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GENETIC, BIOCHEMICAL AND PHYSIOLOGICAL CHARACTERIZATION OF THE WHITE EYE COLOR MUTANT OF THE TSETSE FLY, GLOSSINA MORSITANS SUBMORSITANS NEWSTEAD (DIPTERA: GLOSSINIDAE)

BY

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE.**

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ABSTRACT

A mutant with white-colored eyes was found in the tsetse colony at the Entomology Department of the University of Alberta. It is the first eye color mutant reported for Glossina morsitans submorsitans Newstead. This mutant, designated white (wht), is controlled by an X-linked recessive allele which is tightly linked to two X-chromosome loci, Est-X and Sr. A second mutant, designated pearl (prl), was found shortly after white. Both mutants are phenotypically identical and allelic to one another.

The mutant white is defective in production or deposition of the screening pigment xanthommatin in the compound eyes. In the testes, white males produce 50% more xanthommatin than do wild type males. The formation of xanthommatin in the compound eyes is blocked late in the tryptophan to xanthommatin pathway. During adult development, white accumulates significantly higher levels of 3-hydroxykynurenine than does wild type. 3-Hydroxykynurenine is restricted to the bodies of white flies, whereas wild type flies sequester some 3-hydroxykynurenine in the heads. These properties of white of G. m. submorsitans are compared with those of white (w) of Drosophila melanogaster and white (w) of Lucilia cuprina, and it seems likely that the gene affected in wht is involved with the uptake and storage of xanthommatin precursors.

The bionomic features compared between white and wild type G. m. submorsitans include fertility of males and females, longevity of males and females, fecundity, age at first larviposition, interlarviposition period, male and female puparial weights, male and female puparial periods, emergence rate and sex ratio of offspring. Of these features, emergence rate and fecundity are

slightly reduced in white flies as compared to wild type flies. The only significant difference between the line White and the wild type flies is an increase in the puparial weights of white males.

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INTRODUCTION AND LITERATURE REVIEW

Importance of Tsetse

Tsetse flies (Glossina spp.) transmit African trypanosomes, which are protozoan parasites that cause the often fatal diseases of sleeping sickness in humans and nagana in domestic animals. The presence of tsetse not only produces an obvious health concern, but also has drastic economic impacts. The disease nagana affects the health and numbers of domestic livestock, and also limits the areas where these animals can be raised. The most notorious vectors of nagana belong to the Glossina morsitans species group. They have the highest trypanosome infection rate of the Glossina groups. The insect of study, Glossina morsitans submorsitans Newstead, belongs to this species group (Jordan 1986).

There are potential problems with respect to tsetse and disease control. There is some evidence that the insects may be developing resistance to insecticides (Maudlin et al. 1981), and there is considerable evidence that the disease causing organisms are becoming resistant to chemotherapeutic and prophylactic agents (Jordan 1986). Researchers are now trying to combat insecticide resistance by looking to alternative methods, namely biological control. Genetic control practices show great promise in field situations and mutants can contribute much of the information needed to establish these control practices.

Potential Usefulness of Mutants

The study of eye color mutants can provide us with some of the basic genetic information needed to establish a reliable genetic control program. They may aid in identifying genes that affect fertility, fecundity, or lifespan; elements that are desirable in control programs. They can also provide scientists with a more extensive genetic map to locate other genes. This type of information may be useful for any future work on genetic engineering or identification of important genes such as antitrypanosomal genes. Jordan (1986) identified six factors that influence the vectorial ability of tsetses, and that are potentially affected by mutations: "Sex, genetic differences between species or within species, behavior, physical and biochemical state of the fly, and concurrent infections of the fly with organisms such as viruses, bacteria and fungi". Any or all of these factors are potentially affected by an eye color mutation.

Eye color mutants may also be used as visual markers in field studies of population genetics, for surveying the success of insect control operations, or as markers for genetically engineered insects. To be used successfully in such applications, however, mutants need to have fitness equal, or nearly equal, to that of the wild type. To evaluate fitness, it is essential to have information on pleiotropic effects, ie. other repercussions of a mutation. Many eye color mutants are known to have marked physiological and behavioral alterations associated with the mutation. These changes are related to the changes in eye pigment levels and may be related to the resulting levels of pigment precursors in the body.

Unfortunately for biological control purposes, there are very few mutants known in Glossina, and very little information is available on the genetics of

these insects. For this reason, it is imperative that we study mutants carefully and extract as much information as possible from each.

Mutants can also be studied for purely scientific interest, in attempts to understand how insects function. Research on eye color mutants allows us to expand our knowledge about ommochrome biochemistry (a system which is still incompletely understood) and to relate the process of eye pigment development to other physiological and behavioral features of insects.

The appearance of a white eye color mutant in the tsetse fly, G. m. submorsitans presents an opportunity to acquire some much needed genetic information, to study eye color development in this species, and to assay factors that influence the ability of the fly to transmit trypanosomes. From this information we may be able to ascertain the potential use of the mutant in field studies, future laboratory work, and in control programs.

Scope of Literature Review

It has been known since 1938 that the wild type eye color of Dipterans is produced by two classes of screening pigments (Phillips and Forrest 1980); pteridines which are red and ommochromes which are brown. Pteridines are the final products of purine metabolism, specifically that of guanosine triphosphate. Ommochromes are products of tryptophan metabolism (Linzen 1974).

My main focus is on the ommochrome eye pigment, xanthommatin, the main screening pigment found in Dipteran eyes (Linzen 1974; Summers et al 1982), and the only ommochrome found in the eyes of cyclorraphan flies (Kayser 1981). With regard to the other type of eye pigment, the pteridines, no work was done. Before describing the experimental work, I will review tryptophan (the precursor of xanthommatin) and its metabolism, ommochromes

and their function, previous work on ommochrome mutants in general, and Glossina eye color mutants in particular.

Tryptophan and Tryptophan Metabolism

Tryptophan

Tryptophan, an essential amino acid for insects, is used as a precursor to proteins, hormones, pigments, and the neurotransmitter serotonin (Linzen 1974). It is highly hydrophobic, and has the highest molecular weight (204.2 g/Mol) of all the amino acids. It is one of only three amino acids with aromatic side chains, and is capable of donating electrons. Tryptophan is also one of the rarest amino acids, making up 0.5% to 1% by weight of most animal proteins and between 0.25 and 0.5 % by molarity, depending upon insect species and stages of development (Linzen 1974).

Tryptophan metabolism in general

Although an essential amino acid, tryptophan is fairly toxic, and therefore controlling its concentration is imperative for the insect's survival. Elevated levels of tryptophan are known to retard developmental processes, induce tumors, and cause morphological deformations (Linzen 1974). Most mammalian and many non-mammalian metabolic systems metabolize tryptophan by the glutarate pathway, and/or the nicotinic acid pathway. These two pathways each produce important cofactors in oxidative metabolism (acetyl CoA and carbon dioxide, and NAD and NADP respectively), and at the same time break down the benzene nucleus of tryptophan, a potentially detrimental compound (Linzen 1974).

Terrapins and arthropods do not utilize the glutarate pathway to oxidize the benzene ring of tryptophan to CO_2, and none of the enzymes of this

pathway, (3-hydroxyanthranilic acid oxidase, picolinic carboxylase, and α-aminomuconic semialdehyde) are found in arthropods (Lan and Gholson 1965).

Tryptophan metabolism in insects

In order to control the levels of tryptophan, insects use mechanisms other than the two pathways described above. Metabolism and direct excretion seem to be the two most prominent mechanisms. Tryptophan metabolism in insects can generally take two forms. The first involves the conversion of tryptophan to kynurenine or 3-hydroxykynurenine to store for later excretion. The second involves the conversion of these two intermediates to a multitude of compounds, the most prominent, in the Diptera, being the ommochromes.

The inability of insects to degrade tryptophan by the glutarate or nicotinic acid pathways increases the functional significance of the formation of ommochromes (Brunet 1965; Lan and Gholson 1965; Martel and Law 1992). Brunet (1965) suggests that the ommochrome pathway was preferentially "selected" for, over the nicotinic acid and glutarate pathways. Although ommochromes are not essential for the survival of the insect, as proven by the viability of ommochrome deficient mutants, their presence and function gives them a definite visual advantage under natural conditions.

Biology of Ommochromes

Ommochrome function

Xanthommatin, the most abundant ommochrome in dipterans, is produced by a series of enzyme-catalyzed steps called the tryptophan to xanthommatin pathway (Figure 1). The details of this pathway are described in Chapter 3. Ommochromes have been identified in a number of invertebrates in

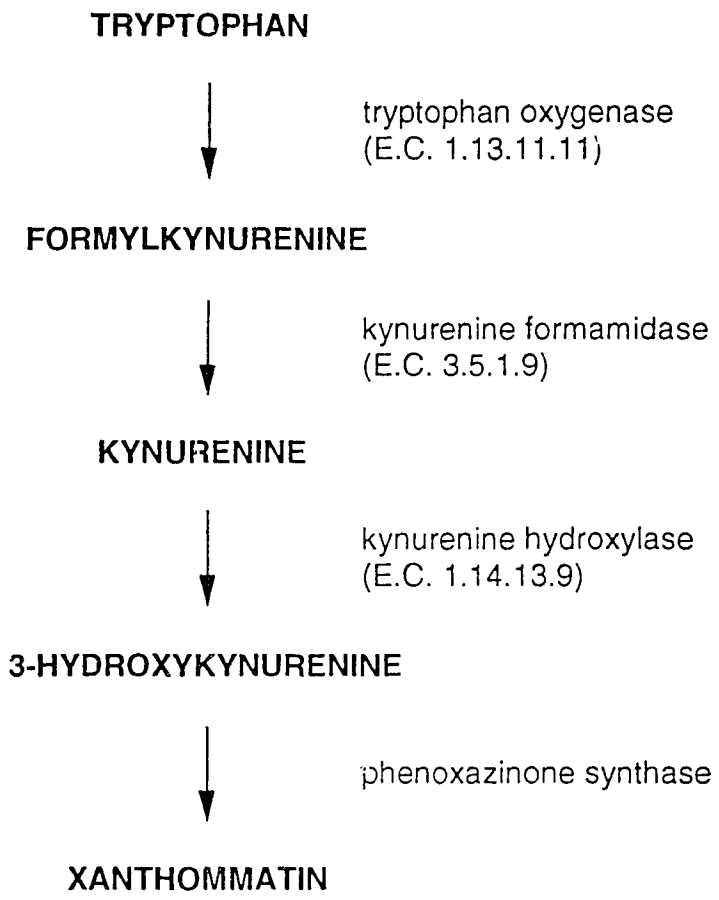

Figure 1 - Tryptophan to xanthommatin pathway.

such taxa as the Anthomedusae, Polychaeta (Linzen 1974), Echiurida, Cephalopoda and Arthropoda (Brunet 1965; Phillips and Forrest 1980). In insects they are mainly found in cuticle, internal organs, and eyes; with the latter playing a key role in storage and utilization of ommochromes.

Within the compound eyes ommochromes are located in primary and secondary pigment cells surrounding the retinula cells of each ommatidium (Langer 1975). Xanthommatin is bound to protein, in intracellular granules (0.4-0.6 μm diameter) in these cells. In these pigment cells they are located in large membrane-bound granules called type I granules (Linzen 1974).

The screening pigments, both ommochromes and pteridines, function to form a light directional screen. They affect light perception by reducing the light energy hitting the receptors, increasing acuity (unidirectional light) by preventing lateral light scattering, and enhancing contrast sensitivity (Linzen 1974; Phillips and Forrest 1980; Summers et al. 1982).

Flies without screening pigments take in more light and have lower thresholds for light detection, and may be blinded by high light intensities (Davis and Gooding 1983). There can be many problems associated with the absence of screening pigments. Activities which are visually stimulated or visually dependent, such as mate or host finding, can be drastically affected by the lack of these pigments (Sappington 1991). As early as 1936, the importance of vision in tsetse was documented. Eltringham believed that vision plays a much more important role than olfaction (Eltringham 1936; Muirhead-Thomson 1982).

Ommochromes exhibit redox behavior. The oxidized and reduced forms of xanthommatin have different colors; the reduced form, the most abundant, is red and the oxidized form is yellowish-brown (Langer 1975). This difference is thought to be produced by the binding of dihydroxyxanthommatin to proteins in the pigment granules (Linzen 1974). It has been suggested that in some

insects this redox behavior allows for a respiratory function (electron accepting and donating system) (Linzen 1974).

Eye Color Mutants

Some of the ground-breaking work on eye color mutants of insects resulted from efforts to understand mechanisms of gene action and the relationships of genes to the biochemical synthesis of eye pigments (Beadle and Ephrussi 1936). The earliest biochemical studies of ommochromes began with the work of E. Becker in the late 1930's and were taken over by Butenandt in the early fifties (Linzen 1974). The most prominent studies of eye color mutants come from work on the Dipterans Drosophila (Drosophilidae), Lucilia (Calliphoridae) and Musca (Muscidae). All three genera have a multitude of eye color mutants available for study. To date, there are 83 different eye color mutants in Drosophila melanogaster (Phillips and Forrest 1980), about seven in Lucilia cuprina (Whitten et al. 1974), and at least 21 in Musca domestica (Laudani and Grigolo 1969). Only a few of these have been studied in detail biochemically, but these have revealed, to a great extent, the process by which eye pigments are developed. For example, in D. melanogaster the vermilion and cinnabar mutants were used to identify tryptophan and kynurenine as precursors involved in pigment formation. It is with the work on these and other mutants that I will compare my findings.

Eye Color Mutants of Glossina

There are five eye color mutants in Glossina (Diptera: Glossinidae). Three are in members of the morsitans group: salmon in G. morsitans morsitans Westwood (Gooding 1979), pink in G. morsitans centralis Machado (Rawlings 1985), and white in G. morsitans submorsitans Newstead (present study). The

fourth and fifth mutants, tan (D'Haeseleer et al. 1987) and brick (van den Abeele and D'Haeseleer 1989), belong to G. palpalis palpalis in the palpalis group. All of these eye color mutants contain lesions in the tryptophan to xanthommatin pathway and are deficient, to a greater or lesser degree, in xanthommatin. Here I review four of these mutants and present information on the origin of the white eyed mutant.

I) salmon in G. m. morsitans

The salmon eye color mutant was the first eye color mutation studied in tsetse. The original mutant individual, a male, was found in May of 1977 at the University of Alberta, Department of Entomology. The G. m. morsitans colony, in which the mutant occurred, was established in July, 1973 from material supplied by the Tsetse Research Laboratory, University of Bristol, England (Gooding and Rolseth 1976).

The salmon mutant has salmon colored eyes and ocelli, rather than the normal dark brown-black color. The mutation results in a lighter coloration of the testicular sheath, but does not affect the color of the spermatheca. The salmon allele is X-linked and recessive to wild type (Gooding 1979), with its locus (designated sal) mapped to a location between the ocra (body color) and arginine phosphokinase loci (Gooding 1981).

The change in compound eye and ocelli coloration is a result of the low quantities of xanthommatin in these structures; salmon mutants contain 2.7% of the xanthommatin found in wild type G. m. morsitans (Gooding and Rolseth 1984). This mutation does not interfere with pteridine production, as there is no significant difference in the levels of pteridines in heads of salmon and wild type flies (McIntyre 1993). There is little if any tryptophan oxygenase produced by the mutants, and consequently they accumulate and excrete tryptophan. This is

in contrast to wild type flies which do not excrete tryptophan (Gooding and Rolseth 1984).

Physiological manifestations of the salmon mutation include decreases in longevity, fertility and fecundity. The maternally influenced semi-lethal nature of the mutation has made it impossible to establish a "pure" mutant line. Matings between sal/sal females and sal/Y males produce offspring with a less than 20% eclosion rate, and those that survive live for only a few weeks (Gooding 1979). The mutation also increases the flies' ability to transmit Trypanosoma brucei brucei (Makumyaviri et al. 1984) and Trypanosoma congolense (Distelmans et al. 1985). This precludes use of this mutant in field control practices.

With respect to behaviour, the salmon flies are less aggressive in mating than are wild type flies (Gooding 1979). This behavior, and possibly also the life span effects of the mutation, may be a consequence of changes in the fly's vision. Tests on the visual responses of salmon show that the mutants "have lower light detection thresholds and increased flicker fusion frequency at light intensities above the sensitivity threshold" (Davis and Gooding 1983). This change in light threshold may cause sal to be blind at light levels found in their normal habitat. As a result, the mutants may have problems locating mates or hosts, under field conditions.

II) tan mutant in G. p. palpalis

In March 1983 a pink eyed male was found in a colony of G. p. palpalis maintained at Rijksuniversitair Centrum Antwerpen (RUCA), Belgium. This particular colony was established in 1974 with flies from Nigeria. The eyes of the tan mutants are pink in live flies, but turn to a tan color in dead, dried flies. Testes of tan have nearly the same color (chestnut brown) as those of the wild

type males. The tan allele, like that of salmon, has an X-chromosome locus and is recessive to the wild type allele.

The tan mutants, like salmon mutants, contain very little xanthommatin in their heads (0.8% of that found in wild type flies). This deficiency is restricted to the head; the testes of the tan males contain unusually high levels of xanthommatin (150% that of wild type flies). The pteridine levels are only slightly lower than those of wild type flies (McIntyre personal communication). Adults with tan colored eyes have nearly normal levels of tryptophan oxygenase (75%) and kynurenine formamidase (79%), and subsequently produce kynurenine and 3-hydroxykynurenine. Like wild type G. p. palpalis, tan mutants excrete kynurenine. The lesion is thought to occur late in the tryptophan to xanthommatin pathway, possibly involving the transport of xanthommatin precursors or affecting the binding of xanthommatin in the eyes, but not the testes (D'Haeseleer et al. 1987). Biochemically, this mutant may be comparable to the scarlet mutant of D. melanogaster (Sullivan and Sullivan 1975; Phillips and Forrest 1980).

Physiologically, the tan mutation is not nearly as detrimental as the salmon mutation, affecting neither life span, fecundity, nor eclosion rate. However, D'Haeseleer et al. (1987) observed an effect on the vision of mutant flies, which appeared "partly blind" (no direct studies were conducted) and "their movements are at least strongly inhibited at normal light intensities".

III) pink mutant in G. m. centralis

A pink eyed male G. m. centralis was discovered at the Tsetse Research Laboratory in June, 1984 (Rawlings 1985), in a colony originating from Tanzania in July, 1980. Phenotypically, this recessive, X-linked mutant closely resembled the salmon mutant in G. m. morsitans, but unlike salmon mutants,

pink mutants did not produce "congenetively abnormal offspring". Pink eyed flies had normal emergence rates but tended to produce low numbers of offspring, whether the female was homozygous or heterozygous (Rawlings 1985). Unfortunately, the pink mutants are no longer available for study, as the colony either died out or was terminated.

IV) brick mutant in G. p. palpalis

A mutant male was found in the G. p. palpalis colony at RUCA, Belgium, in September 1986 (van den Abeele and D'Haeseleer 1989). The colony originated in Bas-Zaire, and has been maintained as an autonomous colony since 1974. The brick allele is recessive to the wild type allele, has an X-chromosome locus, and is nonallelic with tan (van den Abeele and D'Haeseleer 1989).

The mutants have brick-red compound eyes and ocelli, and the eyes of males have a notably darker color than those of females. The color of the testicular sheath and the spermatheca are identical to those of the wild type flies. Little or no xanthommatin is present in the heads of mutants, with no significant difference between females and males (personal observations). Mutants and wild type flies have similar levels of pteridines (McIntyre personal communication).

Physiologically, the mutation has no effect on puparial weight, puparial duration, emergence rate, or ability to transmit Trypanosoma spp.. The mutation does, however, affect fecundity of females, feeding response, and mortality rate (homozygous females having a higher mortality rate than do heterozygous females) (van Den Abeele and D'Haeseleer 1989).

v) white mutant in G. m. submorsitans

In September, 1992 I found a white eyed male while working with R. H. Gooding's experimental line "90-26-S" . This line was originally established from G. m. submorsitans females from a colony originating in Burkina Faso and Nigeria mated to G. m. centralis males from a colony originating in Tanzania (Gooding 1993). The F_1 hybrid females and females in the next three generations were each backcrossed to males from the G. m. submorsitans colony. The white eyed mutant male was from the fourth backcross generation.

The male had wild type body color but very white eyes (with a pink hue) and wild type ocelli. The white eye color changed to yellow after the mutant was dead and dried. Dissections of subsequent white eyed flies showed wild type testicular color (chestnut brown), wild type spermathecal color (brown), and slightly lighter than wild type Malpighian tubule color (light yellow).

The original male seemed healthy and fed frequently. After seven days the male mated readily with a virgin female G. m. submorsitans (Bristol-4; of Nigerian origin). After two days the female was dissected to confirm the insemination ability of the white male. On eight occasions, at two to three day intervals, the male was mated with 2 to 3-day-old virgin G. m. submorsitans females from the Gms-8 colony which is of Burkina Faso and Nigerian origin. All the females became pregnant and were pooled; progeny of these females were designated as the line "White" of G. m. submorsitans, and all white mutants used in subsequent experiments were descendants of this male.

In order to verify the subspecies identity of the original white eyed mutant, three other matings were done; two with G. m. centralis (line Cent-4) virgin females and one with a G. m. morsitans (line 165) virgin female. The females were kept twenty-eight days (enough time for two larvipositions to occur) to see if they would become pregnant. They did not become pregnant, but all three

had been inseminated. This observation, along with the knowledge of fertility of inter-subspecies crosses involving G. m. submorsitans males (Gooding 1982; Gooding 1985), confirmed that the white eyed male was G. m. submorsitans.

Details about this mutation are the subject of this study and are described in subsequent sections of this thesis.

vi) pearl mutant in G. m. submorsitans

A second white eyed male, phenotypically identical to the first, was found in line "90-26-S" in October, 1992. The original pearl male seemed healthy and fed frequently. After seven days the male mated readily with a virgin female G. m. submorsitans (Bristol-4; of Nigerian origin). After two days the female was dissected to confirm the insemination ability of the pearl male. On six occasions, at two to three day intervals, the male was mated with 2 to 3-day-old virgin G. m. submorsitans females from the Gms-8 colony which is of Burkina Faso and Nigerian origin. All the females became pregnant and were pooled; progeny of these females were designated as the line "Pearl" of G. m. submorsitans.

In order to verify the subspecies identity of the original pearl eyed mutant, three other matings were done; two with G. m. centralis (line Cent-4 and Cent-6) virgin females and one with a G. m. morsitans (line 165) virgin female. The females were kept twenty-eight days to see if they would become pregnant. They were inseminated but did not become pregnant. The second mutant male was also verified to be G. m. submorsitans.

Although similar, white and pearl were treated as founders of two distinctly different lines, until it was verified that they were allelic and probably the same mutation. The line set up by the first male was designated White

(locus designated wht), and that set up by the second male was designated Pearl (putative locus designated prl).

The appearance of two phenotypically identical males in the same line, 10 days apart (which is approximately the average interlarviposition period of tsetse), indicates that they may have been brothers. Theoretically, if a female from line "90-26-S" carried the mutation in the heterozygous state (at least in her meiocytes), she could pass it on to her offspring. Finding the trait(s) in males rather than in females suggested that the mutation is recessive and X-linked.

Gynandromorph

In October, 1993, I found a bilateral gynandromorph with one white eye (male side) and one wild type eye (female side). The presence of this mosaic indicated the presence of an autonomous mutant with normal levels of activity in the first 3 enzymes of the tryptophan to xanthommatin pathway. Sturtevant (1920) found that non-autonomous mutants, such as the tryptophan oxygenase mutant vermilion, are incapable of forming this type of mosaic.

Beadle and Ephrussi (1936) determined that in D. melanogaster the mutants vermilion (a tryptophan oxygenase mutant) and cinnabar (a kynurenine hydroxylase mutant) were non-autonomous mutations. When either of the mutant eye discs were transplanted into wild type bodies, a wild type phenotype resulted. Based on this information it was determined that the element missing from, or not being produced by, each mutant, was provided by the wild type host and that these elements were diffusible compounds. These compounds were found to be kynurenine and 3-hydroxykynurenine, respectively. Mutants occurring later in the pathway, including the white mutant of D. melanogaster,

were all found to be autonomous mutants, and did not involve the first three enzymes in the tryptophan to xanthommatin pathway.

Statement of Purpose

The purpose of this study was to determine the genetic nature of the white eye color mutation in G. m. submorsitans, to map the locus in relation to other previously mapped loci, and to determine the relationship of the white mutation to pearl. I wished also to determine the biochemical lesion that caused the white eye color, by assaying the products and intermediates of the tryptophan to xanthommatin pathway in white eyed and wild type G. m. submorsitans. By determining the pleiotropic effects of this mutation, I hoped to determine to what extent the mutation affected fitness and to determine the acceptability of this mutant as a candidate for field studies.

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Chapter 2

GENETIC ANALYSIS OF THE WHITE MUTATION

The most appropriate candidates for intensive genetic work are organisms that produce large numbers of offspring and that have a short generation time (i.e. Drosophila). We do not have that convenience with Glossina. The insect's low number of offspring (six to eight per generation) and relatively long generation time (about 55 days) have deterred many from exploring the genetics of this medically important pest (Bolland et al. 1974).

Modern control methods focusing on genetic control rely on genetic information. Genetic mutations provide information on the structure, position and function of many of the genes controlling biology, physiology and behaviour. Unfortunately there are very few visible and biochemical mutations identified in tsetse. There are seven visible mutations reported for Glossina; the five affecting eye color were reviewed in Chapter 1, ocra (body color, Bolland et al. 1974) and sabr (long scutellar bristles, Gooding 1984). This low number of mutants make it essential to extract as much information as possible from each.

Acquiring basic genetic information using mutants involves understanding the genetic nature of the mutation (chromosomal association and recessiveness/dominance), its location on the chromosome (mapping), and its allelic relationship with other mutants. This study aims to ascertain these aspects of the white mutant in G. m. submorsitans.

MATERIAL AND METHODS

Glossina Life Cycle and Colony Maintenance

The two main lines used in the experiments were Gms-8 (wt) and White (*wht*). The Gms-8 line was established in 1980-1982 by crossing G. *m. submorsitans* from Burkina Faso with G. *m. submorsitans* from Nigeria. The Gms-8 line was subsequently selected for five biochemical marker genes *G6pd*^{b}, *Est-X*^{b}, *Odh*^{a}, *Est-1*^{a}, and *Mdh*^{c} (Gooding 1993). The origin of the White line has been discussed in Chapter 1.

The colonies are kept at 21C to 24C (Gooding 1982) at a moderate but variable humidity (Gooding 1979). Until August 1993, the adult flies were fed six to seven days a week on the ears and backs of cross-breed (Flemish Giant x French Lop-eared) rabbits. Since then they have been fed every other day.

Males become fully fertile about 7 days after eclosion and will mate repeatedly until death. Optimal receptivity for females is between two to seven days after eclosion. After that time, females become refractory and reject males. Once mated, females remain inseminated for life, the sperm being stored in the spermatheca. In our wild type G. *m. submorsitans* colony, mated females are kept three to four months before being terminated. However, due to the constant use of the line White for experimental purposes, the *wht* / *wht* mated females were held until they died. The males were kept no more than three months. Females and males were maintained separately in G-10 cages (16 cm x 8 cm x 5 cm) covered with black terylene bobbinette.

Glossina are larviparous insects, the larvae feeding on milk glands in the mother. At about one week old, the female fertilizes the first egg, and at about 16 days she gives birth to her first offspring, a third instar-larva that pupariates within 30 minutes to two hours. A female deposits her subsequent larvae at 9-

10 day intervals. The skin of the third instar-larva hardens, and 4 days later the true pupa is formed. Eclosion occurs about 30 days after pupariation.

White, an X-linked Recessive Allele

The following five crosses were made to determine the genetic nature of white:

+ / + Gms-8 females x wht / Y (the original mutant male)

+ / wht (F_1 females) x + / Y (F_1 males)

+ / wht (F_1 females) x wht / Y (F_2 males)

wht / wht (females) x + / Y (males)

wht / wht (females) x wht / Y (males)

The resulting offspring were scored for sex and eye color.

Mapping the white Locus

The locus for white was mapped in relation to two other X-linked loci (Est-X and Sr) in G. m. submorsitans (Gooding et al. 1989). In G. m. submorsitans, EST-X has two alleles; $Est\text{-}X^f$ produces a thoracic esterase having an R_f value of 0.17 on a 9% polyacrylamide gel, and $Est\text{-}X^n$, which produces no thoracic esterase at that R_f (i.e. a null allele). G. m. submorsitans carries also two alleles that control sex ratio: males with Sr^d, sire families with fewer than 20% males, whilst Sr^n, males sire families with more than 20% males. The Sr phenotype is expressed in males (Gooding 1986; Gooding et al. 1989; Rawlings and Maudlin 1984). It has been documented previously that Sr and Est-X are separated by less that 2.2 recombination units (Gooding et al. 1989). Results presented above showed that the white locus is on the X-chromosome.

Two lines were available for mapping wht, Est-X and Sr: the "White" line which is homozygous for wht, $Est\text{-}X^f$, and Sr^n and the line "Brist-5" which has

two common male genotypes, + Est-X^{f} Sr^{n} / Y and + Est-X^{n} Sr^{d} / Y and may have two rare genotypes, + Est-X^{f} Sr^{d} / Y and + Est-X^{n} Sr^{n}. Ten Brist-5 males were each mated with three to seven white females (wht / wht). The males were electrophoresed to confirm their Est-X genotype. All the females mated to an individual male were pooled and their offspring were collected and scored for eye color and sex to determine the Sr genotype of the father. The F_1 females whose fathers were + Sr^{d} Est-X^{n} / Y, were backcrossed to white males (wht / Y), and the other families were discarded. F_2 males were scored for eye color, Est-X genotype, and their ability to sire normal (Sr^{n}) or sex ratio distorted families (Sr^{d}). To score Sr, each male was mated with five to seven G. m. submorsitans females. The females were held until each family produced twenty puparia and the adults that emerged from the puparia were scored to determine the sex ratio. Due to the unavailability of large numbers of females, only forty males were tested for Sr, Est-X, and eye color. Of the remaining F_2 males, 93 were scored for Est-X and eye color.

Allelism of pearl and white

Ten white female (wht / wht) were mated with pearl males (prl / Y). The females were kept in isolate cages, their puparia were collected, and the emerging progeny were scored for sex and eye color. The reciprocal cross was conducted using ten pearl females and ten white males. All females were maintained until they died.

Visual Chromosome Anomalies on Polytenes of Heterozygous Females

Seventeen day old puparia from a wht / wht female x + / Est-X^{n} / Sr^{d} / Y male cross were used to extract polytene chromosomes from the trichogen and tormagen cells on the dorsal surface of the thorax (Southern et al. 1973). The

puparia were dissected and the pharate females with wild type eyes (heterozygous females) were selected for polytene staining. These flies contain X-chromosomes with the genetic sections for white and wild type alleles. An inversion, or a deletion may produce a loop in the chromosome that can be visualized. If either change in chromosomal structure occurred in white, then I may be able to locate, using banding patterns, the region of the X-chromosome containing the white locus.

RESULTS AND DISCUSSION

White an X-linked Recessive Allele

Table 1 summarizes the results of the five aforementioned crosses.

The segregation patterns of the wht gene (Table 1) are consistent with the hypothesis that the white allele is recessive to the wild type allele and has an X-chromosome locus. The formation of heterozygous female offspring (F_1), from the cross + / + x wht / Y, prevented the expression of the white allele. If the allele were dominant all F_1 females would have had white eyes. As predicted in Chapter 1, with the appearance of two mutant males, it was in the F_2 generation that the white-eyed phenotype reappeared, and then in males only. The cross between heterozygous females and white males, as expected for a recessive, X-linked allele, produced approximately equal numbers of white and wild type females. The cross between white females and wild type males, as expected produced only white males and wild type females. The final cross (wht / wht x wht / Y) in Table 1 showed that the allele is fully penetrant, and no back mutations have been observed.

No phenotypically white females appeared in the F_2 generation, which is what would have occurred had the gene been located on an autosome. The χ^2

Table 1 - Crosses showing the X-linked, recessive nature of the white locus (wht) in G. m. submorsitans.

Putative Parental Genotype		Number of Progeny				
Female	Male	Females		Males		
		wild type	white	wild type	white	χ^2
+ / +	wht / Y	36	0	20	0	N.C.[a]
+ / wht	+ / Y	71	0	28	25	0.08[b]
+ / wht	wht / Y	130	125	119	128	0.26[b]
wht / wht	+ / Y	30	0	0	32	N.C.
wht / wht	wht / Y	0	121	0	103	N.C.

[a] N.C. = not calculated

[b] χ^2 calculated for data on males; critical value (1 d.f.) for p = 0.05 is 3.841

values were calculated assuming a 1:1 segregation of the sexes. I know that the homozygous state for white is not lethal (later observations), so the absence of phenotypically white females in the F_2 generation was not due to pre-adult mortality. Therefore, I conclude that the locus controlling the white eye color is found on the X chromosome, not on an autosome.

Mapping the white Locus

Of the ten Brist-5 males scored, six were Est-X^n, and four were Est-X^f. Those families sired by Est-X^f males were discarded. Of the six Est-X^n males, one was sterile, and another sired too few offspring to permit continuation of that particular family/pedigree. The four remaining Est-X^n males were Sr^d. The F_1 female offspring from these families were mated to wht males.

As shown in Tables 2 and 3, there was very tight linkage between the loci white and Est-X. Of the 133 males that have been electrophoresed, none were recombinants. This seems to be the case also for the loci Est-X, Sr and white; none of the 40 males tested were recombinants.

Allelism of pearl and white

The results in Table 4 show that white and pearl are allelic, in other words at the same locus. If they had not been at the same locus, wild type F_1 females would have appeared instead of the mutant females.

Pearl and white may be two different alleles at one locus, or they may be the exact same mutation. The results do not distinguish between these possibilities. The results are not surprising since the original mutants may have been brothers; a relationship suggested by their origin, time of appearance, and identical morphology and genetic nature.

Table 2 - Mapping wht, Sr and Est-X on the X-Chromosome of G. m. submorsitans.

Putative Parental Genotype	Number of F_2 Males
wht Sr^N Est-X^F	22
+ Sr^D Est-X^N	18
Recombinant Genotype	
wht Sr^D Est-X^F	0
+ Sr^N Est-X^N	0
wht Sr^D Est-X^N	0
+ Sr^N Est-X^F	0
wht Sr^N Est-X^N	0
+ Sr^D Est-X^F	0
Total	40

Table 3 - Mapping wht and Est-X on the X-Chromosome of G. m. submorsitans.

Putative Parental Genotype	Number of F_2 Males
wht Est-X^F	64
+ Est-X^N	69
Recombinant Genotype	
wht Est-X^N	0
+ Est-X^F	0
Total	133

Table 4 - Allelism of white and pearl.

White Females X Pearl Males[a]	
Offspring Phenotype[b]	Number of Offspring
mutant male	16
mutant female	14
wild type male	0
wild type female	0
Total	30

Pearl Females X White Males[a]	
Offspring Phenotype[b]	Number of Offspring
mutant male	9
mutant female	12
wild type male	0
wild type female	0
TOTAL	21

[a] Each experiment consists of 10 females x 10 males.

[b] Since white and pearl are indistinguishable in color, they are grouped together and called mutant.

Visual Chromosomal Anomalies on Polytenes of Heterozygous Females

Using the methods of Southern et al. (1973), I found that the polytene chromosomes of G. m. submorsitans were very fragile and tended to break. Therefore, I was unable to obtain preparations that were of sufficiently high quality to determine whether there were any gross chromosomal aberrations, such as inversions or deletions. If any small inversions or deletions existed they could not be seen by light microscopy.

CONCLUSION

Of the five eye color mutants found in the genus Glossina, four of them have been studied previously, and each is caused by a recessive, X-linked allele. The fifth mutation (wht), as with the other four, was observed first in a male. Given that males are the heterogametic sex in Glossina, this form of genetic mutation would most likely manifest itself first in a male. As with the other four mutants, wht was also found to be X-linked and recessive to wild type. The close genomic association, origin, genotypic and phenotypic similarities, suggest that the second white eye mutant, pearl, is the same mutation as white.

The white mutants are descendants of a G. m. submorsitans / G. m. centralis cross (as described in Chapter 1). The original white eyed male contained 97% of its genes from G. m. submorsitans and 3% of its genes from G. m. centralis. Two crosses with G. m. submorsitans females were done in order to establish the pure line. The resulting flies contained approximately 99% of the G. m. submorsitans genotype and 1% of the G. m. centralis genotype. All subsequent generations were inbred.

There have been six loci mapped in G. m. submorsitans; Est-X, G6pd and Sr (linkage group I), Est-2 and Odh (linkage group II), and Mdh (linkage group

III) (Gooding 1993). The three G. m. submorsitans X-chromosomal loci tested did not show recombination. It was not possible to map the relative positions of Sr, Est-X, and wht because of a lack of recombination between these loci. Lack of recombination could be due to very tight linkage, close association of these loci to the centromere, or chromosomal aberrations. All three phenomena are known to drastically reduce the frequency of recombination (Strickberger 1976).

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Chapter 3

BIOCHEMICAL CHARACTERIZATION OF THE WHITE MUTATION

> "Many of the fundamental chemical relationships underlying pigment biosynthesis were derived from attempts to elucidate the mechanism of gene action by the use of eye pigment mutants."
>
> (Phillips and Forrest 1980)

Ommochrome mutants are exceptional candidates for understanding gene-biochemical relationships because the tryptophan to xanthommatin biosynthetic pathway is under tight developmental control and because there are many mutants available for study (Ryall and Howell 1974).

Characterization of the tryptophan to xanthommatin pathway was accomplished, for the most part, by Beadle and Ephrussi and by the chemist Adolph Butendandt between 1935 and 1956 (Linzen 1974). Tryptophan is converted to xanthommatin in a series of enzyme catalyzed steps (Figure 2). Information on the specific intermediates and enzymes of this pathway have been well documented in three major reviews (Linzen 1974; Phillips and Forrest 1980; Summers et al. 1982). Ommochrome mutants were used to elucidate the biochemistry of this pathway, but now I will use the elements of this pathway to understand the biochemistry of the white mutation.

Many insect eye-color mutants have been associated with a particular step in the tryptophan to xanthommatin pathway (Figure 3). Mutations causing an inactivation or partial inactivation of an enzyme, result in a build up of the precursor and a decreased concentration of subsequent metabolites in the pathway. For example, mutants with inactive tryptophan oxygenase (i.e.

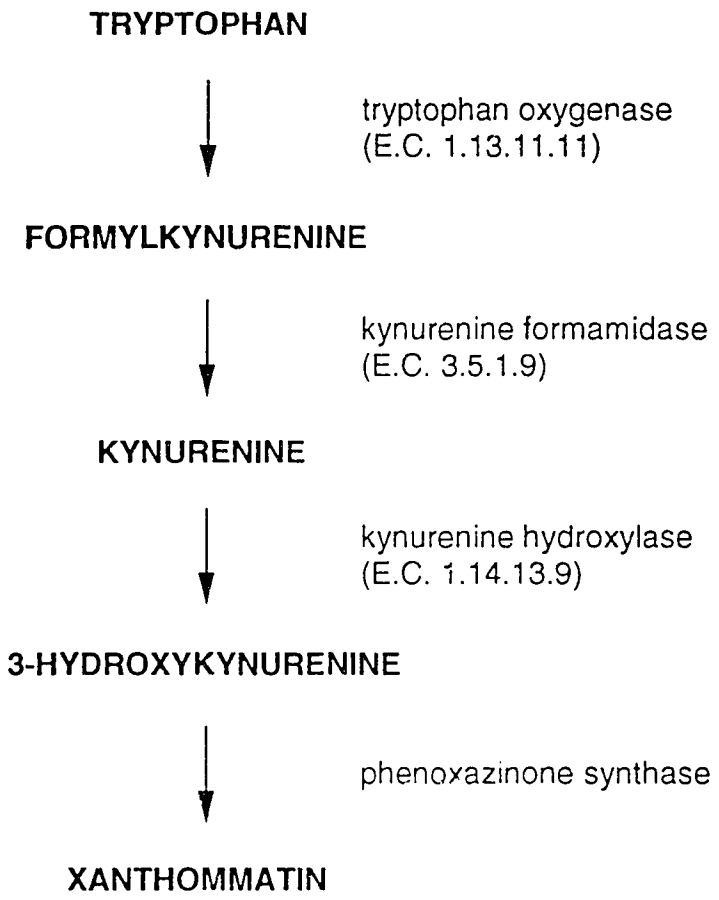

Figure 2 - Tryptophan to xanthommatin pathway.

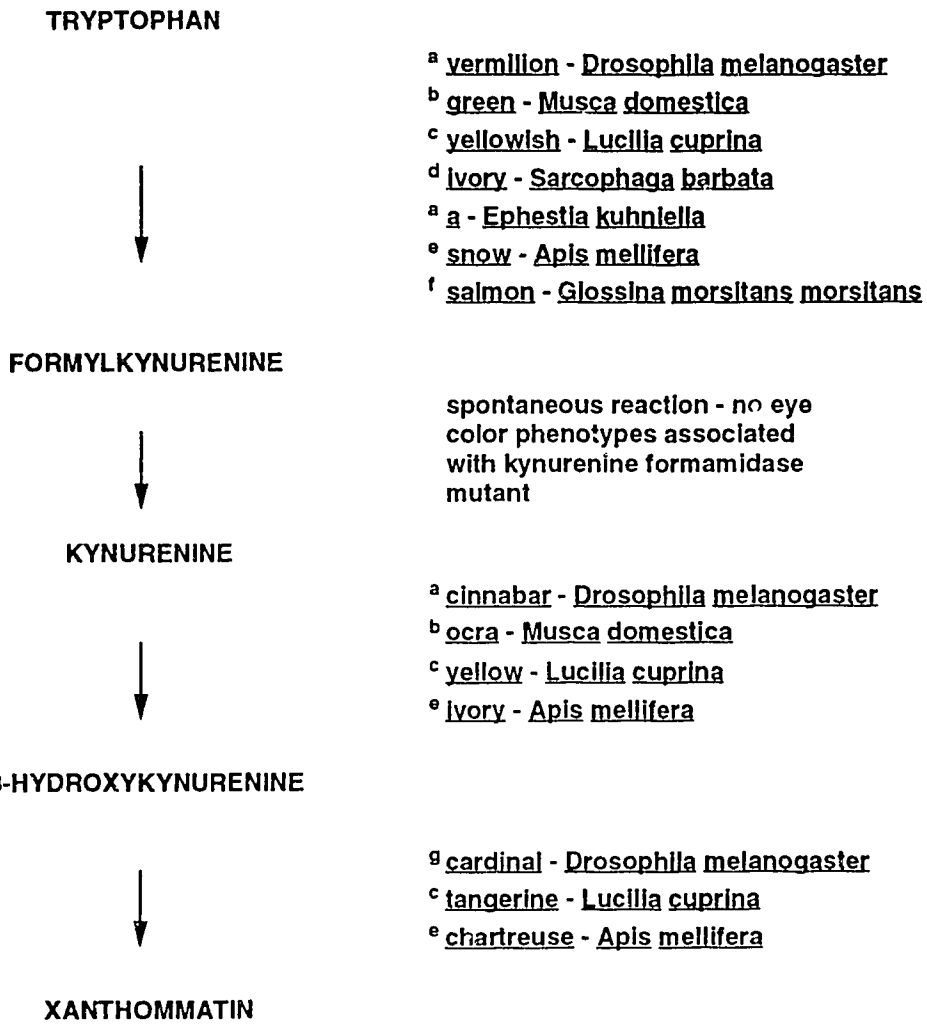

Figure 3 - Enzyme-associated mutants of the tryptophan to xanthommatin pathway.

† a. Linzen 1974; b. Laundani and Grigolo 1969; c. Summers and Howells 1978; d. Trepte 1978; e. Dustmann 1968; f. Gooding 1984; g. Phillips et al. 1973.

vermilion in D. melanogaster Meig. and salmon in G. m. morsitans) fail to produce formylkynurenine and accumulate large quantities of tryptophan. It is this accumulation pattern which implicates the element of pathway directly affected by the mutation.

Potential confusion occurs when mutations affect the last part of the pathway, namely the conversion of 3-hydroxykynurenine to xanthommatin. One of two basic mutation types can exist: a phenoxazinone synthase mutant or a transport mutant. Phenoxazinone synthase is restricted to the head and testes, and mutation affecting this enzyme may cause a decrease in xanthommatin production. Transport mutants are not related to any enzyme of the pathway, but they decrease the production of xanthommatin by causing an inability to transport the pigment precursors into the head.

Here, I present a report on the levels of ommochrome and ommochrome precursors in white (wht) and wild type G. m. submorsitans. These levels suggest that the wht mutation occurs late in the pathway, preventing the production of xanthommatin in the head, most probably at the level of pigment precursor transport.

MATERIALS AND METHODS

Chemicals

L-tryptophan, and 3-hydroxykynurenine were purchased from Sigma ; DL-kynurenine from ICN Pharmaceuticals, trichloroacetic acid from Aldrich Chemical Company, Inc., and $NaNO_2$ from BDH chemicals.

Determination of Dihydroxyxanthommatin

In order to verify that the mutation affects the tryptophan to xanthommatin pathway, I determined the amount of xanthommatin in the heads and testes. The method for quantification of dihydroxyxanthommatin, the reduced form of xanthommatin, was that described by D'Haeseleer et al. (1987). Ten heads from wht females, wild type females, wht males, and wild type males; and each pair of testes from ten wht males, and from ten wild type males were individually homogenized in 400µl of 0.5% ascorbic acid in 1 M HCl. To each homogenate, n-butanol was added (2 ml for heads and 1 ml for testes). The homogenates were centrifuged at 8000g for 10 minutes and the n-butanol fraction was read at 492 nm, the absorbance maximum of dihydroxyxanthommatin, (Dustmann 1968; 1969) in an Hewlett-Packard spectrophotometer.

Assay of Tryptophan and Kynurenine in Feces

To determine whether tryptophan oxygenase was functioning in the flies, the presence of tryptophan and kynurenine were determined in fly feces. This was done following methods modified from Gooding and Rolseth (1984), wherein cellulose plates were used instead of silica gel. Fecal samples were collected from wht females, wht males, wild type G. m. submorsitans (GMS-8) females, and wild type G. m. submorsitans (GMS-8) males daily, and frozen to prevent bacterial degradation. Each 0.5 gram sample of feces was extracted three times with 5 ml of acidified methanol (3 drops 10 M HCl/50 ml methanol). Each extract was centrifuged at 12000g for 10 minutes. The supernatants were pooled and dried in a stream of air. The residue was resuspended with 1200 µl of slightly acidified 80% methanol and recentrifuged for 10 minutes. Cellulose thin layer chromatography plates (Sigma 100 µm) were washed once in water and dried with acetone to make sure water was removed. Two µl of each fecal

preparation, as well as tryptophan and kynurenine standards, were chromatographed using an isobutanol-methanol-water (80: 5: 15) solution as the mobile phase. Each plate was air dried, observed under U.V. light and the location of each fluorescent spot was marked. The plate was then sprayed with 4-dimethylaminocinnamaldehyde spray reagent and baked for 5 minutes at 105C.

Determinations of 3-Hydroxykynurenine

All 3-hydroxykynurenine determinations were done by the nitric acid technique of Inagami (1954) as modified by Howells et al. (1977). The various sample types (I, II, III, and IV) determined are described in detail below. Each sample was homogenized in 4 ml of 5% (w/v) trichloroacetic acid (TCA) and centrifuged at 16000g for 15 minutes. 1.5 ml aliquots of the supernatant were placed into two test tubes and 1.5 ml of distilled water was added to one sample to serve as a blank. To the second 1.5 ml of 0.1% $NaNO_2$ was added. Four minutes after the $NaNO_2$ was added, absorbance was measured at 410 nm in a spectrophotometer. A 3-hydroxykynurenine standard was used to quantify the samples.

I) Assay of feces

3-Hydroxykynurenine was not found in the solute fraction of the previously described fecal extraction. The precipitate remaining from the extraction was further extracted by the nitric acid method described above.

II) Developmental profile

3-Hydroxykynurenine was determined in extracts of whole insects. Each puparial sample (ca. 0.3 grams) contained 10 individuals of unknown sex.

Each sample of teneral and post-teneral flies consisted of five females and five males (0.22g and 0.35 - 0.4g, respectively). Statistical significance was determined using a t-test ($\alpha = 0.05$).

III) Excretion upon eclosion

Samples of 10 puparia of wht and 10 of wild type were held in sterilized glass tubes. After all the flies had emerged, the tubes and their contents were washed with 4 ml of TCA and the level of 3-hydroxykynurenine was measured.

IV) Heads and bodies

3-Hydroxykynurenine content was determined in extracts of wht and wild type teneral flies (1-3 days old) and post-teneral flies (>30 days old). Samples of five males and five females were used in each determination. The flies were frozen at -25 C and decapitated. The heads and bodies were extracted separately.

RESULTS AND DISCUSSION

Determination of Dihydroxyxanthommatin

The heads of male and female wht mutants contained less than 2% of the amount of dihydroxyxanthommatin found in wild types (Table 5).This deficiency in xanthommatin, however, was restricted to the compound eyes. The testes, another site of xanthommatin storage, retained its ability to produce and/or store the pigment. The testes of wht males contained at least 50% more dihydroxyxanthommatin than did those of wild type males (Table 5).

The white mutation caused a deficiency in xanthommatin (Table 5) and pteridines (McIntyre personal communication) in the head of the mutant flies,

Table 5 - Dihydroxyxanthommatin content of white and wild type G. m. submorsitans.

Phenotype [a]	Dihydroxyxanthommatin (µg / sample) [b]	
	Run #1	Run #2
white female head	0.3 ± 0.4	0.8 ± 0.4
wild type female head	43.0 ± 1.7	43.5 ± 1.3
white male head	0.1 ± 0.1	0.7 ± 0.3
wild type male head	43.1 ± 3.4	40.6 ± 1.0
white testes	7.4 ± 0.4	8.4 ± 0.7
wild type testes	3.9 ± 0.3	5.6 ± 0.2

[a] all flies were 30-90 days old.

[b] Measurements are the mean of ten seperate determinations, ± standard deviation.

indicating either a lesion in the tryptophan to xanthommatin pathway (Figure 2) or the transport and/or storage of intermediates of this pathway in the compound eyes. The small amount of xanthommatin found in the heads may be a consequence of the pigment produced in the ocelli or it may be an indication of the lower limit of the sensitivity of the assay procedure.

Assay of Tryptophan and Kynurenine in Feces

Kynurenine was present in extracts of the feces of wht males, wht females, wild type males, and wild type females (Table 6). Tryptophan occurred in trace amounts in one sample from wht males.

Gooding and Rolseth (1984), identified kynurenine as a normal excretory product of post-teneral wild type Glossina. In contrast, the tryptophan oxygenase mutant salmon in G. m. submorsitans is unable to convert tryptophan to kynurenine and consequently large quantities of tryptophan are excreted. The absence, or very low quantities, of tryptophan and the presence of kynurenine indicated that tryptophan oxygenase was active in white eyed G. m. submorsitans.

Determinations of 3-Hydroxykynurenine

I) Assay of feces

The levels of 3-hydroxykynurenine excreted by wht males and females were indistinguishable from those excreted by wild type (Table 6). Of the 0.5 gram sample of fecal material used in each of the readings of wht and wild type, almost 600 µg was found to be 3-hydroxykynurenine.

Levels of 3-hydroxykynurenine in the fecal material of post-teneral wht mutants, that were similar to levels in wild type flies, demonstrated adequate levels of activity of kynurenine hydroxylase for production of pigment.

Table 6 - Intermediate metabolites found in the fecal material of white and wild type G. m. submorsitans.

Intermediate Metabolites	white	wild type
Tryptophan	-	-
Kynurenine	+	+
3-Hydroxykynurenine	+	+

II) Developmental profile

Levels of 3-hydroxykynurenine during adult development are shown in Figure 4. In wild type flies the level of 3-hydroxykynurenine was maximal about midway through the puparial life; it declined precipitously until the time of eclosion. At adult emergence the level decreased drastically then increased as the flies aged.

One to three days into the puparial life, the level of 3-hydroxykynurenine in wht flies was found to be insignificantly lower than that of the wild type. By midway through puparial life (days 18 to 20) the level had increased to levels similar to those in the wild type. In wht, 3-hydroxykynurenine levels reached their maximum at the end of the puparial life (days 27 to 29), retaining more than two times the wild type level at this age. As with wild type flies, there was a substantial decrease in 3-hydroxykynurenine 1 to 3 days after eclosion, but the level of 3-hydroxykynurenine was still significantly higher than that of the wild type. Unlike wild type flies, however, the level continued to decrease with the age of the fly (>30 days old), the final levels in wht and wild type having no significant difference.

The decline of 3-hydroxykynurenine in wild type developing adults, midway through puparial life, coincided with the formation and deposition of xanthommatin (Gooding and Rolseth 1984). The continuing rise in 3-hydroxykynurenine in the latter part of the puparial period of wht mutants, indicated that either the 3-hydroxykynurenine present was not being converted into xanthommatin (defective phenoxazinone synthase, transport, or other problem), or that kynurenine hydroxylase was more active in wht than wild type developing adults. The latter theory is not supported by the lower than wild type levels seen in early (1 to 20 days) wht adult development.

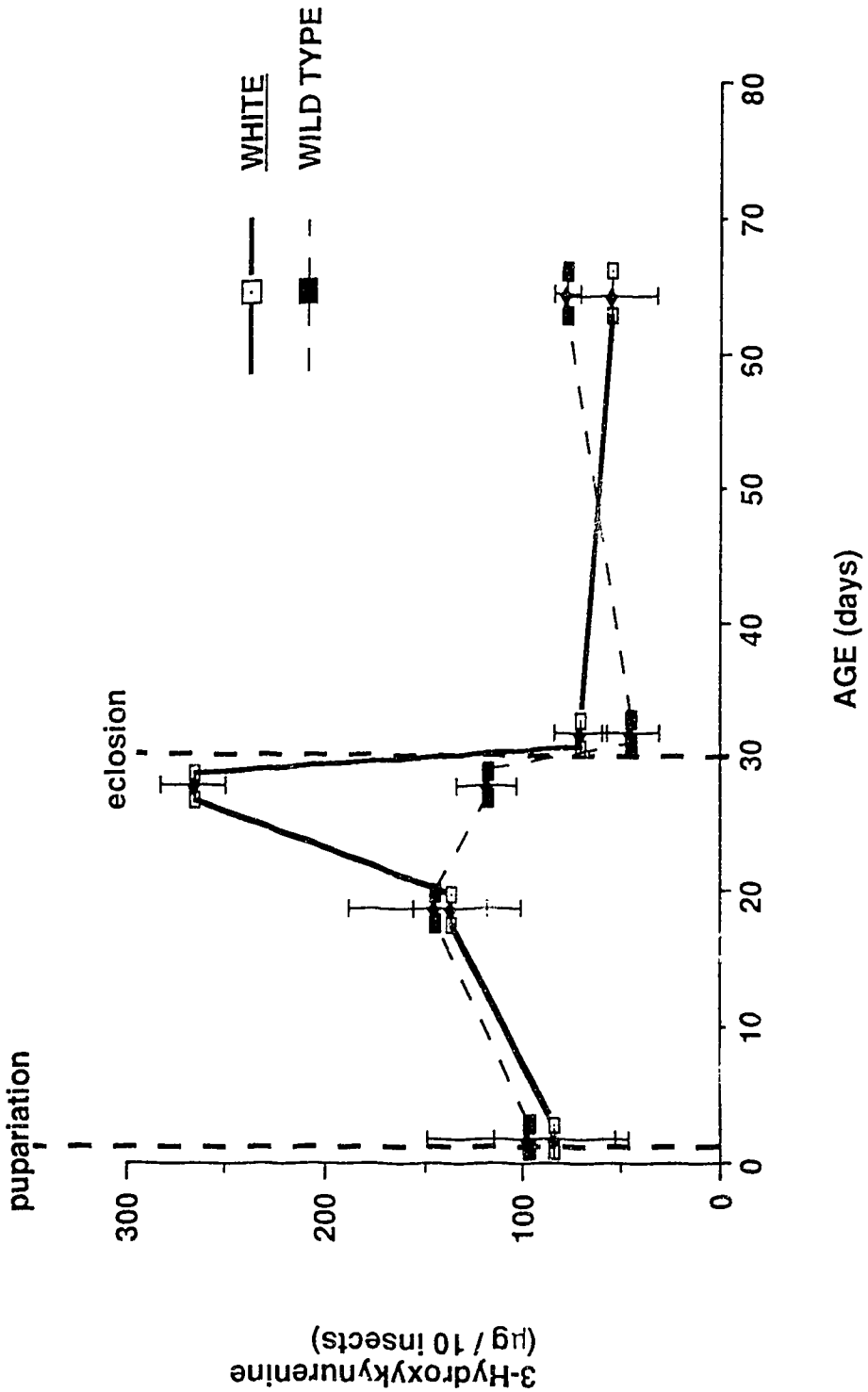

FIGURE 4 - Levels of 3-OH-kynurenine during adult development in wild type, ■; and white, □. Points are the mean of five seperate determinations, ± standard deviation. Each pupal and teneral fly sample was collected over a 3 day period.

The 3-hydroxykynurenine that accumulated during puparial life of wht and wild type flies was either metabolized or excreted. Since the (post-teneral) adults of both wht and wild type flies excreted similar levels of 3-hydroxykynurenine (Table 6), the pupal cases and meconium of teneral flies were assayed for 3-hydroxykynurenine.

III) Excretion upon eclosion

Analysis of material excreted at the time of eclosion, showed that wht mutants excreted more than four times the amount of 3-hydroxykynurenine that wild type flies excreted (Figure 5). This would explain the sudden drop in 3-hydroxykynurenine levels of both wht and wild type at the time of eclosion (Figure 4).

IV) Heads and bodies

The amount of 3-hydroxykynurenine in the heads of wild type teneral flies was almost twice the amount that was in the bodies (Table 7). This distribution changed with age (Table 7). In post-teneral wild type flies (>30 days old), the heads contained only one-tenth the amount found in the bodies.

In contrast, wht teneral flies had most of the 3-hydroxykynurenine in their bodies, greater than eleven times that found in the heads. Unlike wild type flies, the distribution did not change with age. The 3-hydroxykynurenine that was found in the heads of the teneral flies was either utilized or expelled from the head, for none was found in the heads of the post-teneral mutants. The level in the body did not change drastically.

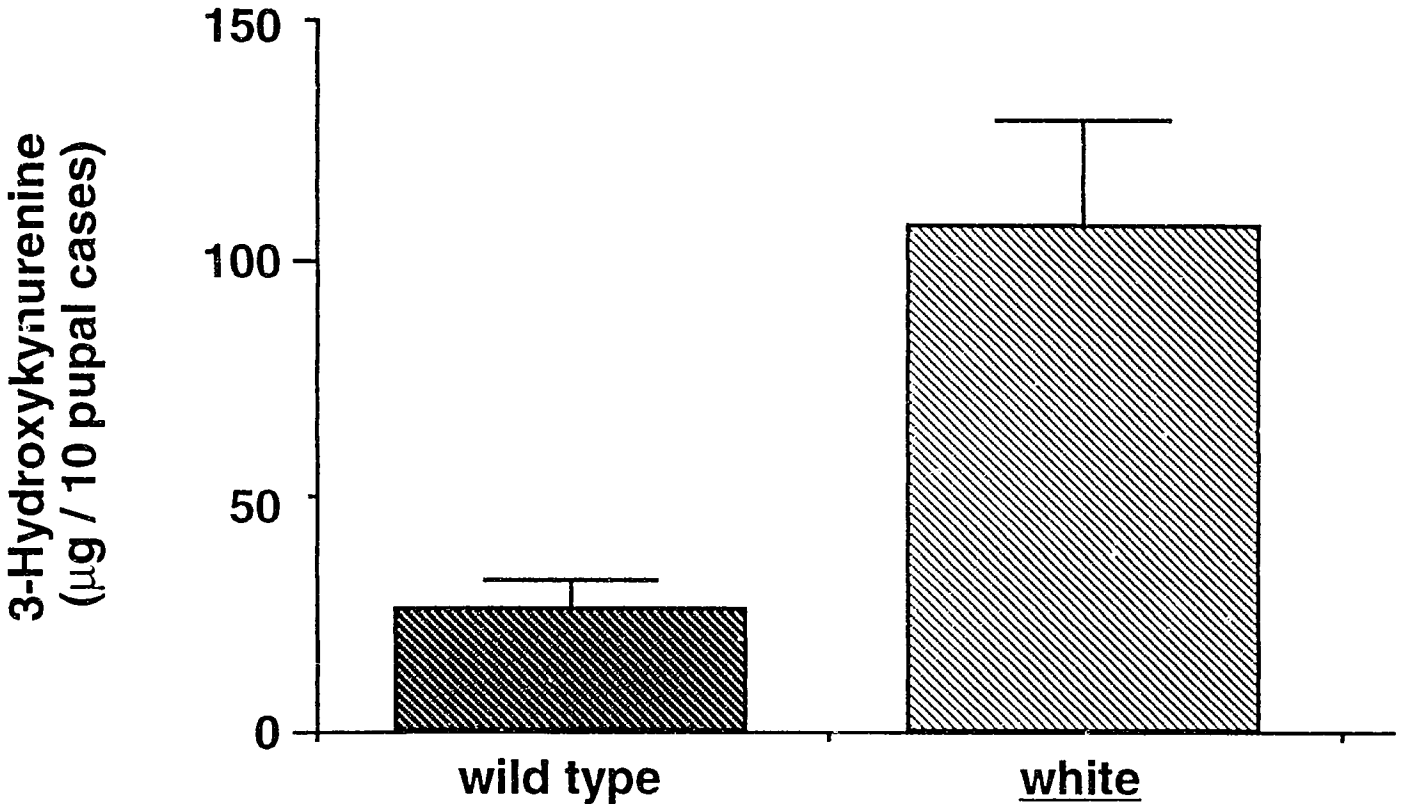

Figure 5 - Amount of 3-hydroxykynurenine excreted by wild type and white teneral flies. Points are the mean of five separate determinations, ± standard deviation. Each sample contains 10 puparia.

Table 7 - 3-Hydroxykynurenine levels in white and wild type G. m. submorsitans.

Tissue Sample	3-Hydroxykynurenine (µg / sample) [a]	
	white	wild type
teneral heads	5.7 ± 1.6	43.5 ± 6.8
teneral bodies	66.9 ± 31.4	23.4 ± 6.6
post-teneral heads	0 ± 2.3	8.2 ± 1.5
post-teneral bodies	63.6 ± 40.8	89.6 ± 15.4

[a] Values ± standard deviation are the means of 5 separate determinations. Each sample contained 10 heads or 10 bodies (5 male and 5 female).

CONCLUSION

The white-eye color phenotype has been identified in many dipteran species: white of D. melanogaster (Beadle and Ephrussi 1936), white of M. domestica L. (Laudani and Grigolo 1969), chalky of Calliphora erythrocephala Meig.(Langer 1975), white of Lucilia cuprina Wied. (Whitten et al. 1974), and white of Anopheles gambiae Giles (Mason 1967; Zheng et al. 1993). The most prominent characteristic of the white phenotype is the absence of both types of screening pigments, the ommochromes and the pteridines (although this has not been determined for Anopheles). Many eye color mutants are known to alter the production and/or accumulation of both classes of pigments to a greater or lesser degree, but others are not as extreme as the white mutants (Summers, et al. 1982). Summers et al. (1982), suggested that this results from an uptake problem for both pteridines and ommochromes.

Recently this hypothesis was verified. Ewart et al. (1994) identified the gene products of white (devoid of ommochromes and pteridines), brown (devoid of pteridines) and scarlet (devoid of ommochromes) loci of D. melanogaster. All three were found to "belong to the Traffic ATPase superfamily of transmembrane proteins involved in transporting guanine (the precursor of pteridines), and tryptophan (the precursor of ommochromes)". Structural changes in the protein interrupt the transport of the precursors.

The presence of kynurenine in the excreta, and 3-hydroxykynurenine in the body and excreta of wht mutants of G. m. submorsitans, showed that the mutation affected the latter part of the tryptophan to xanthommatin pathway. It is highly probable that transmembrane proteins and mechanisms, similar to those discussed in the above paragraph, are involved in the wht mutant of G. m. submorsitans. The mutation caused a deficiency in xanthommatin (Table 5)

and pteridines (McIntyre personal communication) in the compound eyes of the mutant flies, but no deficiency of xanthommatin in the testes (Table 5).

If the mutation is the result of structural changes in the transmembrane proteins, it does not affect xanthommatin production in the testes of wht males. Production of xanthommatin in the testes seemed to compensate for some of the lack of production/storage in the eyes. The overproduction of xanthommatin in the testes may have been a result of 3-hydroxykynurenine build up during the puparial stage. Because the eyes no longer remove 3-hydroxykynurenine from the body, the testes may have had more precursors to produce the higher than normal levels of xanthommatin. This higher than wild type level of testicular xanthommatin has been reported also for tan (in G. p. palpalis), another mutant which affects the latter part of the tryptophan to xanthommatin pathway (D'Haeseleer et al. 1987).

Biochemical information on the wht mutant of G. m. submorsitans is similar to the transport mutants, white of D. melanogaster (Howells et al. 1977) and white of L. cuprina (Summers and Howells 1978, 1980a). All three were devoid of both types of screening pigments, but had the ability to produce kynurenine and 3-hydroxykynurenine. These mutants demonstrated an altered 3-hydroxykynurenine accumulation pattern.

The phenoxazinone synthase mutant cardinal of D. melanogaster (Nolte 1951) and tangerine of L. cuprina (Summers and Howells 1978, 1980a, 1980b) have lower levels of xanthommatin in the heads than do wild type flies, but neither affect the production and deposition of pteridines.

At pupariation, the 3-hydroxykynurenine levels were slightly lower in wht than in wild type flies (Figure 4). Like the transport mutants of D. melanogaster and L. cuprina, this may be a consequence of excreting large quantities of 3-hydroxykynurenine before pupariation (Howells et al. 1977; Summers and

Howells 1980a). Tsetse larvae have two pairs of Malpighian tubules and discharge accumulated waste just prior to pupariation (Langley 1977). Unfortunately, it is technically not feasible to document accumulation of 3-hydroxykynurenine in the Malpighian tubules of tsetse larvae because the larvae are in utero.

The elevated level of 3-hydroxykynurenine in late puparial stages of wht, were characteristic of white and topaz of L. cuprina (Summers and Howells 1978), and cardinal of D. melanogaster but not of white in D. melanogaster (Howells et al. 1977; Phillips et al. 1970). The similarity in levels of 3-hydroxykynurenine in white and topaz of L. cuprina and wht of G. m. submorsitans may be a result of the closer taxonomic relationship shared by these two genera, as compared to Drosophila (Gillott 1980).

Like white of D. melanogaster, the accumulated 3-hydroxykynurenine was excreted upon eclosion, and was found in the meconium and puparial cases (Howells et al. 1977).

The possibility that wht had the same defective transport and storage phenotype as D. melanogaster, was supported by the levels of 3-hydroxykynurenine found in the heads and bodies. Like white of D. melanogaster (Sullivan and Sullivan 1975) and L. cuprina (Summers and Howells 1980a), the wht mutants of G. m. submorsitans had very low levels of 3-hydroxykynurenine in the heads but accumulated large quantities of 3-hydroxykynurenine in the bodies. The highest kynurenine hydroxylase activity occurs in the developing eyes of the fly (Sullivan et al. 1973), therefore this distribution suggests a problem in the mutant's ability to transport 3-hydroxykynurenine or kynurenine (to be converted to 3-hydroxykynurenine) into the head. It is clear that the elevated levels of 3-hydroxykynurenine seen in adult development were not sequestered in the eyes of wht. In contrast, the

heads of the phenoxazinone synthase mutants of D. melanogaster and L. cuprina (cardinal and tangerine, respectively) have levels that are indistinguishable from those in wild type flies (Sullivan and Sullivan 1975; Summers and Howells 1980a).

The exact location of the small amounts 3-hydroxykynurenine found in the heads of wht (Table 7) was uncertain. If the mutants were incapable of transporting the metabolites into the pigment cells, it may have been, as proposed by Summers and Howells (1980a), present in the intercellular space and not directly in the cells.

One surprising difference between wht in G. m. submorsitans and the white mutant of D. melanogaster, was the presence of pigment in the ocelli and the testes of [illegible]. Pigmentation of gonads and ocelli are often affected by mutations that cause abnormal eye color (Summers et al 1982). Gonads of wild type D. melanogaster are yellow but those of white mutants are colorless (Summers et al. 1982); ocelli of white D. melanogaster are colorless (Phillips and Forrest 1980). The gonads of wht mutants of G. m. submorsitans were unaffected; ocelli and gonads have the wild type color.

Since the wht mutant of G. m. submorsitans, like white-eye color mutants of other Diptera, affects also the accumulation of pteridines, it seems less likely that the enzyme phenoxazinone synthase is solely involved. If only the enzyme were defective, one would expect to see pteridine production and accumulation, and possibly the lack of xanthommatin in the testes (if the same phenoxazinone synthase is responsible). The alleged phenoxazinone synthase mutant of Drosophila melanogaster (cardinal), contain pteridines but not ommochromes (Ferre et al. 1986).

These results suggest that the wht mutant is a transport mutant, but we do not know whether it is homologous with the white gene of D. melanogaster.

Future work comparing the white gene from D. melanogaster may verify the gene to be homologous with wht of G. m. submorsitans.

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Chapter 4

PHYSIOLOGICAL EFFECTS OF THE WHITE MUTATION

Pleiotropic genes, genes that produce more than one distinct phenotypic effect, are associated with mutations of all kinds. The multiple phenotypic effects are called pleiotropic effects. With regard to Drosophila eye color mutants, most show complex pleiotropic interrelationships with other systems (Ferré et al. 1986). Some of the pleiotropic effects associated with eye color mutants in general, include changes in longevity, fertility, fecundity, periods of adult development, as well as drastic changes in mating and food acquisition behaviour. The former may be a result of the biochemical interactions, deficiencies, or excesses. The last two are most likely a consequence of changes in the insect's vision, although courtship behaviors in Drosophila are also thought to be sensitive to metabolic defects (Cook 1980; Sappington 1991; Hall 1994). Some body pigment mutants have been associated with neurochemical problems (Black 1988; Hall 1994).

Previous studies on tsetse eye color mutants have demonstrated a wide range of pleiotropic effects. The salmon mutant of G. m. morsitans is a semilethal mutation, producing congenitally abnormal offspring in a pure line. The presence of the mutation causes a decrease in longevity, fertility and fecundity (Gooding and Rolseth 1987). Less drastically affected is the brick mutant, where only the lifespan and fecundity are affected (van den Abbeele and D'Haeseleer 1989). By contrast, the tan mutant is relatively unaffected by the disruption of the tryptophan to xanthommatin pathway (D'Haeseleer et al. 1987; Gooding and Rolseth 1987).

Pleiotropic genes give us insight into how genes and their products work and how they interact with other systems. The interactions of systems within the body of an insect are amazingly complex, and little is understood about how one system depends on another to function normally.

In order to use mutants for purposes other than extracting pure genetic information, or understanding gene-enzyme interactions, one must evaluate the fitness of the mutant. It is important to understand all the repercussions of a mutation before the mutant can be used in any field or control work. The mutant in question must be able to survive and/or reproduce in natural field conditions.

Pleiotropic effects can be advantageous or detrimental depending on what use is to be made of the mutant. For control practices one looks for factors that limit the reproduction and survival of the insect in natural populations (Pal and Whitten 1974). Control practices involving releasing mutant insects into natural populations, to incorporate into natural populations detrimental genes or genetic markers, depends on this information to evaluate the likelihood of success of any control program or population assessment. For example, the salmon mutant has many promising attributes for use in control programs. This semi-lethal mutant might have been an acceptable genetic control agent until it was determined that it has a higher vectorial competence for Trypanosoma spp. than do the wild type G. m. morsitans (Makumyaviri et al. 1984; Distelmans et al. 1985).

The purpose of this report was to assess the fitness of the white G. m. submorsitans lab colony, relative to that of the wild type, and to determine the other physiological repercussions of this mutation. These repercussions may then be used to assess the potential use of white in field or control practices. In order to ascertain the status of the mutant I looked at factors which qualify

fitness. Many of the parameters monitored are commonly used to measure health of laboratory colonies (D'Haeseleer et al. 1987).

MATERIALS AND METHODS

Laboratory conditions and feeding regime were as described in Chapter 2.

I set up matings of ten white female x white male, and from the G. m. submorsitans line Gms-8, ten wild type female x wild type male. The wild type matings served as the control, assuming that the wild type line was healthy and was representative of G. m. submorsitans. Each mated female was kept in a cage (3.5 cm x 3.5 cm x 5 cm) in a soap dish and was monitored daily. I recorded her age (in days) when she deposited her first offspring each gestation period thereafter, and the total number of offspring produced. The males were kept in separate cages of the same size. Both females and males were monitored for 90 days, and within this period, each fly was measured for lifespan, and fertility.

Male fertility was assessed by mating each male to two other virgin G. m. submorsitans females, and checking the females for insemination and pregnancy. Female fertility was based on ability to produce offspring. If a female did not produce any offspring, she was dissected at the time of death or at 90 days of age to see if she had been inseminated. Females which did not produce offspring by twenty-eight days (enough time for two larvipositions to occur) and were inseminated, were scored as infertile.

Pregnant females, one to two days before they were to deposit (* black lobe stage), were placed in a glass tube with 1-3 cm of sand. Once the larvae was deposited, the female was returned to her original cage. As each puparium

was deposited it was placed in an individual container. At no time were the puparia touched by human hands. Latex gloves were used to prevent contracting of oil and dirt from the hands as these would have altered the weight of the puparia. Each puparium was weighed between twenty-four and forty-eight hours after deposition. As well as the puparial weights of females and males, puparial periods of females and males were measured, and emergence of offspring was recorded.

The study was replicated.

Due to the small sample sizes, in most cases, a Bootstrap permutation test was implemented to determine the statistical significance of the difference between samples. Each test involved 10,000 random permutations of the original samples. T-tests ($\alpha = 0.05$) were also used to determine statistical significance in some cases; these are indicated in Tables 8 and 9.

RESULTS AND DISCUSSION

Parental Parameters

Table 8 summarizes the parameters measured for white and wild type adult G. m. submorsitans. These include the percent fertility of males and females, the percentage of males and females surviving the 90 day test period, fecundity, age at first larviposition, and interlarviposition period.

Under laboratory conditions, it was found that fertility and longevity did not differ significantly between mutant and wild type adults. Wild type and white

*The black lobe stage is when "the polyneustic lobes of the third-instar larva have hardened and darkened, and the in-utero larva is within 48 h. of natural parturition" (Langley 1977).

Table 8 - Biological parameters in white and wild type G. m. submorsitans colonies maintained by feeding every two days. Sample sizes are reported in parentheses.

Run #1	Colonies		
Parameter measured	White	Wild type	P, T-value
% fertility of females	90 (10)	100 (10)	——
% fertility of males	100 (10)	100 (10)	——
% surviving of females (90 days)	70 (10)	80 (10)	——
% surviving of males (90 days)	40 (10)	40 (10)	——
Puparia per female (90 days)	3.0 ± 2.3 (30)	4.1 ± 2.0 (41)	1.13 [c]
Initial gestation period (days)	27.2 ± 13.4 (9)	22.2 ± 6.1 (9)	0.28
Interlarviposition period (days)	13.6 ± 5.8 (21)	11.6 ± 2.9 (31)	0.13

Run #2	Colonies [a, b]		
Parameter measured	White	Wild type	P-value
% fertility of females	100 (9)	100 (10)	——
% fertility of males	100 (10)	100 (10)	——
% surviving of females (90 days)	60 (10)	70 (10)	——
% surviving of males (90 days)	60 (10)	50 (10)	——
Puparia per female (90 days) [c]	4.5 ± 1.9 (45)	4.9 ± 1.9 (49)	0.48 [c]
Initial gestation period (days)	21.4 ± 5.7 (9)	20.8 ± 10.1 (9)	0.86
Interlarviposition period (days)	12.9 ± 4.3 (34)	12.6 ± 5.3 (38)	0.77

[a] data given are means ± standard deviation.

[b] data are for 10 individually mated pairs.

[c] t-test value

females showed no significant differences in initial gestation period or in interlariposition period. For both white and wild type females, there was an increase in the overall number of puparia produced in run #2 as compared to run #1, but this increase was not statistically significant. Neither run showed significant differences between white and wild type fecundity, although the mean was consistently lower for white females.

The difference in longevity between males and females was expected for it has been previously reported that females live significantly longer than males (Rawlings and Maudlin 1984).

The initial gestation period was significantly longer and more variable than the interlariposition period in both white and wild type females. This was expected for work by Mellanby (1937) demonstrated that the ripening period of the egg (minimum of eight days), delay in ovulation and/or delay in fertilization all contributed to the irregularity and variability in initial gestation period.

Offspring Parameters

Table 9 summarizes the parameters measured on the offspring of white and wild type G. m. submorsitans. These include weights of male and female puparia, male and female puparial periods, and percent emergence.

The presence of the mutation did not significantly affect the female puparial weights, development time of females, or the emergence rate. With regard to male puparial weight, run #1 did not reveal any significant difference between white and wild type. In contrast, run #2 did show a significantly higher weight in puparia of white males. The larger number of males produced in the run #2, may provide a more accurate representation of male puparial weights.

In both run #1 and run#2 the sex ratio did not differ significantly from a 1:1 ratio of males to females (run #1: 9 white males, 16 white females, χ^2 =

Table 9 - Bionomic parameters in white and wild type G. m. submorsitans colonies maintained by feeding every two days. Sample sizes are reported in parentheses.

Run #1	Colonies[a, b]		
Parameter measured	White	Wild type	P-value
Weight of female puparia (mg)[c]	32.2 ± 3.7 (16)	31.0 ± 4.8 (23)	0.38
Weight of male puparia (mg)[c]	30.8 ± 2.7 (10)	31.6 ± 3.5 (12)	0.53
Female puparial period (days)	29.8 ± 0.6 (16)	30.0 ± 0.8 (23)	0.18
Male puparial period (days)	31.9 ± 0.6 (9)	32.8 ± 0.7 (12)	0.0006
% emergence	83.3	87.5	——

Run #2	Colonies[a, b]		
Parameter measured	White	Wild type	P-value
Weight of female puparia (mg)[c]	31.4 ± 4.8 (18)	32.0 ± 3.5 (26)	0.62
Weight of male puparia (mg)[c]	34.0 ± 2.7 (18)	31.6 ± 2.2 (20)	0.002
Female puparial period (days)	29.5 ± 0.8 (18)	29.9 ± 0.9 (25)	0.093
Male puparial period (days)	32.3 ± 0.7 (18)	32.4 ± 0.7 (20)	0.73
% emergence	83.7	95.7	——

[a] data given are means ± standard deviation.

[b] data are for 10 individually mated pairs.

[c] pupae are weighed 48 hours after deposition.

0.96; 12 wild type males, 23 wild type females, $\chi^2 = 2.86$; run #2: 18 white males, 18 white females, $\chi^2 = 0.0$; 20 wild type males, 25 wild type females, $\chi^2 = 0.54$).

The overall lower fecundity (Table 8), compounded with the overall lower emergence rate (Table 9) did not significantly affect the total production of white adults (critical t-value (1 d.f.) for $\alpha = 0.05$ is ± 2.101). In run #1 twenty-five white adults and thirty-five wild type adults were produced (t=1.03). In run #2 thirty-six white adults and forty-five wild type adults were produced (t=-1.02).

Previous observations showed that a greater number of white larvae become entrapped in the cage netting when they were deposited. This phenomenon may have resulted from the greater weight of white males (Table 9), or it may have resulted from changes in neural activity and behaviour associated with increased levels of kynurenine and 3-OH-kynurenine, as occurs in Apis mellifera (Lopatina et al. 1994).

CONCLUSION

General Comparison of white and Wild Type G. m. submorsitans

The work described here pertains to bionomic characters of white and wild type G. m. submorsitans in laboratory colonies and demonstrated that the white mutant does not differ drastically from the wild type fly. The only significant difference is seen in male puparial weights.

Comparison of white and other Tsetse Mutants

Unlike sal, wht mutants were virtually unaffected with regards to longevity, fecundity, and emergence rate. It was in these ways that white resembled physiologically the tan mutant of G. p. palpalis. Both mutations act

late in the tryptophan to xanthommatin pathway, and neither have drastic pleiotropic effects. Both mutants had a build up of xanthommatin precursors, but the additional loss of pteridines in white (McIntyre personal communication), and its consequent increase in pteridine precursors may account for the minor difference, namely the increased male puparial weight, seen in the white mutants.

Comparison of white of G. m. submorsitans and white of Anopheles

Although there is a great deal of information on the biochemistry of the white eye color phenotype in other insect species, very little work has been reported on its pleiotropic effects. Mason's work (1967) on the white eye color mutant of Anopheles gambiae provided some information with which to compare white of G. m. submorsitans. Mason found that white females produce one-third fewer offspring than do wild type females, but the offspring that are produced are healthy. He found also that the presence of the mutation decreases the longevity of the adults. Studies on the white mutant provided no evidence to demonstrate that fecundity or longevity was drastically affected. The difference in effect on longevity may have resulted from biological differences between the insects, or the results with white for G. m. submorsitans may have been biased by the arbitrary choice of 90 days as the time to terminate the experiment, rather than continuing it until each female and male died. The 90 day experimental period was modeled after Gooding and Rolseth (1987).

Future Work

The results of this work may or may not have represented the same characteristics of the flies under the natural conditions of the field. For example,

Mellanby (1937) reported that the pupae of laboratory raised tsetses are on the average smaller than those found in the field. Further work under field conditions will be needed to confirm these findings.

Work must still be done to determine the mutation's effect on behaviour, namely the mating and feeding responses. Determination of the trypanosome transmission capabilities of white will also be necessary in order to determine the true potential for use of this mutant in control practices or population assessments.

With regards to the physiological parameters studied, the pleiotropic effects of the white allele are minimal. In the event that white is not a better vector than wild type, and that white is able to survive and/or reproduce in natural field conditions (yet to be determined by behavioral studies), it's use in control practices will be limited. The fecundity and lifespan of the mutant are not affected enough to implement white in control practices which solely rely on factors, namely the mutation, that limit the reproduction and survival of the insect.

The white mutant has the potential to be used for population assessments as well as control practices that involve releasing marked insects into the natural population to incorporate detrimental genes. In these situations, the white mutant would have many advantages over biochemical mutants. In release recapture and monitoring experiments, white would be easier to score, decrease chances of error in scoring, consume less time to score, and would be more economical with regards to the man hours and equipment.

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Chapter 5

GENERAL CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

It has been suggested by some tsetse researchers (Langley 1977; Jordan 1986) to investigate the use of molecules of metabolic significance to the normal insect as a control technique. One can do this by manipulating the levels of these molecules either by topical application or by genetic manipulation (production and/or use of genetic mutants). In order to do these types of programs successfully we must increase our understanding of the genetics and physiology of the target organism.

Genetics

This study was done to characterize the white eye color mutant (wht) genetically, biochemically, and physiologically. The mutant white, is the first visible marker reported for G. m. submorsitans. Genetic studies revealed that the wht mutant, like the other eye color mutants reported for G. m. morsitans and G. p. palpalis, was X-linked and recessive to wild type. The white mutant was allelic to pearl, a variant that is phenotypically identical to white and which was found shortly after white was found. I was unable to map the location of wht, relative to the two previously mapped X-chromosome loci, Est-X and Sr, because of the lack of recombination. This was unfortunate, because knowledge of gene locations and the manifestations of mutations at these locations would allow us to apply genetic engineering in attempts to control the insect population.

Once antitrypanosomal genes or detrimental genes (i.e. second generation lethals or sterility factors) are discovered, they can be incorporated

into the tsetse genome. In order to do this successfully, scientists need a sufficient map of the tsetse genome. Mapping visual markers such as white help to enrich our knowledge of the tsetse genome and provide reference points with which to locate other genes.

A single gene mutation can be formed in a variety of ways, and under certain circumstances can affect other genes. The genes affected may account for some of the phenotypic effects seen. For example, the formation of the original mutation wht, may have been due to a chromosomal anomaly, possibly affecting nearby genes. Likewise, multiple mutations in a small region of a chromosome may also indicate a mutational "hotspot". If the mutation occurred in such a region, other genes in the vicinity may have been affected also, but not detected. With regard to white, there was no evidence to indicate that a chromosomal aberration was involved. If the lack of recombination amongst the three X-chromosome loci, wht, Est-X and Sr, was due to very tight linkage, the presence of three genetic mutants in such close proximity might indicate a mutational "hotspot" region.

Biochemistry

Like most other white eye color mutants reported for Diptera, the mutation prevents the production or deposition of both xanthommatin and pteridines. The white mutant of G. m. submorsitans is unusual in that it affects only the compound eyes and not the ocelli or testicular sheath. The focus of my study was on the production of xanthommatin through the tryptophan to xanthommatin pathway.

The accumulation of large quantities of 3-hydroxykynurenine demonstrated that the first three enzymes in the pathway were sufficiently active to produce xanthommatin. The high levels of 3-hydroxykynurenine in the

bodies, but the lack of this precursor in the heads (in contrast to the situation in wild type flies), supports the hypothesis that it could not be transported into the compound eyes to be converted to xanthommatin.

Physiological Effects

Many ommochrome mutants have been reported as pleiotropic mutants. The effects of one mutation can affect a whole array of biological factors. They can have other genetic consequences, as well as biochemical, physiological and behavioral repercussions.

The associations or interactions of biochemicals within the insect body are largely unknown, and consequently, alterations to this normal balance may have unknown effects. If chemicals from one pathway normally interact with metabolites or cofactors in another, a mutation may disrupt the association and produce multiple phenotypic effects. Also, research has shown that the build up of certain chemicals in the body produce toxic or carcinogenic effects (Linzen 1974).

Previous studies showed that elevated levels of ommochrome precursors, namely tryptophan, have detrimental effects on developmental processes causing retardation of larval growth, prolongation of adult development, reduction of size, and the induction of tumors (Sullivan et al. 1973; Linzen, 1974). The white mutant did not produce elevated levels of tryptophan, but did produce elevated levels of 3-hydroxykynurenine. The pleiotropic effects resulting from these elevated levels were not as detrimental to the white mutant in G. m. submorsitans, as were the elevated levels of tryptophan found in the salmon mutant in G. m. morsitans (Gooding 1979; Gooding and Rolseth 1987). Other effects of tryptophan with regard to the above mentioned parameters, have not yet been reported for salmon.

Physiological manifestations of the mutation, other than the obvious lack of screening pigments in the compound eyes, are minimal. Although the differences in bionomic parameters compared between white and wild type adults were not statistically significant, an overall lower mean in fecundity and higher means in initial gestation period and interlarviposition period (Table 8) of white were seen. These three parameters may have been influenced by the somewhat unique larviparous nature of Glossina. Potential toxic effects resulting from the production and accumulation of 3-hydroxykynurenine in the developing larvae (Figure 4), may have been borne by the mother. The toxicity of these precursors did not influence the survival of white females, but may have led to the minor changes in fecundity, initial gestation period and interlarviposition period (Table 8).

One of the important pleiotropic effects to mention, is vision. All eye color mutants are variably affected in this area. The lack of screening pigments, especially of the ommochromes, cause a lowering of the light detection thresholds, and a decrease in acuity and contrast sensitivity. Mutants of this type take in more light, and consequently may be blinded at normal light intensities. Casual observations of white suggest this may be the case. When released from their cage, they tended to migrate to darker areas of the room, whereas wild type flies were attracted to lights. This possible blindness at normal light intensities was also observed in the tan mutant of G. p. palpalis (D'Haeseleer et al. 1987) and the salmon mutant of G. m. morsitans (Davis and Gooding 1983) and may have been more enhanced in white which lack pteridines also. The potential behavioral and physical disabilities, under laboratory light conditions, might impede efforts to utilize the mutant in field studies, where light intensities are much higher. These disabilities may have

detrimental effects on the individual's ability to procure food and mates, as well as locate places to oviposit.

Future Work

This study provides some basic information about the white mutant of G. m submorsitans, but more work must be done in order to understand fully the mutation and it's potential for field application. Future attempts to identify the wht locus should be directed at determining the genes homology with the white mutant of Drosophila. This homology, and the chromosomal location of wht may be established by using the previously sequenced white gene of Drosophila and in situ hybridization techniques. Once the gene is identified, it can be sequenced. This sequence may reveal similarities in the gene products of wht of G. m. submorsitans and white of Drosophila (Ewart et al. 1991).

The full capabilities of this mutant will not be known until its vectoring abilities are determined. Of the six factors that influence the vectoring ability of tsetse (Jordan 1986), the wht mutation altered the genetic status, the physical and biochemical, and possibly the behavioral (no direct evidence) state of the fly. It will be interesting to see what influence, if any, the wht mutation has on trypanosome transmission. With regard to T. b. brucei and T. congolense, the salmon mutant of G. m. morsitans is a much more potent vector (Makumyaviri 1984; Distelmans 1985). It has been reported that trypanosomes utilize tryptophan (Hall et al. 1981), but the functional significance and pathogenic contribution of tryptophan are yet undefined. The increased vectorial capability of the salmon mutant may be a direct result of this utilization of excess tryptophan by trypanosomes, and may indicate the significance of the metabolite in vectorial capability.

As for the white mutant, it would be interesting to see if elevated levels of 3-hydroxykynurenine have a similar effect. I would predict that white will be similar to tan of G. p. palpalis, for they both act late in the tryptophan to xanthommatin pathway and neither have accumulated levels of tryptophan. Future studies on the effects of elevated levels of tryptophan and other ommochrome precursors have on trypanosomes and trypanosome transmission may provide pertinent information to establish new control practices. For example, if utilization of tryptophan or other metabolites are essential for the pathogenisity and development of trypanosomes, development of methods which decrease these metabolites in the tsetse host could decrease disease transmission.

In order to control an insect population we must understand how the insect functions. Eye color mutants allow us to investigate the inner workings of an insect. The white mutant has given me the opportunity to expand the genetic information available on tsetse, to study the process of eye pigment formation, and to relate the genetic and biochemical consequences of the mutation to other physiological processes.

As well as using fitness information for judging potential candidates for control, understanding how systems, biochemical or other, depend on one another, may inspire new and innovative ideas for insect control. By knowing the effects of elevated levels of biochemicals (i.e. tryptophan), or effects produced by the lack of others (i.e. ommochromes), we may be able to manipulate the accumulation or depletion within the wild type population and possibly offer ideas to supplement other control strategies.

Unfortunately the elevated levels of 3-hydroxykynurenine had no detrimental effect on the insect itself. This precludes the use of the metabolite for insecticidal purposes. However, the possibility exists of using white if the

elevated levels of 3-hydroxykynurenine interfere with the development and transmission of trypanosomes. This could make white a good candidate for a sterile release program because there would be no threat of increasing the incidence of disease transmission.

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