported were from crosses that produced no 'yellow-eye' progeny. In retrospect, the 'wild-type' adults used in these crosses were assumed to be homozygous for the 'wild-type' phenotype. Attempts to isolate and establish a homozygous 'wild-type' strain are in progress.

Table 2. Preliminary comparisons of biological data for tobacco budworm progeny resulting from crosses within each of the parental 'wild-type' and 'yellow-eye' strains.

Strain	n[a]	Mean Developmental Period[b]	Fecundity[c]	n[d]	%Egg Hatch[e]
Wild-type	25	34.9 ± 4.33	317 ± 40	25	68
Yellow-eye	25	31.5 ± 2.53	302 ± 61	25	64

[a]Total numbers of larvae used to determine mean developmental times.
[b]Mean developmental times (days ± s.e.) From egg hatch through adult.
[c]Numbers of eggs oviposited per female ± s.e.
[d]Total numbers of eggs used to determine % egg hatch.
[e]Percentage of eggs hatched ± s.e.