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Genetic studies of the oriental fruit fly, *Bactrocera dorsalis* (Hendel), (Diptera: Tephritidae): Description and linkage analysis of genetic markers

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GENETIC STUDIES OF THE ORIENTAL FRUIT FLY, BACTROCERA DORSALIS (HENDEL), (DIPTERA: TEPHRITIDAE): DESCRIPTION AND LINKAGE ANALYSIS OF GENETIC MARKERS

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BY

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Dedicated to the memory of Dr. Michael D. Johnson, Professor of Biological Sciences at DePauw University, whose death on November 24, 1991 left a void at my alma mater. Without his guidance at a critical point in my life, I would not have chosen to obtain a graduate degree in entomology. Thank you, Mike, for many insightful discussions, for your enthusiastic teaching, and for your encouragement. You are missed by those who had the good fortune of knowing you.

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My husband, Lawrence Mordan, and my daughter, Sarah Mordan-McCombs, have allowed me to devote my energies to studies and work when they may have preferred that my attention be on other matters. Larry 's encouragement and understanding of my academic pursuits have given me the freedom to fulfill the long held desire to complete my Ph.D. Sarah has been a wonderful child, not always understanding what I'm doing, but knowing that my work is important to me. Thank you both for all of your emotional support during the past five years.

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My advisor, Stephen Saul, encouraged me to return to graduate school to obtain a Ph.D. He has allowed me to work independently and find my own niche in the world of fruit fly genetics. Stephen has facilitated my professional growth by providing opportunities for research, writing, and travel. Thank you for the confidence that you have had in my abilities and your unwavering support.

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ABSTRACT

The oriental fruit fly, *Bactrocera dorsalis* (Hendel), is an important agricultural pest in the Pacific Basin and a constant threat to the United States mainland. The extensive economic losses caused by this polyphagous species have been the impetus for research directed towards developing effective non-toxic control measures.

A library of genetic markers was constructed by isolating morphological variants in a series of mutagenesis trials using ethyl methane sulfonate and formaldehyde, and by screening of laboratory and geographic populations. Twenty markers were described, eye color: cherry, copper, gold-grey, Grape, mandarin red, white eye, and yellow eye; eye structure: amethyst, Azure, Furrow, matte, and notched eye; puparium color: melanistic and white puparium; puparium morphology: elongate puparium and robust puparium; and wing structure: crossveinless, curled, notch wing and small wing.

The mode of inheritance of marker genes was determined by reciprocal single pair crosses between mutant and wild-type flies. The F_2 progeny numbers were analyzed by chi-square test for goodness of fit to proposed models for autosomal or sex-linkage, recessive or dominant inheritance. All genes were autosomal in nature. Furrow was inherited as a dominant gene, Grape and Azure as incompletely dominant genes and all others were inherited as recessive traits.

F_2 progeny numbers from reciprocal crosses with the marker genes in the repulsion configuration were analyzed to assign 15 markers to the five autosomal linkage groups. Map distances were determined from recombinant F_2 progeny numbers from crosses in coupling. Total mapping units (m.u.) were equal to the mean recombination frequency times 100.

The linear order of genes in linkage group A was given with mandarin red at position 0, robust puparium at 5.4 m.u., melanistic at 14.1 m.u., matte at 26.7 m.u., and elongate puparium at 30.2 m.u. Genes in linkage group C were mapped relative to the

white puparium locus, amethyst at 23.4 m.u. from white puparium, gold-grey at 6.7 m.u. and white eye at 31.2 m.u. Grape and small wing in linkage group D were 43.2 m.u. apart. Yellow eye and notched eye were the only markers in linkage groups B and E, respectively. Five markers, Azure, curled, crossveinless, Furrow and notch wing remain unmapped.

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am	amethyst gene; the strain homozygous for the amethyst gene
Az	Azure gene; the strain homozygous for the Azure gene
°C	Degrees Celsius
cd	curled gene; the strain homozygous for the curled gene
ch	cherry gene; the strain homozygous for the cherry gene
cu	copper gene; the strain homozygous for the copper gene
cv	crossveinless gene; the strain homozygous for the crossveinless gene
d	day(s)
DNA	deoxyribonucleic acid
EMS	ethyl methane sulfonate
ep	elongate puparium gene; the strain homozygous for the elongate puparium gene
F	filial generation
Fr	Furrow gene; the strain homozygous for the Furrow gene
gg	gold-grey gene; the strain homozygous for the gold-grey gene
Gr	Grape gene; the strain homozygous for the Grape gene
h	hour(s)
kV	kilovolts
ma	mandarin red gene; the strain homozygous for the mandarin red gene
me	melanistic gene; the strain homozygous for the melanistic gene
mt	matte gene; the strain homozygous for the matte gene
m.u.	map unit(s)
ne	notched eye gene; the strain homozygous for the notched eye gene
nw	notch wing gene; the strain homozygous for the notch wing gene
RH	relative humidity

rp	robust puparium gene; the strain homozygous for the robust puparium gene
SIRM	Sterile Insect Release Method
sw	small wing gene; the strain homozygous for the small wing gene
µm	micron(s)
USDA/ARS	United States Department of Agriculture/Agricultural Research Service
v	volume
we	white eye gene; the strain homozygous for the white eye gene
wk	week(s)
wp	white puparium gene; the strain homozygous for the white puparium gene
wt	weight
ye	yellow eye gene; the strain homozygous for the yellow eye gene

CHAPTER 1. INTRODUCTION

1.1 BACKGROUND

1.1.1 Distribution and host range

The oriental fruit fly, *Bactrocera dorsalis* (Hendel) was introduced to Hawaii in 1946 by armed services personnel returning from Saipan (Fullaway 1946). The species has been recorded from at least 300 host fruits (Hardy & Delfinado 1980), with the common yellow guava (*Psidium guajava* L.) and red strawberry guava (*Psidium littorale*) as the primary wild hosts in Hawaii. The oriental fruit fly is of Indo-Malayan origin and is currently considered to be one of approximately fifty species in the *dorsalis* complex (Drew & Hardy 1981) which is distributed throughout the Oriental region and numerous Pacific islands (Hardy 1977, Hardy & Foote 1989). Drew (1989) has revised the subfamily Dacinae using a classification based on the shape of the male surstylus and abdominal sternum V. The revised classification divides the subfamily into two genera. *Dacus* is the African group with fused abdominal terga and *Bactrocera* is the Oriental/Pacific group with free abdominal terga. Morphology, host preference, and response to lures are parameters being considered to ascertain the status and relationships within the *dorsalis* complex (Drew *et al.* 1981, Drew 1989).

1.1.2 Economic impact

The economic losses caused by this multivoltine species represent a continuum of damage ranging from a decrease in storage potential of the fruit to its total destruction. The female stings the fruit as she inserts her ovipositor to deposit eggs beneath the skin. This oviposition puncture opens the fruit to microbes, both bacteria and fungi, which are responsible for decreasing the storage potential of the fruit. Larval development within, and concomitant destruction of, the fruit pulp renders the fruit unmarketable. Larvae proceed through three larval instars in the fruit, with the mature third instar popping out

of the fruit to pupate in the soil. After several days the adults emerge to continue the cycle of damage.

The extensive damage and wide host range of the oriental fruit fly become obstacles to agricultural diversification in areas with large populations of this species. For example, loss of potential agricultural development in Hawaii has been estimated to account for 90% of the economic impact of fruit flies (Nakahara 1977). New crop systems such as exotic fruits have been seen as unsuitable due to the cost of measures to control fruit flies and the expense of commodity treatment to meet quarantine regulations for export to markets in non-infested areas.

1.1.3 Quarantine regulations

Quarantine regulations mandated by the U.S. Department of Agriculture Hawaiian Fruits and Vegetables Quarantine No. 13 (Code of Federal Regulations 1988) have been enacted to prevent introduction of the four pest species of tephritids found in Hawaii to the U.S. Mainland. Inspection of the baggage of domestic airline passengers, postal shipments and commercial shipments of treated commodities are expensive, labor-intensive procedures utilized to enforce these quarantine regulations.

1.1.4 Commodity treatment

Commodity treatments determined by quarantine regulations must attain Probit 9 mortality (Baker 1939) of immature stages without altering the commodity. Proposed protocols include the use of fumigants, lethal temperatures, gamma irradiation, physical barriers and insect growth regulators. The fumigant ethylene dibromide (EDB) was widely used for papaya treatment until its registration was cancelled in 1984 by the U.S. Environmental Protection Agency (Anonymous 1984). Alternative measures based primarily on thermal tolerance of the insect and commodity include vapor heat (Baker *et al.* 1944), combination hot-cold (Couey *et al.* 1984), two-stage hot water (Couey & Hayes 1986), hot water (Sharp *et al.* 1989), high-temperature forced air (Armstrong *et*

al. 1989) and cold treatments (USDA/APHIS 1985a, 1985b). Gamma irradiation administered at dosages necessary to kill all stages of fruit flies results in injury to the commodity, thereby making it unacceptable as a quarantine treatment. Plastic shrinkwrap film has been shown to reduce, but not eliminate, larval infestations of papaya (Jang 1990) making it unacceptable for meeting quarantine regulations. Finally, the insect growth regulator (IGR) methoprene allows larvae in treated fruit to pupate but prevents adult eclosion (Saul *et al.* 1987, Saul & Seifert 1990).

1.1.5 Control measures

The expense of control measures directed at the oriental fruit fly is an important component of its economic impact (APHIS (KETRON) 1980). Control measures include cultural techniques, natural enemies, bait sprays, male annihilation and the sterile insect release method. Cultural control practices are the least expensive and have been an integral part of successful fruit fly control efforts in California. Fruit picking, field sanitation and host elimination require the cooperation of commercial growers and private citizens in order to have an impact in fruit fly control efforts.

1.1.5.1 Natural enemies

Natural enemies to control the oriental fruit fly were introduced to Hawaii soon after the species itself (Clausen *et al.* 1965, Haramoto & Bess 1970). Three braconid wasps: *Biosteres arisanus* (Sonan)(=*Opius oophilus*), *B. vandenboschi* (Fullaway), *Diachasmimorpha longicaudata* (Ashmead)(=*Biosteres longicaudata*=*Opius longicaudata*), and *Psyttalia incisi* (Silvestri) (=*Opius incisi*) are the most successful natural enemies of the oriental fruit fly in Hawaii. *Biosteres arisanus* oviposits in eggs of the host, *B. vandenboschi* in first instar larvae and *D. longicaudata* in second or third instars. The parasitoids complete their development in the host and emerge from the host puparium. *B. arisanus* accounts for up to 80% of the total parasitization, *D.*

longicaudata 10%, *P. incisi* 10%, and *B. vandenboschi* 0.25% (Wong *et al.* 1984b, Wong & Ramadan 1987, Stark *et al.* 1991)

Other, less effective, natural enemies of tephritid fruit flies include predators, pathogens and nematodes. Three species of ants: *Pheidole megacephala* (Back & Pemberton 1918; Willard 1927), *Iridiomyrex humilis* (Wong *et al.* 1984a), and *Solenopsis geminata* (Wong & Wong 1988); earwigs (Back & Pemberton 1918; Willard 1927) and two species of crabronine wasps (Willard 1927) have been observed as predators of fruit flies. Other animals such as birds, toads, geckos and spiders also ingest fruit flies (Mitchell & Saul 1990). Pathogens which cause mortality in tephritids, but have not been developed for control, include the bacterium *Serratia marcescens* (Moore & Nadel 1961), the fungus *Beauveria bassiana*, and the microsporidian *Nosema tephrititae* (Steiner & Mitchell 1966, Fujii & Tamashiro 1972). Oriental fruit fly larvae are susceptible to infection by the entomogenous nematode, *Steinernema feltiae* (Lindegrin & Vail 1986). However, the concentrations of nematodes necessary to achieve LC_{95} are so high as to be cost-prohibitive for control.

1.1.5.2 Bait sprays

Bait sprays incorporate protein hydrolysate to attract and a toxicant, usually malathion, to kill both sexes (Steiner 1952, 1954, 1957). Environmental and human health hazards associated with the use of malathion have had a negative impact in California (Troetschler 1983) and limit the use of this control strategy in Hawaii (APHIS (Ketron) 1980).

1.1.5.3 Male annihilation

The primary means of population control, male annihilation, also involves the use of toxicants, usually naled, mixed with the synthetic lure methyl eugenol to attract and kill males (Steiner *et al.* 1965, Koyama *et al.* 1984, Sonda & Ichinohe 1984). This method results in population decline by effectively reducing the number of mates

available for females. The continued use of this method in Hawaii may be threatened by the potential carcinogenicity of methyl eugenol (Miller *et al.* 1983) and the fact that the lure is not registered for use in the State. Environmental side effects of insecticides on insect pollinators and fledgling birds (APHIS(Ketron) 1980) are also a negative feature of the male annihilation tactic.

1.1.5.4 Sterile insect release method

The most attractive alternative control measure is the sterile insect release method (SIRM) (Steiner *et al.* 1970, APHIS (Ketron) 1980). This method involves inundative releases of laboratory reared sterile male flies to mate with indigenous females which subsequently produce infertile eggs. Releases of sterile males at a ratio of 100 sterile to one indigenous male over a period of weeks to months result in population decline. The major disadvantage of SIRM as applied to the oriental fruit fly lies in the inability to separate the sexes prior to the adult stage so that both males and females are reared and released into the targeted area (Saul 1986). The co-released females have a negative impact on the efficiency and acceptability of SIRM due to the incidence of sterile to sterile matings, whether random or non-random, and sting damage.

1.1.5.4.1 Non-random mating

Non-random mating occurs when the sterile males mate preferentially with co-released females. This has been shown to reduce the efficiency of SIRM by an estimated 50-100% in the Mediterranean fruit fly (Robinson *et al.* 1986). Increasing the efficiency of SIRM by removal of females from the released flies could reduce the need for preliminary insecticide spraying to reduce the target population. The removal of co-released females may improve the efficiency of SIRM sufficiently to reduce the number of males necessary for control, thereby eliminating the need for building additional rearing facilities in Hawaii to meet the demands of an eradication program.

1.1.5.4.2 Sting damage

The acceptability of SIRM would be enhanced by elimination of the sting damage caused by thousands of sterile females as they attempt to oviposit. The economic losses resulting from this sting damage become devastating in targeted control areas and may actually be worse than the original infestation. The cost of laboratory production would be significantly decreased and the effective rearing capacity would be increased, if a sex sorting system could be employed to eliminate females at an early developmental stage. A sex sorting system would also greatly reduce the risk of accidentally releasing fertile females which could themselves start a serious outbreak.

1.1.5.4.3 Markers of released populations

The availability of an accurate system for identification of released and wild flies captured in traps is of primary importance in the implementation and evaluation of sterile insect release programs (Cunningham *et al.* 1980). The present method of marking tephritid pupae is to coat the external surface of the puparia with a mixture of dye and dry powder such as Day-Glo fluorescent dye (Switzer Bros., Inc., Cleveland, OH) (Schroeder *et al.* 1972). The bodies of the eclosing adults pick up dye particles adhering to the puparium exterior or on surrounding dyed pupae (Schroeder & Mitchell 1981). Grooming activities remove dye particles from the body surface, but those adhering to scales on the ptilinum remain. The head capsule must be crushed to release the dye for positive identification under ultraviolet light. Other technologies such as radioisotope and biochemical methods are restricted because there is considerable lag time in analyzing results from large numbers of specimens.

Genetically marked flies, such as eye color mutants which are easily distinguishable, are promising for rapid field identification (Saul & McCombs 1992). Genetic markers should be detectable for weeks after death and in the dried state examined from traps. The white thoracic marking strain in the oriental fruit fly was used

as a genetic marker, but the lack of permanence of the color differences made it unsuitable in trap collected adults. The yellow markings of the wild-type flies faded to a light yellow or white after death (Hart & Steiner 1972) so that the wild-type was not distinguishable from the released white strain. In addition, this white thoracic strain was not competitive with wild flies in mating.

1.2 PREVIOUS GENETIC RESEARCH

This work draws heavily on previous genetic studies in *Drosophila melanogaster* Meigen (Lindsley & Grell 1968, Ashburner 1989a, 1989b) and the Mediterranean fruit fly (Saul 1986). It is not possible to directly transfer information from one species to another, but previous studies do provide a guide for formulating research and interpreting results.

1.2.1 Drosophila melanogaster

The extensive knowledge base available from over 80 years of studies on *Drosophila melanogaster* provides numerous models for studies on insect pest species. Much of our understanding of basic genetic principles is due to the pioneering work by T.H. Morgan and his students, Bridges, Muller and Sturtevant (Suzuki *et al.* 1986). Models for determining the mode of inheritance of genes, linkage analysis, mapping of linked genes and epistasis are due in a large part to the initial studies of these individuals. Mutagenesis, biochemical, and molecular studies developed in *Drosophila* (Ashburner 1989a) provide model systems for understanding the biology of, and developing genetic control measures for, insect pest species (Louis *et al.* 1986).

1.2.2 Ceratitis capitata

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is the only species closely related to the oriental fruit fly on which classical genetic studies have been conducted. This important agricultural pest has been studied genetically since the mid-1970's in the hope of gaining a better understanding of the

biology of the species and to develop and/or enhance genetic control techniques (Saul 1986). Described visible genetic markers in the Mediterranean fruit fly can be divided into five major types: eye color (Rössler & Koltin 1976; Rössler & Rosenthal 1988; Saul 1982, 1985a, 1985b; Sharp & Chambers 1973), eye structure (Carante 1982, Saul & Rössler 1984a, 1984b; Lifschitz 1985; Rössler & Rosenthal 1988), puparium color (Rössler & Koltin 1976, Rössler 1979a), puparium shape (Lifschitz 1985), and wing structure (Rössler & Rosenthal 1988). Electrophoretic variation and its relationship to population structure was reviewed by Saul (1986).

Linkage maps for the Mediterranean fruit fly were initially based on the labelling system of Saul and Rössler (1984a) which used capital letters to refer to the linkage groups, but not to specific chromosomes. This system was revised as cytological correlation assigned markers to specific chromosomes (Saul 1986, Zapater & Robinson 1986).

A primary goal of genetic studies on the Mediterranean fruit fly has been the development of a genetic sex sorting system to enhance the sterile insect release method (Saul 1986). The basic method involves a dominant selectable marker that can be linked, by traditional or molecular techniques, to the male-determining chromosome. In such a system, males carry the wild-type gene and are viable under restrictive conditions whereas females carry the defective gene and are killed (Saul 1984). The most efficient sexing system would be one in which the selection occurs at an early developmental stage. The removal of females early in development would result in an effective doubling of the rearing capacity of present facilities, this is of primary importance in control and eradication programs.

Genetic sexing systems proposed for the Mediterranean fruit fly which are based on resistance to insecticide (Busch-Petersen & Wood 1983, Wood *et al.* 1985), ethanol (Robinson & Van Heemert 1982a, 1982b; Rössler 1985a) or purine (Saul 1982) require

large amounts of expensive selective chemicals in the mass rearing system. A genetic sexing system based on pupal color dimorphism described in the Mediterranean fruit fly (Rössler 1979a, b; Robinson & Riva 1983; Robinson *et al.* 1986) requires expensive pupal sorting machines. Saul (1990) has described a genetic sexing system based on a translocation stock of a flightless mutant in which males are wild-type and females are flightless. This system essentially removes the deleterious effects of the females who are unable to disperse from the release site and requires no toxic selective chemical or mechanical sorting device. However, the females would still have to be reared, irradiated and shipped to the release site.

A potential problem in translocation-based sexing systems is the apparent instability of translocation lines (Rössler 1982a,b, 1985b; Robinson 1984). Recombination in males does breakdown the Y-autosome translocation on which genetic sex sorting stocks are based. This makes constant monitoring of mass rearing stocks imperative to prevent possible problems. In addition to recombination, one possible mechanism for strain breakdown is transposable elements in the medfly genome integrating at the marker gene locus.

1.2.3 Bactrocera dorsalis

Hart and Steiner (1972) described an oriental fruit fly strain with white thoracic markings instead of the bright yellow markings of the wild-type. This white strain was used as a marker of the mass-rearing colony at USDA/ARS Tropical Fruit and Vegetable Laboratory in Honolulu, Hawaii. The genetic basis of the white thoracic markings trait has not been defined, but appears to be polygenic in nature possibly involving two loci one of which is the major gene (Donald McInnis, personal communication). The frequency of this trait in native populations from Hawaii was approximately 2-4% (Hart & Steiner 1972).

1.2.4 Other pest species

Genetic studies of *Lucilia cuprina* (Weidemann) (Diptera: Calliphoridae) (Whitten *et al.* 1975, Foster *et al.* 1980) and *Cochliomyia hominivorax* (Coquerel) (Diptera: Calliphoridae), the screwworm fly, (Taylor & Martinez 1986, Taylor 1989) have been conducted primarily to develop or improve genetic control measures.

Genetic studies lead to the construction of compound chromosomes in *L. cuprina.* Theoretically, the compound chromosome strain should, if released in sufficient numbers, be able to replace the wild population. A compound chromosome strain was field tested in Australia (Foster *et al.* 1985), but it was not successful in replacing the wild population. Even though the compound chromosome strain did overwinter, it was eliminated from the population in the spring. Breakdown of the system was attributed to reduced fitness of the released strain and a deficiency of hybrid matings.

The sterile insect release method is the primary means of population control for the screwworm fly in Central and North America (Taylor 1989). Genetic research on this species has as a primary goal the development of a genetic sexing mechanism. The elimination of females from the mass-rearing procedure to increase production efficiency. Genetic markers are necessary for the development of a genetic sexing system and studies of behavioral genetics. Visible and electrophoretic markers (Taylor & Martinez 1986, Taylor 1989) were isolated and mapped.

1.3 EYE COLOR DETERMINATION

There are two components to eye color in insects, pigmented compounds and reflective properties of the corneal lens. Pigments present in the compound eye are of two major types, the brown ommochromes and the red pteridines. These pigments are produced via biosynthetic pathways that are most clearly understood from studies of eye color genes in *Drosophila melanogaster* (Summers *et al.* 1982).

Each step in the pathways is mediated by a specific enzyme and mutations in genes encoding these enzymes or enzyme co-factors may result in mutant eye color phenotypes. The two pathways interact when enzymes or products from one pathway influence the control and availability of cofactors in the other (Summers *et al.* 1982). Abberrant eye colors may also result from defective transport or uptake of pathway precursors (Tearle 1991).

1.3.1 Ommochrome pathway

Tryptophan is converted to the brown pigment xanthommatin via the ommochrome biosynthetic pathway. There are four enzymatic steps (Fig. 1) that have tissue and developmental specificity. Tryptophan in the hemolymph is taken up by the Malpighian tubules and fat body during the larval stage. Two intermediates in the pathway are synthesized and stored in these tissues, 3-hydroxykynurenine in the Malpighian tubules and kynurenine in the fat body (Beadle 1937 a,b; Nissani 1975). These stored intermediates are released into the hemolymph during pupariation at which time they are absorbed into the compound eyes (Beadle & Law 1938). Xanthommatin can be synthesized from tryptophan alone, indicating that all of the necessary enzymes are present in the compound eye (Nissani 1975). Tissue specific regulation of genes encoding the enzymes has been inferred from the differential expression of pathway mutants in tissues such as the eye, ocelli, Malpighian tubules and fat body (Tearle 1991).

Tryptophan oxygenase catalyzes the conversion of tryptophan to formylkynurenine. The structural gene for this enzyme, *vermilion*, has been identified and cloned in *Drosophila melanogaster* (Searles & Voelker 1986, Walker *et al.* 1986). The structural gene for the enzyme that catalyzes the conversion of formylkynurenine to kynurenine, kynurenine formamidase, has not been identified (Tearle 1991).

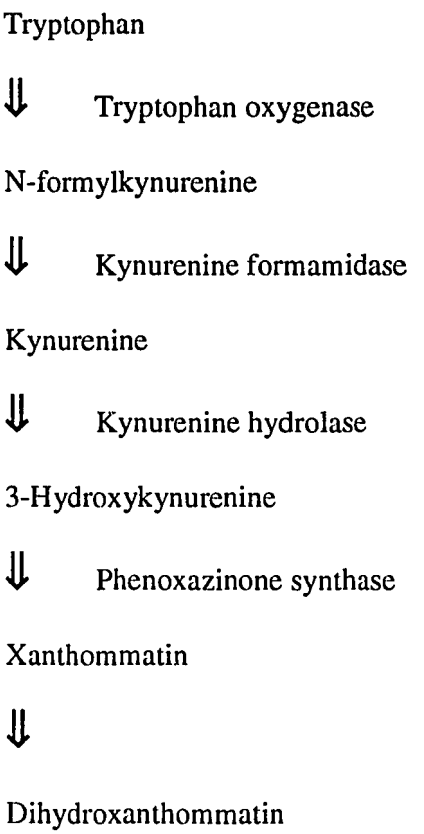


Fig. 1. Ommochrome biosynthetic pathway (Summers *et al.* 1982).

Cinnabar of *Drosophila* has been identified as the structural gene of kynurenine hydroxylase, the enzyme that converts kynurenine to 3-hydroxykyrurenine (Summers *et al.* 1982). The final step in the ommochrome pathway, 3-hydroxykynurenine to xanthommatin, is catalyzed by phenoxazinone synthase. The structural gene for this enzyme has not been identified, though several mutants do show reduced levels of the enzyme and its product (Summers *et al.* 1982).

Two genes affecting the ommochrome pathway, white (*w*) and scarlet (*st*), were cloned and sequenced in *D. melanogaster* (O'Hare *et al.* 1984, Tearle *et al.* 1989). Amino-acid homology to components of bacterial permeases indicate that the gene products may function in transport of pigment precursors (Sullivan & Sullivan 1975). Tearle (1991) proposed a model with three sites of action of the *w* and *st* gene products. The first site of action is at the plasma membrane for transport of tryptophan into the cell. Second, the gene products are required for transport of kynurenine across the outer mitochondrial membrane to interact with kynurenine hydroxylase to form 3-hydroxykynurenine. The final proposed site of gene product action is at the pigment granule membrane for transport of 3-hydroxykynurenine into the pigment granule where xanthommatin is formed.

1.3.2 Pteridine pathway

The pteridine pathway is not well defined in insects. The proposed pathway (Fig. 2) by which guanosine triphosphate is converted to pteridines has branched and interrelated biosyntheses. Pteridines have been found in the larval Malpighian tubules and are concentrated in the head of adults, indicating that pteridines are taken up from the hemolymph and transported to the pigment granules of the compound eyes (Summers *et al.* 1982).

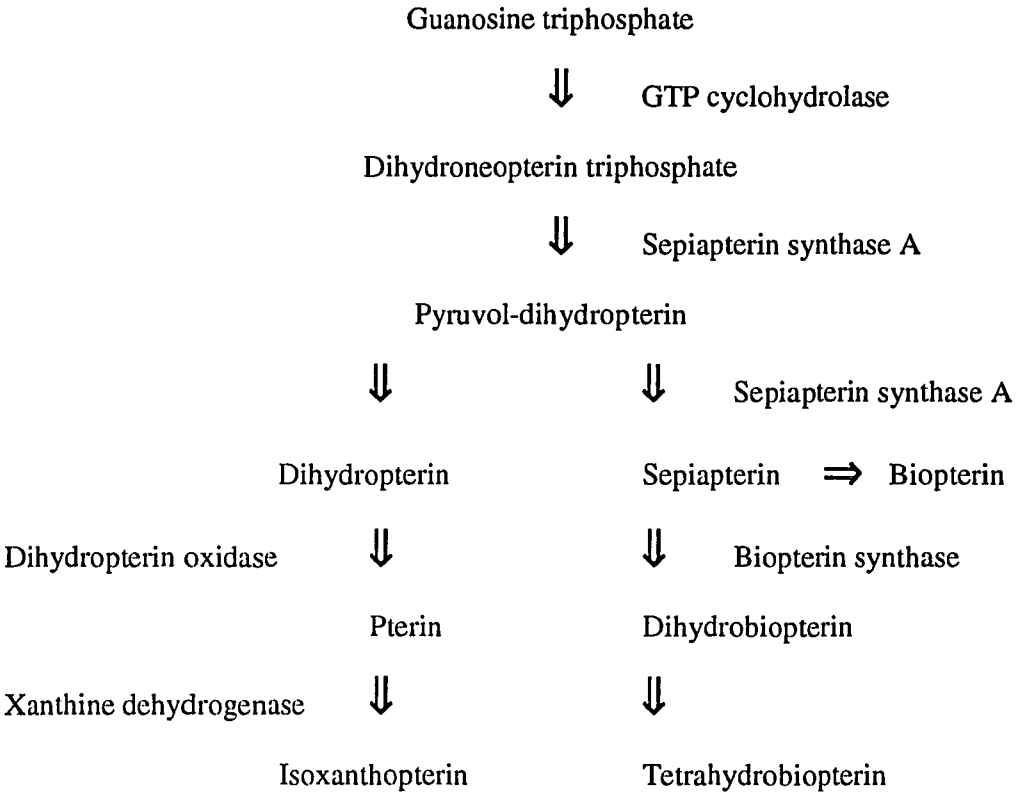


Fig. 2. Proposed pteridine biosynthetic pathway (Summers *et al.* 1982).

The first step in the pathway is the conversion of guanosine triphosphate (GTP) to dihydroneopterin triphosphate in a reaction mediated by GTP cyclohydrolase. Two mutants in *Drosophila melanogaster*, raspberry (*ras*) and prune (*pn*) have reduced GTP cyclohydrolase levels, but neither appears to be the structural gene (Zeigler & Harmsen 1969). The steps from dihydroneopterin to pterin have not been identified in insects, but are given in Figure 2 as determined in *Escherichia coli* (Summers *et al.* 1982). Xanthine dehydrogenase catalyzes the conversion of pterin to isoxanthopterin at the time of pupariation. The structural gene for XDH in *Drosophila*, *rosy* (Gelbart *et al.* 1974), also reduces the activity of GTP cyclohydrolase (Evans & Howells 1978). This suggests that products of an XDH catalyzed reaction may play a role in determining GTP cyclohydrolase activity.

The second branch of the proposed pteridine pathway, from dihydroneopterin triphosphate to biopterin are not well characterized. Sepiapterin synthase A converts dihydroneopterin triphosphate to an unstable intermediate which is then converted to sepiapterin by sepiapterin synthase B. In *Drosophila*, the structural gene may be at the *purple* locus, as this mutant has reduced levels of sepiapterin synthase A. Sepiapterin is then converted to dihydrobiopterin and dehydrosepiapterin to biopterin in independent reactions catalyzed by biopterin synthase (Fan & Brown 1979).

Sepiapterin is the proposed precursor of the drosopterins which consist of at least six separate compounds that confer the red coloration of the *Drosophila* eye (Zeigler & Harmsen 1969). The exact reaction sequence and participating enzymes have not been identified. It was proposed that the reactions occur on the pigment granule membrane and involve enzymes that convert the single pteridine ring to a dipteridine (Summers *et al.* 1982). Zeigler and Feron (1965) did not detect drosopterins in the eyes of *Ceratitis capitata*, identifying only sepiapterin masked by ommochromes. This is problematical because several mutants in this species do have a 'red' phenotype (Saul 1986). This red

coloration could result from ommochromes, but these are primarily brown pigments. Alternatively, there may be compounds that are related to the drosopterins which have not been detected in the Tephritidae.

1.3.3 Interference filters

Corneal interference filters are located in the outer epicuticle of the ommatidial lens (Hinton 1976). The interference filters are composed of a specialized system of alternating dense and rare quarter wavelength layers (Bernard & Miller 1968). The interference filters are responsible for the iridescent quality of insect eyes, brighter reflected patterns of the eye are associated with filters having more layers present. There is evidence that the corneal filters are optical components of the eye that have a contrast filtering function (Miller *et al.* 1968, Bernard 1971). The corneal filters function to enhance the contrast of colored objects in a background of dissimilar colors (Bernard & Miller 1968).

1.4 STRUCTURE OF THE COMPOUND EYE

The peripheral retina of the insect compound eye is composed of individual ommatidia. Each ommatidium has three major components: the dioptric apparatus, retinula cells, and pigment cells (Agee *et al.* 1977, Summers *et al.* 1982, Davis *et al.* 1983, Wu *et al.* 1985). The dioptric apparatus consists of the corneal lens, the pseudocone and four Semper cells. Eight retinula cells, comprising the photoreceptor cells of the eye, run from the base of the Semper cells to the basement membrane. The axons of these cells pass through the basement membrane to synapes in the lamina. The retinula cells contain the visual pigment rhodopsin on microvilli which form the rhabdomere (Chapman 1982).

Primary and longitudinal pigment cells surround the dioptric apparatus. Twelve longitudinal pigment cells extend to the basement membrane, separating the retinula of each ommatidium (Agee *et al.* 1977, Davis *et al.* 1983). Basal pigment cells, or Semper

cell pigment bodies, that function to regulate the quantity of reflected light at the basement membrane have been described in some diptera (Summers *et al.* 1982, Davis *et al.* 1983, Wu *et al.* 1985).

The peripheral retina begins development during the late third instar and proceeds through early and late pupal periods in *Drosophila* (Cagan & Ready 1989). The retinal pattern forms in the eye disc during the third instar. Morphogenesis proceeds in a posterior to anterior direction as marked by the presence of the morphogenic furrow. The retinula cells differentiate in a fixed sequence in which cell fates are determined by sequential cycles of cell induction (Basler & Hafen 1991).

The Semper cells are formed early in the pupal stage, as are the primary pigment cells that enclose them. The longitudinal pigment cells are derived from a pool of cells that are in contact with the retinula cells. In the late pupal stage the rhabdomeres form and the retina lengthens. Pigment granules appear in the pigment cells at this time. The corneal lens is secreted by the Semper cells, primary and secondary pigment cells in the late pupal stage. Finally, the pseudocone is secreted by the Semper cells, pushing these cells away from the lens.

1.5 SIGNIFICANCE

The work reported in this dissertation encompasses the initial genetic studies on the oriental fruit fly, *Bactrocera dorsalis* (Hendel). The construction of a library of genetic variation in the oriental fruit fly provides valuable reference material for understanding and manipulating this important agricultural pest. Genetic variants can be used in the development of field management techniques, to investigate gaps in our knowledge of the basic biology of the species and in molecular studies. Expanded knowledge in these areas may lead to more effective and environmentally safe control practices.

1.5.1 Field management practices

Basic genetic information is essential for the development of field management practices to enhance the sterile insect release method. Eye color mutants are of special importance in this work because similar phenotypes in *Drosophila* result from biochemical changes in the pathways which produce the pigmented compounds of the compound eye (Summers *et al.* 1982). Some of these changes involve loci encoding critical enzymes or co-factors of enzymes and may, therefore, function as selectable markers (Gelbart *et al.* 1976). The genetic sexing strategy is to link a dominant selectable marker to the male-determining chromosome by a translocation (Saul 1982). Such a strain would have females carrying a gene that encodes a defective enzyme so that they are killed under restrictive conditions, but the males have a wild-type gene and survive under those same conditions. Conditional lethal genes, such as temperature or chemical sensitivities, could provide the means of designing a system to remove females at an early developmental stage. Sex sorting systems may also be applied at the pupal stage using puparium color markers (Rossler 1979a) or at the adult stage using flightless mutants which are unable to disperse from the release site (Saul 1990, McCombs & Saul 1992a).

An accurate system of identifying the released population is essential for the evaluation of sterile insect release populations. Visible genetic markers provide permanent, easily identifiable markers of released populations (Saul & McCombs 1992).

1.5.2 Basic biology

Gaps in our knowledge of basic biological processes cover a range of areas from biochemical to behavioral. Eye color mutants can be used to investigate pigment biosynthetic pathways (Summers *et al.* 1982). Studies of mating behavior could be facilitated by mutants with impaired vision and restricted wing movement. Sperm competition studies can employ pupal color mutants that allow unambiguous assignment

of paternity (Saul *et al.* 1988). Morphological mutants provide material for developmental studies.

1.5.3 Molecular studies

The development of genetic control systems based on molecular techniques requires an understanding of the target species. Genetic variants provide a basis for application of the genetic engineering techniques developed in *Drosophila* to a real pest species. For example, genetically unstable eye color mutants can be used to screen for transposable elements (TEs) as was done in *Drosophila* (Rubin *et al.* 1982). Isolation of TEs could lead to the development of a gene transfer system which would allow precise genetic manipulation of pest tephritids (Louis *et al.* 1986).

1.6 SPECIFIC OBJECTIVES

This work has three primary objectives, which include:

Objective I. Construct a library of genetic markers

- Specific Aim 1. Screen strains for genetic variation
- Specific Aim 2. Conduct mutagenesis trials
- Specific Aim 3. Establish pure-breeding strains of isolated mutants

Objective II. Characterize marker loci

- Specific Aim 1. Determine the mode of inheritance of each marker gene
- Specific Aim 2. Describe the phenotype of each marker gene.

Objective III. Construct a linkage map of marker loci

- Specific Aim 1. Determine linkage relationships
- Specific Aim 2. Determine map distances
- Specific Aim 3. Construct multiple marker strains

CHAPTER 2. MATERIALS AND METHODS

2.1 REARING PROCEDURES

Fundamental to fulfillment of the objectives of this study was the availability of techniques for consistent single pair mating and larval rearing. The critical factors were oviposition stimulus and larval rearing media.

2.1.1 Egg collection

Several fruit preparations were tested and a thin layer of undiluted guava nectar (Hawaiian Sun Products Inc., Honolulu HI) applied inside the oviposition vial proved to be the most reliable stimulus for egg collection from isolated females and stock cages. Oviposition vials were inserted in cages containing females of at least 2 wk of age. After 24 hr, the vials were removed and the eggs were placed on larval media with a camel hair brush. The larval food cups were held in 0.27 L clear plastic cups with 2 cm of vermiculite as a pupation substrate. After 18 d the pupae were sifted from the vermiculite, transferred to a 0.02 L paper cup, and held in a 0.432 L cage. Adults were allowed to feed at will and water was supplied via a 0.06 L water cup and lid fitted with a 0.95 x 3.8 cm dental roll.

2.1.2 Larval medium

Larval rearing media previously formulated for the Mediterranean fruit fly (Saul 1982) proved to be suitable for the oriental fruit fly. The diet consisted of 1200 g of wheat bran (Arrowhead Mills, Hereford TX), 600 g torula yeast (United States Biochemical Co., Cleveland OH), 600 g granulated sugar (C & H Sugar Co., Aiea HI), 48 g Vanderzant insect vitamins (United States Biochemical Co., Cleveland OH), 2400 ml of distilled water, and 48 ml of mold inhibitor (saturated solution of methyl paraben and sorbic acid in 95% ethanol). This wheat-based diet retains its integrity in rearing large numbers of larvae for population cages as well as small numbers for genetic analysis and sensitivity testing.

2.1.3 Adult nutrition

Adults were fed on a mixture of 864 g of granulated sugar (C & H Sugar Co., Aiea HI), 144 g enzymatic yeast hydrolysate (United States Biochemical Co., Cleveland OH), 144 g enzymatic casein hydrolysate (United States Biochemical Co., Cleveland OH), and 3 g L-methionine. This mixture was spread in trays at a depth of 1 cm and held at ambient temperature until it hardened by absorbing moisture from the atmosphere. Flies had access to small portions placed on the nylon screen forming the top of the adult cage.

2.1.4 Origin of Strains

Stocks used in this study were derived from two sources. The laboratory colony was originally obtained from the USDA/ARS Tropical Fruit and Vegetable Laboratory Honolulu, Hawaii where it was maintained for several years as a mass rearing colony. Geographic strains were derived from adults and larvae collected from two sites on Oahu: Aiea Loop Trail and Kahuku; three sites on Maui: Pukalani, Kula and Makawao; Volcano on Hawaii; and Hanapepe on Kauai.

2.2 MUTAGENESIS

2.2.1 Mutagens

Mutagenesis trials were conducted using ethyl methane sulfonate (EMS) (Sigma Chemical Co., St. Louis, MO) and formaldehyde (Fisher Scientific Co., Santa Clara, CA). EMS is an alkylating agent which appears to alkylate keto groups at the number six position of thymine and guanine, resulting in single base pair alterations in the DNA (Suzuki *et al.* 1986). Formaldehyde is an apparent inducer of large deletions in the DNA (Suzuki *et al.* 1986).

2.2.2 Screening procedures

2.2.2.1 EMS mutagenesis

EMS was administered to adult males less than three hours of age by exposing them to a 0.03 M solution in sucrose (Busch-Petersen *et al.* 1986). Treatments were conducted in a fume hood with strict safety precautions. All contaminated items were soaked in a 5% (v/v) solution of thioglycolic acid for 24 hr and discarded. The males were held for 3 h in 10 dram plastic vials lined with filter paper saturated with the EMS-sucrose solution. Treated males were transferred to 3.45 L cages for 24 hr and mass-mated to virgin females. Subsequent generations were mated in isolated pairs (Fig. 3). Screening for variants was conducted at the F_4.

2.2.2.2 Formaldehyde mutagenesis

Formaldehyde was administered as a 2% concentration (v/wt) in larval media so that larvae ingested the mutagen throughout their larval development (Ashburner 1989a). All generations were mated in isolated pairs with screening at the F_3 (Fig. 4).

This work was extremely labor intensive and involved hundreds of single pair matings and the screening of thousands of individual flies for variations. Pure-breeding lines were established for each identified variant by breeding the flies expressing the particular variation if both sexes were present or by crossing variants to wild-type siblings if only one sex was available. Three or more subsequent generations were screened for the variant and the line was purified by a series of single pair matings covering three generations.

2.3 GENETIC CROSSES

2.3.1 Mode of inheritance

Parental crosses of mutant strains were made by isolating male and female flies in separate containers within 24 h of eclosion, placing pairs of adults together in a single container after 4 d and collecting eggs 7 to 10 d later. Ten replicates of each

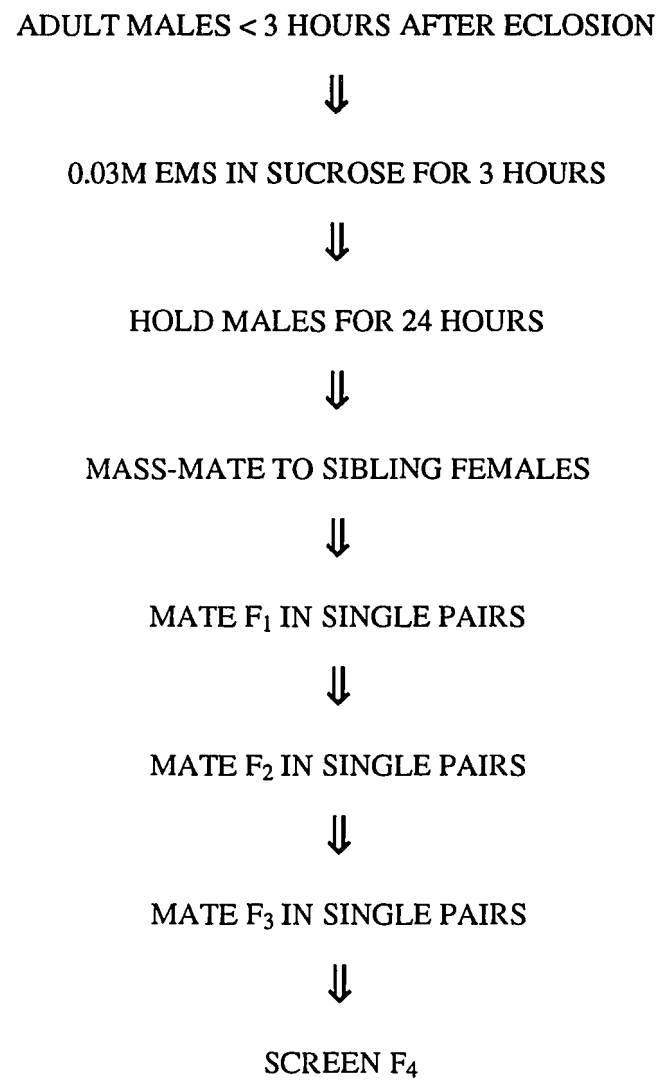


Fig. 3. Protocol for ethyl methane sulfonate (EMS) mutagenesis.

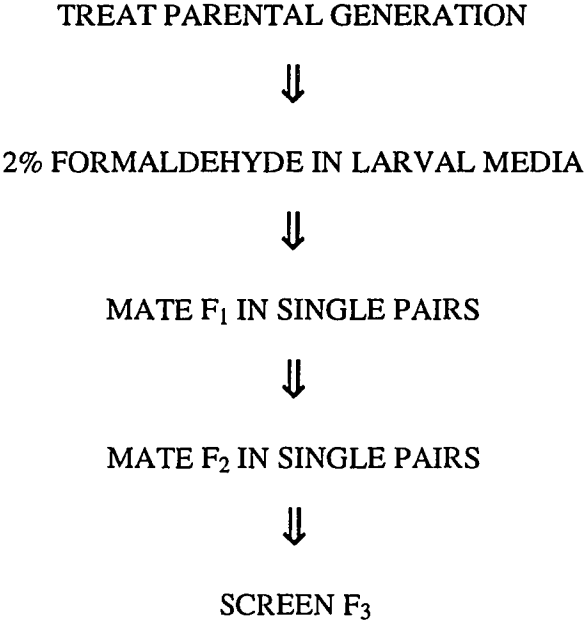


Fig. 4. Protocol for formaldehyde mutagenesis.

reciprocal cross were made: wild-type male x mutant female and mutant male x wild-type female (Fig. 5). The F_1 was screened and the sex and phenotype were recorded. The F_2, produced by allowing the F_1 to mass-mate, was screened for sex and phenotype. The F_2 progeny numbers were analyzed by chi-square test for goodness of fit to proposed models for autosomal or sex-linkage, dominant or recessive inheritance (Saul & Rössler 1984a).

2.3.2 Linkage analysis

Linkage relationships were determined from analysis of reciprocal crosses involving the marker genes in the repulsion configuration (Fig. 6). The F_2 generation was produced by mass-mating of the F_1 flies. The F_2 progeny numbers were analyzed by chi-square test for goodness of fit to proposed models for linkage, non-linkage and epistasis (Saul & Rössler 1984a) (Appendix D). The F_3 was obtained by breeding flies from each F_2 phenotypic class to determine true-breeding status and for detection of recombinants.

2.3.3 Mapping studies

Map distances were determined by analysis of F_2 progeny numbers from reciprocal crosses with the marker genes in the coupling configuration (Fig. 7). The map distance was equal to the mean recombination frequency times 100 (Suzuki *et al.* 1986). The map distance for each single pair mating was calculated and the mean distance distance and standard deviation was reported for each type of cross.

Several mutants have reduced viability and are underrepresented in progeny numbers. This results in significant deviations from the expected ratios in crosses to determine linkage and map distances. The Fisher Product Method formulae (Immer 1930, Weir 1990) which are disturbed very little by differential mortality, were used to calculate linkage intensities and crossover values in these instances.

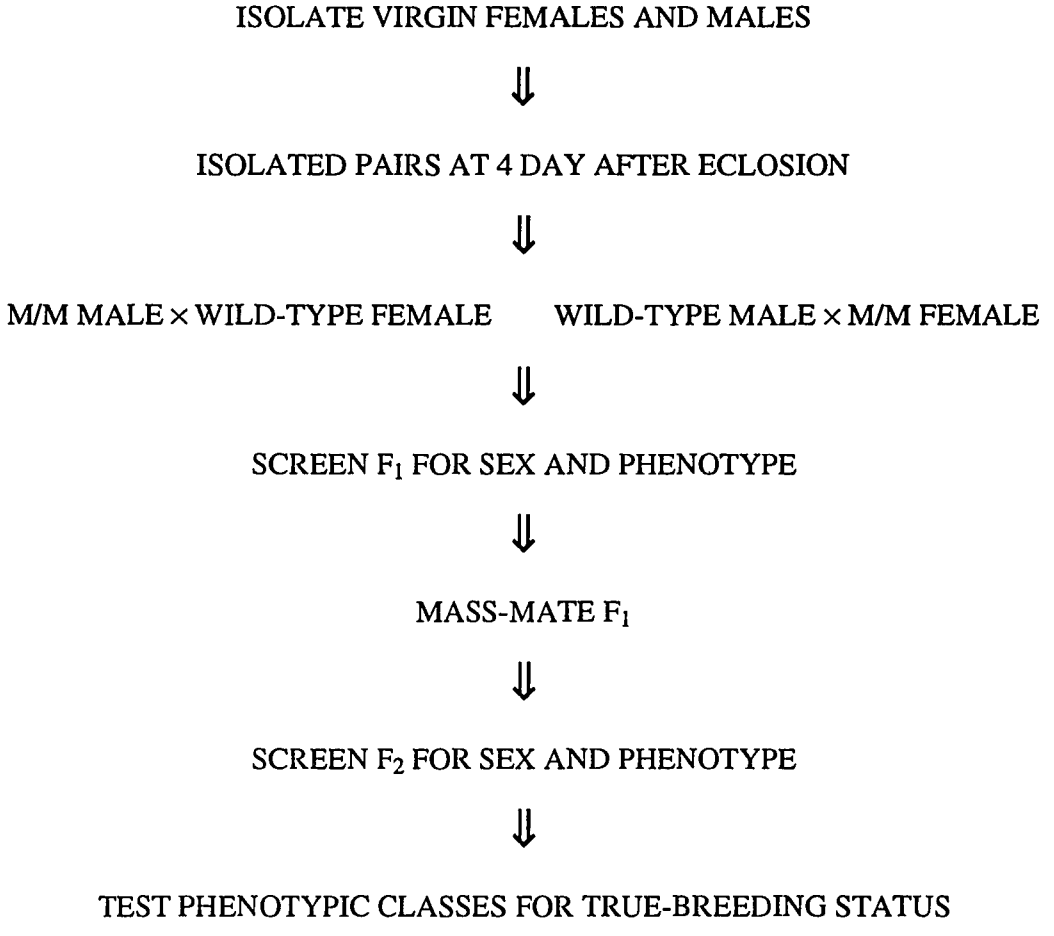


Fig. 5. Scheme for crosses to determine the mode of inheritance of isolated genes. M/M is used as a symbol for the phenotype of the parental genotype used in each cross.

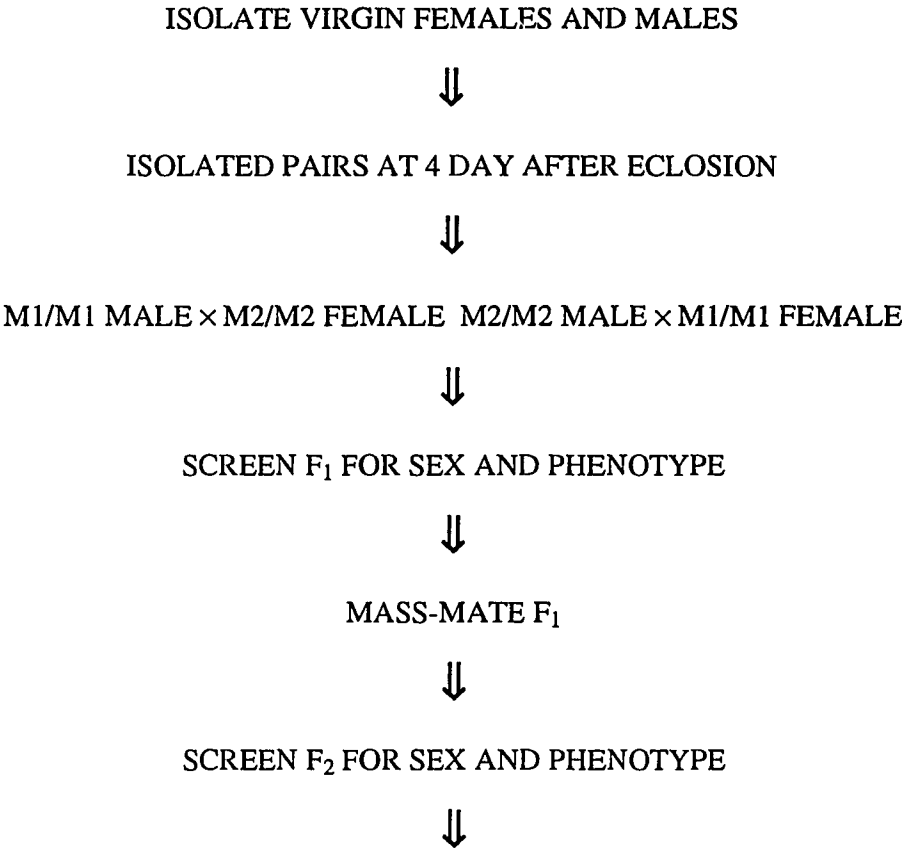


Fig. 6. Scheme for crosses in the repulsion configuration to determine the linkage relationships of isolated genes. M1/M1 and M2/M2 are used as symbols for the phenotypes of the male and female parental genotypes used in each cross.

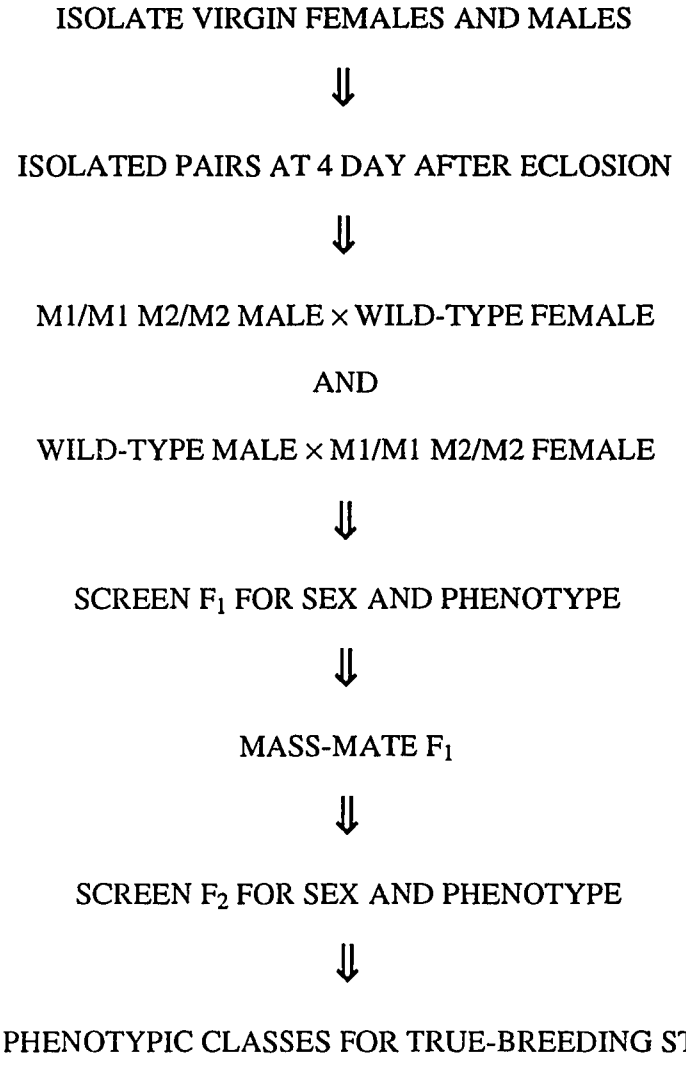


Fig. 7. Scheme for crosses in the coupling configuration to determine map distances between genes within the same linkage group. M1/M1 M2/M2 is used as the symbol for the phenotype of the double homozygote of the parental genotype used in each cross.

2.4. MULTIPLE MARKER STRAINS

Multiple marker stocks were constructed by isolating individuals homozygous for two or more marker loci. These stocks were selected from the F_2 or F_3 of crosses in the linkage studies and purified by single pair matings. Additional markers were incorporated into the stocks by crossing to other strains. The goal was to construct stocks that had markers assigned to each of the autosomal linkage groups. Two three-marker and one four-marker strains were constructed for use in mapping studies, allowing characterization of newly isolated mutants in two simple steps, instead of the time consuming process of separately crossing to individual markers on the linkage groups (Saul & Rössler 1984a).

2.5 AGE-SPECIFIC MORTALITY

Viability studies were conducted by separate egg hatch and larval viability-adult eclosion trials.

2.5.1 Egg hatch

Eggs for hatch rate studies were collected from 2 wk old adults over a period of 2 hours. Eggs were held for 24 h and then placed onto green blotter paper in a humidified dish, 100 eggs per replicate. The humidified dish consisted of a plastic petri dish lined with distilled water saturated filter paper and two dental rolls. The number of larvae hatching was counted daily for 2 d.

2.5.2 Larval viability and adult eclosion

Larval viability trails were conducted by placing 100 newly hatched first instar larvae on the surface of 25 g of larval medium in a 0.02 L cup, 4 replicates per strain. Pupae were recovered and counted after 18 d. Pupae were placed in a 0.432 L cage for adult emergence. Total number of adults eclosing for 7-10 d and sex ratio were recorded. Analysis of variance was used to determine whether significant differences were detectable between strains for these parameters and Scheffe's multiple range test

was used for unplanned comparisons between means (Statgraphics ®version 5, Statistical Graphics Company, Rockville, MD).

2.6 HISTOLOGY

Histological techniques were used to study several mutant strains to more fully describe the phenotype of morphological variants. Standard procedures for fixation in Carnoy's solution (Gray 1964), dehydration in a butanol series and embedding in Paraplast Plus (Oxford Labware St. Louis, MO) (melting point 56^{O} C) were used (Appendix C). Tissues were sectioned at 7μm using a rotary microtome, affixed to albumin-subbed glass slides, and stained with hematoxylin and eosin. Cover slips were affixed with Permount (Fisher Scientific Co.).

Preparations of eyes were made from light-adapted adults that were ether anesthetized and decapitated. Heads were fixed and dehydrated as above. Sections were stained with hemotoxylin and eosin or cleared of paraffin in xylene and mounted in Permount. Histological preparations were examined with a Leitz compound microscope fitted with an ocular micrometer for measurements.

2.7 SCANNING ELECTRON MICROSCOPY

Samples were prepared from ether immobilized flies by decapitation and placement of the head on an aluminum stub with an adhesive. Specimens were coated with gold-palladium in a sputter coater (Technics Inc.) and examined immediately in a Cambridge S500 or Hitachi field emission scanning electron microscope at 10 kV. Photographs were taken to document these studies using Polaroid Type 55 positive/negative film.

2.8 DISSECTION OF INTERNAL ORGANS

Internal organs were removed from larvae and adults by dissection in Ringer's solution (Ashburner 1989b) in a depression slide. Tissues were examined with a fiber optic light of color intensity 3350°K under a Wild stereomicroscope.

CHAPTER 3. RESULTS AND DISCUSSION

3.1 STRUCTURE OF THE COMPOUND EYE

The compound eye of the oriental fruit fly is roughly hemispherical in shape (Plate I a) It is rounded at the posterodorsal margin and the frontal margin tapers from the antennae to the genal spot. There are approximately 4500 ommatidia that range in length from 150 μm in the posterior to 195 μm in the anterior region. Each ommatidium consists of a dioptric apparatus, eight retinula cells, and their associated pigment cells. The dioptric apparatus is composed of the corneal lens, pseudocone, and four Semper cells. The lenses are square along the equator (Plate I c), but are 24 μm wide hexagons (Plate I b) over the remainder of the eye. Corneal nipples are present on the surface of the corneal lens. The cup-shaped pseudocone, 19 μm in length, sits directly proximal to the corneal lens and appears to have no cellular organelles.

Four wedge-shaped Semper cells sit directly proximal to, and are responsible for the production of, the pseudocone (Plate II c) (Chapman 1982, Cagan & Ready 1989). Each Semper cell has a large nucleus located at the periphery of the cell. Wu *et al.* (1985) reported the presence of Semper cell processes in electron microscopy studies of the oriental fruit fly, however they were not observed in this study. Semper cell basal pigment bodies (Plate II e) were observed beneath the rhabdom at the distal end of the ommatidal cavity.

The retinula is composed of eight photosensitive retinula cells whose cell bodies surround the ommatidal cavity from the Semper cells to the basement membrane. Slender processes of each retinula cell pass through the basement membrane to synapses in the optic ganglion (Plate II e). Nuclei of retinula cells one to seven occur in the distal third of the cell body and that of the eighth retinula cell is in the proximal third (Plate II b). The retinula cells form an open rhabdom structure with a superior seventh retinula cell and an inferior eighth retinula cell (Wu *et al.* 1985).

The ommatidial cavity is bordered distally by the rhabdomere cap just below the Semper cells (Plate II c). The retinula cells form the lateral border and the Semper cell basal pigment bodies form the proximal end.

Two primary, or corneal, pigment cells surround the pseudocone from the corneal lens to the Semper cells (Plate II c). Two nuclei are evident, but as has also been observed in the Mediterranean fruit fly and Caribbean fruit fly (Davis *et al.* 1983, Agee *et al.* 1977) the presence of a membrane is not. They contain numerous pigment-filled vacuoles.

There are 12 shared longitudinal pigment cells (LPC) surrounding the retinula cells of each ommatidium, running from the corneal lens to the basement membrane (Plate II b and d). Thin projections of the LPC pass through the basement membrane with the retinula cell axons (Plate II e). The LPC nuclei are located proximal and the retinula cell nuclei in the distal third of the ommatidium (Plate II b). There are numerous pigment-filled vacuoles in the cytoplasm.

The compound eye of the wild-type oriental fruit fly is a deep purple (B60 Y40 M99) (Kueppers 1982) with blue-green iridescence at the teneral stage (Plate III a) and it develops a reddish iridescence with age (Plate III b). The primary pigment cells contain yellow sepiapterin and orange ommochrome pigments (Plate II a). The LPC, their projections through the basement membrane, and the basal pigment cell bodies contain deep reddish-orange ommochrome pigments (Plate II d).

3.2 DESCRIPTION OF GENETIC MARKERS

3.2.1 Eye color markers

3.2.1.1 Cherry

The cherry (*ch*) trait appeared during routine screening of the mandarin red (*ma*) strain. The cherry phenotype is characterized by a bright red background pigmentation with a blue iridescence (Plate IV a). The cherry eye color darkens with age to a deep red

with dark blue irdescence (C60 Y00 M99) (Plate 4 b). This is distinguishable from all other eye color phenotypes described in this study. There is no black pigmentation at the base of the front-orbital bristles, vertical bristles, ocellar bristles, nor on the frons.

3.2.1.2 Copper

The copper (*cu*) trait was found during routine screening of the yellow eye (*ye*) strain. The copper phenotype is characterized by pale orange eye color upon eclosion (Plate V a) which darkens to brown with a green iridescence (B00 Y99 M40) (Plate V b). There is no black pigmentation at the base of the front-orbital bristles, vertical bristles, ocellar bristles, nor on the frons.

Orangish-yellow pteridine pigments are concentrated in the peripheral retina area at the primary pigment cells and at the area of the basement membrane (Plate VI A). Pigmentation in the longitudinal pigment cells is diffuse and orangish-yellow. Larval and adult Malpighian tubules are pale yellow in contrast to the bright yellow of the wild-type.

3.2.1.3 Gold-grey

Gold-grey (*gg*) appeared in a light eye color line (coral) which was isolated during screening of laboratory colonies treated with EMS. The gold-grey phenotype is a greyish brown color with gold iridescence (Y99 M99 C50) (Plate VII). There is no black pigmentation at the base of the front-orbital bristles, vertical bristles, ocellar bristles, nor on the frons. Scanning electron micrographs (Plate VIII) show that the surface of the corneal lens has the impression of the Semper cells on the surface. This indicates that the phenotype may be due, in part, to an alteration of the corneal lens.

Histological studies indicate that the gold-grey has normal morphological features (Plate IX). The primary pigment cells contain deep yellow pigments (Plate IX a) and the longitudinal pigment cells (LPC) contain reddish-orange pigments (Plate IX d). Cross section of the LPC show that the intensity of these pigments is reduced in comparison to

the wild-type (Plate II d). The post-basement membrane projections of the LPC contain a reddish-orange pigment (Plate IX e), in contrast to the bright red pigment seen in the wild-type (Plate II e)

3.2.1.4 Grape

The Grape (*Gr*) phenotype was detected in a mandarin red line as a very dark red eye. When a line pure-breeding for this phenotype was crossed to wild-type flies, the F_2 progeny had four phenotypic classes: wild-type, Grape, mandarin red and individuals like the original isolation in a 9:3:3:1 ratio. This indicates that the individuals originally isolated were double homozygotes for *Gr* and *ma*. The pure-breeding *Gr* strain was established from the *Gr/Gr* flies isolated in these crosses.

The Grape phenotype is characterized by a deep purple pigmentation (C90 Y90 M99) and a dull colorless reflectance (Plate X). Histological studies of the Grape strain indicate that the primary pigment cells contain a bright yellow pigment (Plate XI a and c). The LPC have a purplish pigment concentrated at the proximal and distal ends (Plate XI a,c, and d) and more diffusely surrounding the retinula (Plate XI d). This purplish pigment contrasts with the more reddish pigment observed in the wild-type (Plate II).

3.2.1.5 Mandarin red

The mandarin red (*ma*) trait appeared in the F_4 of an isofemale line from mutagenesis trials with EMS (McCombs & Saul 1989). The mandarin red phenotype is characterized by a bright red background pigmentation with gold iridescence (B20 Y50 M99) (Plate XII). This contrasts sharply with the teneral adult wild-type and is distinguishable at all adult stages. There is no black pigmentation at the base of the front-orbital bristles, vertical bristles, ocellar bristles, nor on the frons. In contrast to the bright yellow Malpighian tubules of the wild-type larvae and adults, those of mandarin red larvae are light yellow and of adults are very pale yellow.

The primary pigment cells contain a diffuse yellow pigment (Plate XIII a). A red pigment was observed in the LPC, running from the corneal lens to the post-basement membrane area (Plate XIII a). The red pigment did not have the brown hue observed in the wild-type (Plate II). The LPC surrounding the retinula of the mandarin red eye (Plate XIII d) were not as deeply pigmented as those in the wild-type (Plate II d).

3.2.1.6 White eye

The white eye (*we*) trait appeared as a spontaneous mutation in 5 males from a laboratory colony (McCombs & Saul 1992b). The pure-breeding strain was established from white-eyed F_2 progeny of these males mated to sibling females. The eye is a bright white and clearly distinguishable from the wild-type and other eye color mutants described in this study (Plate XIV). There is no black pigmentation at the base of the front-orbital bristles, vertical bristles, ocellar bristles, nor on the frons. No pigment was detected in the eye and no pigment granules were visible in histological sections of the white eye strain (Plate XV).

The mean hatch rate of the white eye strain was 0.38 (±0.21) which was significantly lower than the wild-type mean hatch rate of 0.75 (±0.05) (Table 1). The larval viability and adult eclosion rates were not significantly different than the wild-type (Table 1, Table 2, Table 3). The lower hatch rate indicates that the white eye strain is associated with a sub-vital character or has reduced mating ability.

3.2.1.7 Yellow eye

The yellow eye (*ye*) phenotype appeared as a spontaneous mutation in 3 males and 5 females from a laboratory colony and the pure strain was established by breeding these flies (McCombs & Saul 1992b). The bright yellow eye color of the yellow eye strain is clearly distinguishable from the wild-type and all other eye colors described in this study (Plate XVI). There is no black pigmentation at the base of the front-orbital bristles, vertical bristles, ocellar bristles, nor on the frons.

The primary pigment cells contain a light yellow pigment (Plate XVII a). No pigment was detected in the LPC (Plate XVII c and d).

3.2.2 Eye morphology markers

The morphology of the wild-type compound eye as studied with the scanning electron microscope is characterized by regularly spaced hexagonal facets (Plate XVIII a). The fine structure is as described above (Plate II).

3.2.2.1 Amethyst

Amethyst (*am*) was observed in male and female flies in the F_4 of EMS treated lines (McCombs & Saul 1992b). A pure-breeding amethyst phenotype is characterized by an irregular blue sparkling iridescence and a deep purple pigmentation (Plate XIX). The amethyst phenotype is distinguishable at all adult stages from that of the wild-type. The sparkling quality results from a disruption of the normal facet pattern (Plate XVIII a) by irregular enlargements of the facets and numerous bubbles on and between the facets as seen by examination with a scanning electron microscope (Plate XVIII b). This phenotype differs from that of sparkling in the Mediterranean fruit fly (Saul & Rössler 1984b) which results from facets of uneven shape and irregular spacing.

The amethyst strain has a mean hatch rate of 0.50 ($\pm$ 0.12) that is lower than the wild-type (Table 1), resulting in an overall fitness of 0.34 (Table 3) which is significantly lower than that of the wild-type, 0.62.

3.2.2.2 Azure

Azure (*Az*) appeared as a spontaneous mutation in a laboratory colony. The Azure phenotype is characterized by a uniform sparkling iridescence that gives the eye a bright blue color (Plate XX). The facets appear to protrude in the center, as seen in the scanning electron microscope (Plate XXI a).

The Azure strain has an extremely low mean hatch rate of 0.19 (±0.11) (Table 1), it is significantly lower than all other strains in this study. This results in the lowest overall fitness, 0.09, of the strains described (Table 3).

3.2.2.3 Furrow

Furrow (*Fr*) appeared as a spontaneous mutation in the gold-grey strain. The Furrow phenotype is characterized by a horizontal groove in the compound eye (Plate XXII). Scanning electron microscopy indicates that the facets in the area of the groove are regular hexagons, but indented (Plate XXI b). Histological studies show that the ommatidia are disrupted in the area of the groove (Plate XXIII b).

The Furrow strain has a mean hatch rate of 0.31 (±0.11) (Table 1). The larval viability and adult eclosion rates were not significantly different than the wild-type (Table 1).

3.2.2.4 Matte

The matte (*mt*) phenotype was observed in 3 males and 4 females from the F_3 of a laboratory strain established from field-collected males mass-mated to laboratory females (McCombs & Saul 1992b). The matte phenotype is characterized by an interruption of the normal reflective pattern of the eye. The pigmentation appears to be normal, however when the trait is expressed, the iridescence is absent over an area ranging from a minute spot to the entire eye (Plate XXIV a and b). The trait is incompletely penetrant, with 3-10% of *mt/mt* homozygotes appearing wild-type. The matte phenotype is similar to that of reflectionless eye described by Carante (1982) in the Mediterranean fruit fly, which he attributed to the disruption of the layered system of cuticle comprising the corneal lens. Scanning electron micrographs of the matte eye (Plate XVIII c) provide evidence of a disrupted corneal lens, the transition zone between the matte area (lower right) and the wild-type area (upper left) the outer cuticle appears wrinkled.

3.2.2.5 Notched eye

The notched eye (*ne*) trait is characterized by an indentation of the compound eye along the dorsal-lateral margin at the occipital row (Plate XXV). Examination with the electron microscope revealed the presence of misshapened facets and involution of the eye (Plate XXVI). The notched eye trait has variable expression ranging from a slight disruption of the facets (Plate XXVI a) to extreme indentations (Plate XXVI b).

Histological studies show that the retinal orgaization is entirely disrupted in the notched area (Plate XXIII c). There are numerous spaces and the regular lattice of ommatidia is absent, only irregularly spaced LPC are seen.

The notched eye strain had a mean hatch rate of 0.31 (±0.08) (Table 1) as compared to a value of 0.75 (±0.05) for the wild-type strain. This lower hatch rate results in a lower overall fitness, 0.17, as compared to 0.62 for the wild-type strain (Table 3).

3.2.3 Puparium color markers

The wild-type puparium is medium brown in color and elliptical in shape (Plate XXVII).

3.2.3.1 Melanistic

The melanistic (*me*) phenotype was observed as a spontaneous mutation in a pure-breeding stock of the coral (*co*) phenotype. The melanistic phenotype is characterized by a black puparium (Plate XXVII) and dark pigmentation of the adult (McCombs & Saul 1992c). Malpighian tubules and fat body of larvae and adults do not differ from that of the wild-type, however, the posterior spiracles of the larva are black instead of brown as observed in wild-type larvae. The melanistic pupal and adult phenotypes are similar to those of the dark pupae (*dp*) mutant described by Rössler & Koltin (1976) in the Mediterranean fruit fly.

The melanistic strain has a mean hatch rate of 0.27 (±0.05), as compared to 0.75 (±0.05) for the wild-type (Table 1). The mean adult eclosion rate, 0.67 (±0.07), is lower than that the wild-type value of 0.94 (±0.03). These two lower rates result in an overall fitness of 0.14 which is significnatly lower than the 0.62 value of the wild-type (Table 2, Table 3).

3.2.3.2 White puparium

The white puparium (*wp*) phenotype was independently isolated from a laboratory colony by myself and at least two other investigators (D. McInnis & D. Westcot, personal communication). The phenotype is characterized by a white color (Plate XXVII) that differs noticeably from the brown wild-type puparium and adults are wild-type in appearance (McCombs & Saul 1992b). The *wp/wp* homozygote is 100% penetrant and fully expressed.

3.2.4 Puparium morphology markers

3.2.4.1 Elongate puparium

Elongate puparium (*ep*) appeared as a spontaneous mutation in a laboratory colony. The pure-breeding phenotype is characterized by a pronounced elongation of the puparium and ridges at the anterior end (Plate XXVIII) (McCombs & Saul 1992c). The puparium resembles the mature third instar in size and shape and is significantly longer and narrower than the wild-type puparium (Table 4, Table 5). The *ep/ep* adult appears wild-type except for indentations in the compound eye which result from contact with the ridges at the anterior end of the puparium.

The mean eclosion rate of the elongate puparium strain is reduced with respect to the wild-type (Table 1). The mean adult eclosion rate of 0.41 (±0.01), results from the failure of numerous adults to extract themselves from the malformed puparia. This lowered eclosion rate results in a significantly lower overall and after hatch fitness values of 0.16 and 0.31, respectively (Table 2, Table 3).

3.2.4.2 Robust puparium

The robust puparium (*rp*) trait appeared as a spontaneous mutation in the white puparium (*wp*) stock. The robust puparium is significantly shorter and wider than the wild-type puparium (Table 4, Table 5) and is clearly distinguishable from the wild-type and *ep/ep* (Plate XXVIII) (McCombs & Saul 1992c). The *rp/rp* homozygote is 100% penetrant and fully expressed. Mature third instar larvae of the robust puparium strain attempt, but are unable to, bend and grasp the posterior end with their mouth hooks to execute the jumping behavior of wild-type larvae.

The mean eclosion rate of the robust puparium strain, 0.34 (±0.08), is significantly lower than that of the wild-type strain, 0.94 (±0.03) (Table 1). This results in a lower overall (0.10) and after hatch fitness (0.24) values (Table 2, Table 3).

3.2.5 Wing morphology markers

The wild-type wing is illustrated in Plate XXIX. Terminology used for the Dacinae wing venation and cells follows Shiraki (1933) and Drew (1989).

3.2.5.1 Crossveinless

Crossveinless (*cv*) is characterized by the interruption or absence of the basal crossvein (Plate XXX a and b). Crossveinless was found in males and females during routine screening of the notched eye strain and the crossveinless strain was established by breeding these flies. The trait is variably expressed and incompletely penetrant.

3.2.5.2 Curled

Curled wing (*cd*) occurred as a spontaneous mutation in a laboratory colony. Expression of the curled trait is variable, with the wing turned under or up at the distal end (Plate XXXI). The eye of the curled strain has a sparkling quality that scanning electron micrographs indicate to be the result of the facets that are irregular in shape and size on the dorsal half of the eye (Plate XXXII a and b).

3.2.5.3 Notch wing

The notched wing (*nw*) phenotype was isolated from the amethyst strain during routine screening. The notch wing phenotype is characterized by notches of varying sizes along the wing margin (Plate XXXIII). The trait is incompletely penetrant and has variable expression.

3.2.5.4 Small wing

The small wing (*sw*) phenotype was observed in male and female flies in the F_2 of a cross between cherry (*ch*) and white puparium (*wp*) (McCombs & Saul 1992a). A pure-breeding *sw/sw* stock was constructed from breeding these flies. The small wing phenotype is characterized by an indentation along the costal margin above the first costal cell and the medial vein is incomplete at the proximal end (Plate XXXIV). The distal end of the wing is fragile and often fractured. The wings are reduced in size and held out from the thorax and may droop as the adult ages. Flies with the small wing phenotype are unable to fly. The mean length of the small wing was 69.94 mm, as measured from the proximal end of the costal vein to the wing apex, and the mean width was 33.48 mm, measured as the perpendicular distance from the edge of the anal lobe to the costal vein (Table 7). These values are significantly less than the 94.4 mm length and 35.32 width of the wild-type wing (Table 6, Table 7).

The phenotype of the small wing trait resembles the droopy wing syndrome described in the mass-rearing colony at the USDA/ARS in Honolulu, Hawaii (Ozaki & Kobayashi 1981). The droopy wing resulted from mechanical damage to the indirect flight muscles during the mass-rearing process (Little *et al.* 1981). Histological studies demonstrated missing or detached vertical flight muscles in *Ceratitis capitata* (Little & Cunningham 1978). The indirect flight muscles of the small wing strain were found to be intact, indicating that the wing position and flightless character of the strain did not result from mechanical muscle damage.

3.3 MODE OF INHERITANCE

3.3.1 Eye color markers

3.3.1.1 Cherry

Results of crosses to determine the mode of inheritance (Table 8, crosses 1 & 2) indicate that *ch* is inherited as an autosomal recessive gene (Appendix D, Model 1). The significant deviation from the expected 3:1 ratio in the F_2 of cross 2 results from an underrepresentation of the *ch/ch* double homozygous class. The *ch/ch* homozygous genotype is 100% penetrant and fully expressed.

3.3.1.2 Copper

There were three phenotypic classes identified in the F_2 of crosses between copper and wild-type flies (Table 9, crosses 1 & 2). F_2 progeny numbers did not differ significantly from the 12 wild-type: 3 yellow eye: 1 copper ratio expected for dominant epistasis of two independently assorting genes (Suzuki *et al.* 1986). In this model (Appendix D, Model 6), the wild-type allele of the yellow eye gene is epistatic to the wild-type allele of the copper gene. The copper phenotype would occur only when the genotype was *ye/ye*; *cu/cu*. As expected copper was the only pure-breeding F_2 phenotypic class.

In crosses between yellow eye and copper flies (Table 9, crosses 3 & 4), the F_1 progeny were all yellow-eyed. The F_2 progeny numbers did not differ significantly from the expected 3 yellow eye: 1 copper ratio expected under the dominant epistasis model.

3.3.1.3 Gold-grey

Results of crosses to determine the mode of inheritance (Table 8, crosses 4 & 5) indicate that *gg* is an autosomal recessive gene.

3.3.1.4 Grape

Results of crosses 5 and 6 (Table 10) indicate that *Gr* is an incompletely dominant gene (Appendix D, Model 2). That is, the F_1 phenotype is a distinguishable as intermediate between *Gr* and wild-type and three phenotypic classes are distinguishable in the F_2. The significant deviation from the expected ratio in cross 5 is due to an over abundance of the *Gr*/wild-type intermediate phenotype. This may be due to misidentification as the sum of the *Gr*/wild-type plus the wild-type classes is not significantly different from the value expected for a 3:1 ratio. In cross 6, there is an overrepresentation of the *Gr/Gr* class which results in a significant deviation from the expected ratio ($P \leq 0.05$).

3.3.1.5 Mandarin red

Results of crosses to determine the mode of inheritance (Table 8, crosses 6 & 7) indicate that *ma* is inherited as an autosomal recessive gene (McCombs & Saul 1989). The *ma/ma* homozygous genotype is 100% penetrant and fully expressed.

3.3.1.6 White eye

Results of crosses to determine the genetic basis of the white eye trait shown in Table 8 (crosses 8 & 9) indicate that it is an autosomal, recessive gene (McCombs & Saul 1992b). The *we/we* homozygote is 100% penetrant and fully expressed.

3.3.1.7 Yellow eye

Crosses to determine the genetic basis of the yellow eye trait shown in Table 8 (crosses 10 & 11) indicate that the ratio of wild-type to yellow-eyed progeny in the F_2 did not differ significantly from the expected 3:1 ratio for an autosomal, recessive gene (McCombs & Saul 1992b). The *ye/ye* homozygote is 100% penetrant and fully expressed.

3.3.2 Eye morphology markers

3.3.2.1 Amethyst

Progeny numbers from crosses to determine the genetic basis of the amethyst trait are shown in Table 11 (crosses 1 & 2) (McCombs & Saul 1992b). The presence of *am/am* homozygotes in the F_1 suggests that the *am* gene could have also been present in the wild-type laboratory colony and detected through single pair matings. The *am/am* homozygote has reduced fitness due to low egg hatch and reduced larval viability (Table 1) which may cause the underrepresentation of this class in the F_2 from cross 1. We conclude that *am* is an autosomal, recessive gene.

3.3.2.2 Azure

Progeny numbers from crosses to determine the genetic basis of this trait (Table 10, crosses 1 & 2) indicate that Azure is inherited as an incompletely dominant gene. The F1 progeny have a phenotype that is intermediate between the wild-type and Az/Az and three phenotypes classes are distinguishable in the F_2. F_2 progeny numbers are difficult to interpret because the *Az/Az* homozygous class is approximately half of that expected, 1 wild-type: 2 heterozygous: 1 *Az/Az*. This indicates that the homozygotes are sub-vital. Viability measurements (Table 1) indicate that the *Az* strain has a mean hatch rate of 0.19 (±0.11), which is significantly lower than all other strains described in this study.

3.3.2.3 Furrow

Crosses to determine the genetic basis of the Furrow trait indicate that it is inherited as a dominant gene (Table 10, crosses 3 & 4) (Appendix D, Model 2). The F_1 progeny exhibit low expression of the Furrow phenotype, having only a slight indentation as opposed to the deep groove of the *Fr/Fr* extreme expression. Chi-square values indicate that there is a significant deviation from the 1 wild-type: 3 *Fr/_* ratio expected for a dominant gene. This may be due to the reduced hatch rate (Table 1) of the

Furrow strain or misidentification of the *Fr/_* heterozygotes with low expression of the Furrow trait as wild-type. Furrow is tentatively classified as a semidominant gene.

3.3.2.4 Matte

Results of crosses to determine the mode of inheritance of the matte trait are shown in Table 11 (crosses 3 & 4) (McCombs & Saul 1992b). The ratio of wild-type to *mt/mt* in the F_2 did not differ significantly from the expected 3:1 ratio for an autosomal, recessive gene. The *mt/mt* homozygote is incompletely penetrant and has variable expressivity.

3.3.2.5 Notched eye

Progeny numbers from crosses (Table 1, crosses 5 & 6) indicate that notched eye is inherited as an autosomal recessive gene. The *ne* gene appears to have complete penetrance, but variable expression.

3.3.3 Puparium color markers

3.3.3.1 Melanistic

Results of crosses to determine the mode of inheritance of the melanistic trait are shown in Table 12 (crosses 3 & 4) (McCombs & Saul 1992c). F_2 pupal counts did not differ significantly from the expected 3:1, wild-type to *me*, for an autosomal, recessive gene. However, adults from cross 4 had an overrepresentation of *me/me* in the F_2. The *me/me* homozygote is completely penetrant and fully expressed.

3.3.3.2 White puparium

We have completed crosses to determine that the white puparium trait is inherited as an autosomal, recessive gene (Table 12, crosses 7 & 8) (McCombs & Saul 1992b). The significant deviation from the expected 3:1 ratio in the F_2 of cross 8 resulted from an overrepresentation of *wp/wp*.

3.3.4 Puparium morphology markers

3.3.4.1 Elongate puparium

Results of crosses to determine the genetic basis of the elongate puparium trait are shown in Table 12 (crosses 1 & 2) (McCombs & Saul 1992c). The ratio of wild-type to *ep/ep* pupae in the F_2 did not differ significantly from the expected 3:1 for an autosomal recessive trait. However, there was an underrepresentation of adult *ep/ep* homozygotes in the F_2 from cross 2 which may be due to the low eclosion rate (Table 1) resulting from death of pupae and of emerging adults that are unable to exit the puparium.

3.3.4.2 Robust puparium

Results of crosses to determine the genetic basis of the robust puparium trait are shown in Table 12 (crosses 5 & 6) (McCombs & Saul 1992c). Pupal progeny numbers of the F_2 did not differ significantly from the 3:1 ratio, wild-type to *rp/rp*, expected for an autosomal, recessive gene. However, adult *rp/rp* homozygotes are underrepresented in cross 5. This deviation from the expected 3:1 ratio may result from the low eclosion rate (Table 1) due to the inability of numerous adults to exit the puparium.

3.3.5 Wing morphology markers

3.3.5.1 Crossveinless

Results from crosses to determine the mode of inheritance of the crossveinless trait indicate that it is an autosomal recessive gene (Table 13, crosses 3& 4). The significant deviation from the expected 3 wild-type: 1 *cv/cv* may result from the variable expression and incomplete penetrance of this gene. There was a significant deviation from the expected 1:1 sex ratio within both the wild-type and crossveinless classes. There were significantly more females with the crossveinless trait, indicating that expression of the *cv* gene could be sex-influenced.

3.3.5.2 Curled

F_2 progeny numbers from crosses to determine genetic basis of the curled trait (Table 13, crosses 1 & 2) indicate that it is an autosomal recessive gene. There is a significant deviation from the expected 3 wild-type: 1 *cd/cd* in cross 2 because of the variable expression and incomplete penetrance of the *cd* gene.

3.3.5.3 Notch wing

Results of crosses to determine the mode of inheritance of the notch wing trait (Table 13, crosses 5 & 6) are difficult to interpret due to the variable expression of the notch wing trait. The notch wing class is extremely deficient, resulting in a highly significant deviation from the 3 wild-type to 1 notch wing ratio expected for an autosomal recessive gene. I conclude that notch wing is inherited as an autosomal recessive gene.

3.3.5.4 Small wing

Results of crosses to determine the mode of inheritance of the small wing trait indicate that it is an autosomal, recessive gene (Table 13, crosses 7 & 8) (McCombs & Saul 1992a). The significant deviation from the expected 3:1 ratio in the F_2 of cross 1 is due to the underrepresentation of *sw/sw* homozygotes. The *sw/sw* homozygous genotype is 100% penetrant and variably expressed.

3.4 LINKAGE ANALYSIS

The F_1 phenotype for all crosses was wild-type, unless specifically noted, and the F_2 was produced by mass-mating of the F_1. Flies from each F_2 phenotypic class were intracrossed to determine true-breeding status and for detection of recombinants.

3.4.1 Linkage relationships of amethyst

The low viability (Table 1) and low overall fitness (Table 3) of *am/am* homozygotes must be considered in the interpretation of results from crosses involving

the *am* gene. Progeny numbers from crosses consistently show a deficiency of *am/am* and the double homozygote classes and an excess of wild-type flies.

Amethyst segregates independently from the *ma* and *mt* genes (McCombs & Saul 1992b). The significant deviation from the expected F_2 ratio of crosses with these alleles in the repulsion (Table 14, crosses 1 & 2) and in the coupling configuration (Table 15, crosses 1 & 2) results from the low viability discussed above. The presence of double homozygous flies, which breed true, and poor fit to any other possible models (Saul & Rössler 1984a) indicates that deviations are due to reduced viability and incomplete penetrance of the *am* and *mt* genes, respectively. In addition, linkage intensities were calculated (Immer 1930, Weir 1990) and the crossover values of 0.480 and 0.470 for *am* and *ma* (Table 14, cross 1; Table 15, cross 1) and 0.470 and 0.530 for *am* and *mt* (Table 14, cross 2; Table 15, cross 2) indicate that linkage is not the cause of the observed deviations. We conclude that the *am* gene is in a separate linkage group from *ma* and *mt*.

Amethyst gene is linked to the *we* and *wp* genes (McCombs & Saul 1992b). Results of crosses involving the *am* and *we* genes do not show a significant deviation from the expected F_2 ratio for linkage (Table 14, cross 3). In the F_3, white eye is a true-breeding class and the amethyst class produced 5 white eye and 248 amethyst individuals. These flies could represent *we/we am/am* recombinants in which *we* is epistatic to *am*. We have shown that the *we/we* is epistatic to *ye/ye* and *ma/ma* and that *ye/ye* is epistatic to *am/am* which supports the idea that *we* and *am* are linked and *we* is epistatic to *am*.

The deviation from the expected F_2 ratio in crosses between the *am* and *wp* genes is due to a deficiency of *am/am* and an excess of wild-type flies (Table 14, cross 4). Recombinant *am/am wp/wp* individuals were isolated in the F_3 of both *am* and *wp* classes and were used to establish a double homozygous strain. Crosses in the coupling

configuration confirm that these genes are located in the same linkage group, separated by a distance of 23.9 m.u. (Table 16, cross 1).

Amethyst and yellow eye segregate independently (McCombs & Saul 1992b). Test matings indicate that *ye/ye* is epistatic to *am/am* and the expected F_2 ratio is 9:4:3, wild-type:*ye/ye*:*am/_* (Table 14, cross 5). The yellow eye phenotype is the only pure-breeding F_2 class.

3.4.2 Linkage relationships of mandarin red

Mandarin red is linked with the *mt* gene (McCombs & Saul 1992b). The deviation from the expected F_2 ratio is due to incomplete penetrance and variable expression of the *mt/mt* homozygote (Table 14, cross 6). Recombinant *ma/ma mt/mt* flies isolated in the F_3 of both mandarin red and matte classes were used to establish a pure-breeding double homozygous strain. Results from crosses in coupling (Table 16, cross 6) confirm that these two genes are in the same linkage group and separated by 24.7 m.u.

Mandarin red segregates independently from the *we*, *wp* and *ye* genes (McCombs & Saul 1992b). Test matings show that *we/we* and *ye/ye* are epistatic to *ma/ma* with the expected F_2 ratio being 9:4:3 of wild-type:*we/we*:*ma/_* and wild-type:*ye/ye*:*ma/_*, respectively (Table 14, cross 7 & 9). The white eye and yellow eye phenotypic classes are the only pure-breeding F_2 classes in the respective crosses. F_2 progeny numbers from crosses involving mandarin red and white puparium do not differ significantly from the 9:3:3:1 ratio expected for two independently segregating genes (Table 14, cross 8). The crossover value of 0.490 supports this conclusion.

3.4.3 Linkage relationships of matte

Matte segregates independently from the *we*, *wp* and *ye* genes (McCombs & Saul 1992b). The significant deviation from the expected F_2 ratio in each of these crosses (Table 14, cross 10 & 11; Table 15, cross 6) results from the underrepresentation of the *mt/mt* and double homozygote classes and an excess of wild-type flies. The presence of a

double homozygous class in each cross which is true-breeding and poor fit to any other possible models indicate that the deviation results from the variable expression and reduced penetrance of the *mt/mt* homozygote. Calculated linkage intensities and crossover values indicate that linkage is not responsible for the observed deviations.

3.4.4 Linkage relationships of white eye

Results of crosses with *we* and *wp* in the repulsion configuration indicate a significant difference from the 1 *we/we*: 1 *wp/wp*: 2 wild-type expected for linkage (Table 14, cross 12) (McCombs & Saul 1992b). This is due to an excess of wild-type progeny and may be attributable to reduced fitness of the two mutants (Tables 1, Table 3). Recombinant *we/we wp/wp* flies were isolated in the F_3 from these crosses used to establish a double homozygous strain. Crosses in the coupling configuration (Table 16, cross 10) confirm that these two genes are in the same linkage group separated by a map distance of 31.3 m.u. Backcrosses (Table 17, crosses 5 and 6) confirm the linked status of the *we* and *wp* genes with map distances of 27.0 and 30.4 m.u., respectively.

White eye and yellow eye segregate independently (McCombs & Saul 1992b). Test matings indicate that *we/we* is epistatic to *ye/ye* and the expected ratio is 9:4:3 of wild-type:*we/we*:*ye/_* (Table 14, cross 13). The white eye phenotypic class is the only pure-breeding F_2 class.

3.4.5. Linkage relationships of white puparium

White puparium and yellow eye segregate independently (Table 14, cross 14) (McCombs & Saul 1992b). The F_2 progeny numbers do not differ significantly from the expected 3 *wp/wp*:3 *ye/ye*:9 wild-type:1 *wp/wp;ye/ye* ratio, indicating that the two genes are not linked.

3.4.6 Linkage relationships of elongate puparium

Progeny numbers from crosses involving elongate puparium consistently show a deficiency of *ep/ep* and the double homozygote classes and an excess of wild-type flies

(Table 18, crosses 1 thru 10). The low viability may be attributable to pupal and adult mortality (Table 1, Table 3) and must be considered in the interpretation of cross results.

Elongate puparium is linked to the *ma, me, mt,* and *rp* genes (McCombs & Saul 1992c). F_2 progeny numbers from crosses with these genes in the repulsion configuration (Table 18) differ significantly from the expected 1:1:2 ratio for all but crosses 7 and 8. The absence of a double homozygous class in the F_2 is due to linkage and the absence of recombination in males of this species. Recombinants were isolated from the F_3 of the elongate puparium and mandarin red, melanistic, matte, and robust puparium classes in the respective crosses and were used to establish strains with the genes in the coupling configuration.

Results of crosses with the *ep* and *ma* genes in the coupling configuration (Table 16, cross 2) indicate that the two genes are linked and separated by a distance of 30.2 m.u. Progeny numbers from backcrosses involving the *ep* and *ma* genes further support the conclusion that these genes are linked (Table 17). Crosses 3 and 4 gave map distances of 24.7 and 25.4 m.u., respectively.

F_2 progeny numbers from crosses with *ep* and *me* genes in the coupling configuration indicate that these genes are linked and separated by 15.5 m.u. (Table 16, cross 3). Elongate puparium and robust puparium are also linked (Table 16, cross 4) and separated by a distance of 14.0 m.u.

Elongate puparium is linked with the *mt* gene (McCombs & Saul 1992b). Results of crosses with the genes in the repulsion configuration (Table 18, cross 5 & 6) indicate that the two genes are linked. There is a significant deviation from the expected ratio due to an underrepresentation of the elongate puparium class.

Elongate puparium segregates independently from the *ye* and *wp* genes (McCombs & Saul 1992c). Results from crosses with the *ep* and *ye* genes in the repulsion configuration (Table 18, cross 9 & 10) indicate that elongate puparium is

underrepresented in the F_2 progeny numbers. The crossover values of 0.545 and 0.445 obtained from linkage intensities indicate that linkage is not the cause for the significant deviation from the expected ratio. Results from crosses with the *ep* and *wp* genes in coupling (Table 15, cross 3) do not differ significantly from the expected ratio for non-linkage (Appendix D, Model 7). Results from backcrosses involving the *ep* and *wp* genes (Table 19, crosses 1 & 2) do not deviate significantly from the expected 1:1:1:1 ratio, indicating that the two genes assort independently (Appendix D, Model 17).

3.4.7 Linkage relationships of melanistic

The *me* gene is linked with mandarin red (McCombs & Saul 1992c). Results of crosses in the coupling configuration (Table 16, cross 5) indicate the genes are separated by 14.1 m.u. Progeny numbers from backcrosses 1 and 2 (Table 17) gave map distances of 11.1 and 12.8 m.u., respectively.

Melanistic is linked with the *mt* and *rp* genes (Table 18) (McCombs & Saul 1992c). The significant deviation from the expected 1:1:2 ratio may be due to incomplete penetrance and expressivity of the *mt* gene (crosses 13 and 14) and the low eclosion rate of *rp* (cross 15). No double homozygous class was seen in the F_2 of these crosses, but were recovered from the F_3. Results from crosses in coupling involving the *me/me mt/mt* strain indicate linkage and a map distance of 11.0 m.u. (Table 16, cross 7). Similarly, cross 8 with the *me/me rp/rp* strain indicate linkage of the two genes at a map distance of 8.7 m.u.

Melanistic segregates independently from the *wp* gene (McCombs & Saul 1992c). Test matings show that F_2 progeny numbers from crosses involving the *me* and *wp* genes do not differ significantly from the 9:3:3:1 ratio expected for two independently segregating genes (Table 15, cross 4). The *me/me*; *wp/wp* double homozygote puparium has a distinct greyish phenotype (Plate XXXV) and the adult is darkly pigmented. This

is similar to the dark pupae, white pupae double homozygote in the Mediterranean fruit fly (Rössler 1979b).

3.4.8 Linkage relationships of robust puparium

Robust puparium segregates independently from the *ye* (Table 18, crosses 18 & 19) and *wp* genes (Table 15, cross 5) (McCombs & Saul 1992c). The significant deviation from the expected ratio for non-linkage in the F_2 progeny numbers of crosses involving the *rp* and *ye* genes is attributable to an underrepresentation of the *rp/rp* and *rp/rp;ye/ye* classes. The robust puparium strain does have reduced fitness which may have resulted in lower numbers of progeny in these classes. Linkage intensities of 0.5 and 0.53 (Table 18, cross 18 & 19) indicate that the deviation is not the result of linkage. F_2 progeny numbers from crosses in the coupling configuration involving the *rp* and *wp* genes are significantly different from the expected ratio for non-linkage (Table 15, cross 5). The *rp/rp* class was underrepresented and linkage intensity of 0.515 indicates that the deviation was not caused by linkage.

Robust puparium is linked with the *ma* and *mt* genes (McCombs & Saul 1992c). Results of crosses in the repulsion configuration indicate that robust puparium is linked to mandarin red (Table 18, crosses 11 & 12). There is a significant deviation from the expected ratio in cross 12 which is due to an underrepresentation of the robust puparium class. F2 progeny numbers from crosses between robust puparium and matte (Table 18, cross 16 & 17) differ significantly from the expected 1 *rp/rp*:1 *mt/mt*:2 wild-type ratio for two linked genes. This results from the underrepresentation of both the rp/rp and mt/mt classes. The reduced fitness of the rp/rp and the variable expressivity of mt/mt may be responsible for the observed deviation. Results of cross 9 in the coupling configuration (Table 16) indicate that the two genes are linked and separated by 19.6 m.u.

3.4.9 Linkage relationships of Grape

Grape assorts independently from the *ma* and *wp* genes. In progeny numbers from crosses in the coupling configuration (Table 20, cross 1 & 2) between Grape and mandarin red the wild-type and intermediate phenotypic classes have been combined. This was done because the misidentification of these classes resulted in an underrepresentation of the wild-type class. When the two classes are combined, the progeny numbers do not differ significantly from the 9 wild-type : 3 mandarin red : 3 Grape : 1 Grape-mandarin red ratio expected for two independently assorting genes (Appendix D, Model 14). For linkage, the expectation would be to observe only three phenotypic classes, the double homozygous class would be missing (Appendix D, Model 13).

Crosses in the repulsion configuration involving Grape and white puparium (Table 21, cross 1 & 2) indicate that these two genes are not linked. There was a significant deviation from the expected ratio (Appendix D, Model 12) because the wild-type phenotypic class was overrepresented while the intermediate phenotype was under represented, suggesting that misidentification of this class occurred. All six of the phenotypic classes expected in the non-linkage model were represented in the F_2 progeny numbers. If the two genes were linked, only three phenotypic classes would be observed (Appendix D, Model 11).

Progeny numbers from crosses involving the Grape and amethyst genes in the repulsion configuration differ significantly from the expected ratio for non-linkage (Table 21, cross 3 & 4) (Appendix D, Model 12). Five phenotypic classes were observed in the F_2, the intermediate/amethyst class was not detected and may have been represented in the amethyst class. The double homozygous class was greatly underrepresented in both reciprocal crosses. The Grape class is overrepresented,

suggesting that the double homozygotes may not have been completely distinguishable from Grape.

Grape and notched eye appear to assort independently. Progeny numbers from crosses in the repulsion configuration (Table 22, cross 7 & 8) indicate that the double homozygous class is underrepresented, resulting in a significant deviation from the expected ratio (Appendix D, Model 12). The notched eye trait is variably expressed, making it difficult to detect when the facet disruption is slight.

Progeny numbers from backcrosses involving Grape and white eye (Table 19, cross 3 & 4) were not significantly different from the 1:1:1:1 ratio expected for two independently assorting genes (Appendix D, Model 20). Backcrosses involving the Grape and yellow eye genes (Table 19, cross 5) also fit the non-linkage model (Appendix D, Model 20).

Grape and small wing are linked (Table 22, cross 5 & 6) (Appendix D, Model 11). The significant deviation from the expected ratio was attributable to an underrepresentation of the *sw/sw* class in each cross. There were no grape/small wing double homozygotes observed in the F_2 of crosses in the repulsion configuration, but this class was recovered from the F_3. Progeny numbers from crosses in coupling (Table 23, cross 2) indicate that Grape and small wing are 43.7 m.u. apart.

Grape and Furrow are not linked. All phenotypic classes expected for non-linkage of one incomplete dominant and one dominant gene (Appendix D, Model 15) were observed in the F_2 progeny (Table 24). Three phenotypic classes were expected for two linked genes (Appendix D, Model 16). The significant deviation from the expected ratio is due to the overrepresentation of the wild-type and intermediate phenotypic classes and the underrepresentation of the double homozygous class. This pattern was as observed in other crosses involving the Grape gene.

3.4.10 Linkage relationships of notched eye

Notched eye assorts independently from mandarin red. The F_2 progeny numbers from crosses in coupling (Table 20, cross 3) were not significantly different from the expected ratio for two independently assorting genes. Progeny numbers from crosses in repulsion involving the Furrow and notched eye genes (Table 22, cross 1 & 2) differed significantly from the ratio expected for independently segregating genes (Appendix D, Model 9). Four phenotypic classes were observed in contrast to the three classes expected for linked genes (Appendix D, Model 10). The variable expression of both of these traits may account for the observed deviation.

Notched eye assorts independently from robust puparium, yellow eye, white puparium (Table 25). Progeny numbers from crosses between notched eye and a multiple marker strain with *rp/rp;ye/ye;wp/wp* indicate that *ne* is not linked with any of these markers. Notched eye was observed to assort with each of the other genes, as expected for non-linkage.

3.4.11 Linkage relationships of copper

Yellow eye and copper fit a model of dominant epistasis (Appendix D, Model 6), where the wild-type yellow eye allele is epistatic to the wild-type copper allele. This must be considered in the interpretation of linkage analyses involving the copper trait.

Copper is linked to white eye gene. The F_1 progeny were all wild-type in appearance and there were four phenotypic classes in the F_2. The F_2 progeny numbers (Table 26) did not differ significantly from the expected 9 wild-type: 1 copper: 4 white eye: 2 yellow eye ratio expected for a model that considers *cu* and *we* as linked genes, where *we/we* has recessive epistasis to both *cu/cu* and *ye/ye*, and the wild-type yellow eye allele is epistatic to the wild-type copper allele (Appendix D, Model 6).

Copper is linked to gold-grey. The F_1 progeny all had the wild-type phenotype and there were four phenotypic classes in the F_2. The proposed model (Appendix D,

Model 6) is one in which the wild-type yellow eye allele is epistatic to the wild-type copper allele, *we/we* is epistatic to *ye/ye* and *cu/cu*, and *ye/ye;gg/gg* has the gold-grey phenotype. The F_2 progeny numbers (Table 26) in cross 3 did not differ significantly from the expected 9 wild-type: 1 copper: 4 gold-grey: 2 yellow eye ratio expected for this model. Progeny numbers from cross 4 were significantly different from the expected ratio due to an underrepresentation of the gold-grey class.

Copper is linked to white puparium (Table 27). The F_2 progeny numbers from crosses with the genes in the repulsion configuration did not differ significantly from the expected ratio of 9 wild-type: 3 white puparium: 2 yellow eye: 1 yellow and white puparium: 1 copper: 0 copper and white puparium. The proposed model (Appendix D, Model 6) was one in which the dominant epistasis outlined above is operating and copper is linked to white puparium. In a system with no recombination in males, *cu/cu wp/wp* individuals are not expected to occur and none did.

3.4.12 Linkage relationships of gold-grey

Gold-grey is linked to white puparium . F_2 progeny number from crosses in the repulsion configuration involving the *gg* and *wp* genes (Table 28, cross 5 & 6) are not significantly different from the expected ratio for non-linkage. Crosses in the coupling configuration indicated that the *gg* and *wp* genes are separated by 6.7 m.u. (Table 23).

Gold-grey is linked to the *we* gene. The F_2 progeny numbers from crosses in the repulsion configuration do not differ significantly from the expected 1 gold-grey: 1 white eye : 2 wild-type (Table 28, cross 3 & 4) for non-linkage. No recombinants were recovered and crosses in coupling were not made.

3.4.13 Linkage relationships of cherry

Cherry is allelic to mandarin red. The F_1 progeny from crosses in repulsion involving the *ma* and *ch* genes were of the mandarin red phenotype. The F_2 progeny

numbers did not differ significantly from the 3 mandarin red : 1 cherry expected for alleles (Table 28, cross 1 & 2). Mandarin is dominant to cherry.

3.4.14 Linkage relationships of crossveinless

Crossveinless assorts independently from white puparium, mandarin red, and yellow eye. The significant deviation of F_2 progeny numbers from the ratio expected for independently segregating genes (Table 28). The crossveinless trait has highly variable expression and is incompletely penetrant which could account for the underrepresentation of the crossveinless and double homozygous classes.

3.4.15 Linkage relationships of Azure

Azure assorts independently from the *ye* and *ma* genes. Crosses in repulsion involving the *Az* and *ma* genes (Table 21, cross 5 & 6). All expected phenotypic classes for two independently assorting genes were observed in the F_2 (Appendix D, Model 12). Only three phenotypic classes were expected for the linkage model (Appendix D, Model 11). The F_2 progeny numbers from cross 5 were not significantly different from the expected ratio. However, cross 6 F_2 progeny numbers were significantly different from the expected ratios due to an overrepresentation of the intermediate classes and a deficiency of *Az/Az* homozygotes.

Azure and yellow eye appear to assort independently. All phenotypic classes expected for the non-linkage model (Appendix D, Model 12) were observed in the F_2 progeny from crosses in the coupling configuration (Table 29). There was a siginficant deviation from the expected ratio in cross 1due to a deficiency in the yellow/intermediate and yellow/Azure classes and an over abundance of Azure individuals. The F_2 progeny numbers from cross 2 did not differ significantly from the expected ratio for two independently segregating genes (Appendix D, Model 14).

3.4.16 Linkage relationships of notch wing

Notch wing appears to segregate independently from the *ep*, *wp*, and *ye* genes. F_2 progeny numbers from crosses between notch wing flies and the multiple marker strain, *ep/ep;wp/wp;ye/ye*, (Table 25) indicate that the *nw* gene is not linked to these marker genes. Notch wing was observed with each of these phenotypes in the F_2 progeny which would only occur if the genes were not linked.

3.4.17 Linkage relationships of small wing

Small wing is not linked to *ep*, *wp* or *ye*. In the F_2, small wing was observed to assort independently of each marker in the multiple marker strain, *ep/ep;wp/wp;ye/ye*, (Table 25). This is as expected for crosses in the repulsion configuration in a species in which recombination does not occur in males.

3.5 MAP DISTANCES WITHIN LINKAGE GROUPS

I have constructed the first linkage group map for *Bactrocera dorsalis* (Table 30). This linkage map places markers on each of the five autosomal linkage groups. Labeling of the linkage groups is arbitrary and does not imply any correlation with cytological descriptions of the chromosomes (Bhatnagar *et al.* 1980).

3.5.1 Linkage Group A

The various map distances for genes in linkage group A (Table 31) were used to construct a preliminary linkage map. Mandarin red (*ma*) is at position zero and the other markers were given relative to this gene. Cherry is allelic to *ma* and is also at position zero. Robust puparium (*rp*) is given at position 5.4 m.u., melanistic (*me*) at 14.1m.u., matte (*mt*) at 26.7 m.u., and elongate puparium (*ep*) at 30.2 m.u.

3.5.2 Linkage Group B

Linkage group has only one marker, yellow eye (*ye*).

3.5.3 Linkage Group C

Linkage group C has four eye color markers: *am*, *cu*, *gg*, *we*, and a puparium color marker, white puparium. Three of the markers have been mapped relative to white puparium, amethyst (*am*) is 23.3 m.u. from *wp*, gold-grey (*gg*) is 6.7 m.u., and white eye (*we*) is 31.2 m.u. from the *wp* gene (Table 32). Copper (*cu*) has been mapped to this linkage group, but the relative position of this gene was not determined.

3.5.4 Linkage Group D

Grape (*Gr*) and small wing (*sw*) assort independently from markers on linkage groups A, B, and C. The *Gr* and *sw* genes are linked but at a distance of 43.2 m.u. (Table 32). The genes are so widely separated in this linkage group as to bring doubt that the genes are linked. The linkage hypothesis is supported by the fact that crosses with the *Gr* and *sw* genes in repulsion did not have any individulas in the *Gr/Gr; sw/sw* class (Table 22, cross 5 & 6).

3.5.5 Linkage Group E

Linkage group E has only one marker, notched eye (*ne*). Notched eye was observed to assort independently from markers on each of the other linkage groups.

3.5.6 Unmapped markers

Five markers, Azure (*Az*), curled (*cd*), crossveinless (*cv*), Furrow (*Fr*), and notch wing (*nw*), remain unmapped.

3.6 Multiple marker strains

Two multiple marker strains were constructed for use in mapping studies. These strains, *ep/ep;ye/ye;wp/wp* and *rp/rp;ye/ye;wp/wp*, had marker genes for linkage groups A, B, and C, respectively. Mapping of the *ne*, *sw*, and *nw* genes were done, in part, using these strains (Table 25).

3.7 Potential uses of genetic markers

3.7.1 Genetic sex sorting system

Three mutants hold promise for development of a genetic sex sorting system to enhance the sterile insect release method. The two puparium color markers, white puparium and melanistic, could be used to develop a sorting system similar to the translocation-based system described by Rössler (1979a). This sexing system was based on pupal color dimorphism in which males are wild-type and females carry the mutant pupal color gene. The sexes can be separated at the pupal stage using sorting machines.

Small wing could be used to develop a sexing system in which males are wild-type and females are homozygous for the small wing gene and are, therefore, flightless. Saul (1990) described a translocation-based system in the Mediteranean fruit fly based on the flightless mutant v-wing. In this system, the females have the v-wing phenotype and are flightless while males are wild-type at the v-wing locus. Females would be unable to disperse from the release site which should significantly decrease assortative mating and eliminate sting damage.

3.7.2 Markers of released populations

Two eye color markers, white eye and yellow eye, could be used as markers for released populations (Saul & McCombs 1992). The mutant phenotype is distinguishable from the wild-type even when the adults are dead and dried for several weeks. The relative fitness of the eye color markers must be considered before their usefulness can be determined. Eye color variants may be unable to effectively disperse or locate food under field conditions.

3.7.3 Basic biological studies

3.7.3.1 Biochemistry

Variants in eye color provide genetic material for studying the ommochrome and pteridine biosynthetic pathways. Several mutants appear to result from an interruption of

ommochrome biosynthesis. The absence or reduction of ommochromes in the compound eyes of the cherry, copper, gold-grey, mandarin red, yellow eye, and white eye strains results in altered eye color phenotypes. These phenotypes are associated with the absence of dark pigmentation on the head and reduced coloration of the Malpighian tubules. Thin layer chromatography could be used to identify the fluorescent pigments present in each mutant strain. Similar studies of eye color mutants in *Drosophila melanogaster* (Summers *et al.* 1982) were important in understanding the pigment biosynthetic pathways.

Epistatic interactions provide insight into the role of specific genes in the eye pigment biosynthetic pathways. White eye was found to be epistatic to *ye/ye* and *ma/ma.* Yellow eye was found to be epistatic to *ma/ma* and *am/am.* The double homozygous *we/we;Gr/Gr* and *ye/ye;Gr/Gr* phenotypes were similar in color to white eye and yellow eye, respectively. There was a slight darkening and reduced reflective quality that allowed for classification of the double homozygotes. This indicates that the *we* and *ye* genes may also be epistatic to Grape. Gene products of the white eye and yellow eye loci may function in transport, as suggested for the white and scarlet genes of *Drosophila melanogaster* (Sullivan & Sullivan 1975, Tearle 1991).

Eye color phenotypes could result from mutations at loci coding for critical enzymes or co-factors of enzymes of biosynthetic pathways. Typically, enzymes also have a role outside the eye pigmentation pathways and may function as selectable markers (Gelbart *et al.* 1976). For example, in *Drosphila melanogaster* a mutation at the xanthine dehydrogenase structural gene, *rosy*, results in an altered eye color phenotype and an altered ability to metabolize purines.

3.7.3.2 Mating behavior

Pupal color variants allow the unambiguous assignment of paternity for specific mating types in the Mediterranean fruit fly (Saul *et al.* 1988). The melanistic and white

puparium genes in the oriental fruit fly can be used in a similar manner for sperm competition studies and in evaluating mating efficiency and remating rates.

3.7.3.3 Development

Mutants allow for the study of normal developmental processes by providing examples of how these processes can go wrong. Studies of the variants in eye structure: amethyst, Azure, Furrow, matte, and notched eye; could provide insight into morphogenesis of the compound eye.

3.7.3.4 Molecular biology

Spontaneous or genetically unstable mutants could result from the insertion or excision of transposable elements at specific loci. The availability of such mutants in the oriental fruit fly suggests that transposable elements may be present in the genome. Study of the spontaneous eye color mutants, i.e., white eye and yellow eye, may allow for the isolation of transposable elements. This would provide the basis for developing a gene transfer system specifically for the Tephritidae.

CHAPTER 4. SUMMARY

4.1 Described genetic markers

Twenty new genetic markers were described in the oriental fruit fly, *Bactrocera dorsalis* (Hendel). There were five general types of markers described, including eye color: cherry, copper, gold-grey, Grape, mandarin red, white eye and yellow eye; eye structure: amethyst, Azure, Furrow, matte, and notched eye; puparium color: white puparium and melanistic; puparium morphology: elongate puparium and robust puparium; and wing structure: crossveinless, curled, notch wing and small wing.

4.2 Inheritance of genes

All described markers were autosomal genes. Furrow was inherited as a dominant gene and two genes, Azure and Grape, were incompletely dominant. The remaining seventeen traits were inherited as recessive genes.

4.3 Linkage relationships

Linkage group A had six markers including, cherry, elongate puparium, mandarin red, matte, melanistic, and robust puparium. The linear order of genes in linkage group A was given with mandarin red at position 0, robust puparium at 5.4 m.u., melanistic at 14.1 m.u., matte at 26.7 m.u., and elongate puparium at 30.2 m.u. Cherry was a recessive allele at the mandarin red locus.

Linkage group C had five markers including, amethyst, copper, gold-grey, white eye and white puparium. The linear order of these markers could not be determined due to interaction of the eye color genes. The genes were mapped relative to the white puparium locus. Amethyst was 23.4 m.u. from white puparium, gold-grey was 6.7 m.u., and white eye was 31.2 m.u. from the white puparium locus. Copper was not mapped.

Linkage group D had two markers, Grape and small wing which were 43.2 m.u. apart. Yellow eye and notched eye were assigned to linkage group B and E, respectively.

Five markers remain unmapped including, Azure, curled, crossveinless, Furrow, and notch wing.

4.4 Epistatic interactions

White eye was epistatic to *ye/ye* and *ma/ma*. Yellow eye was epistatic to *ma/ma* and *am/am*. The yellow eye gene was dominantly epistatic to copper, so that only individuals of the *ye/ye;cu/cu* genotype expressed the copper phenotype.

APPENDIX A: TABLES

Table 1.
Viability measurements of described strains of the oriental fruit fly, *Bactrocera dorsalis*

Strain	Hatch rate			Larval viability			Adult eclosion		
	No. rep [a]	mean	(SD)	No. rep [b]	mean	(SD)	No. rep	mean [c]	(SD)
am	22	0.50	(0.12)	7	0.87	(0.09)	6	0.79	(0.06)
Az	8	0.19	(0.11)	7	0.73	(0.10)	3	0.65	(0.06)
ch	16	0.49	(0.14)	12	0.81	(0.13)	12	0.63	(0.17)
cu	18	0.49	(0.16)	8	0.86	(0.04)	8	0.86	(0.08)
ep	20	0.52	(0.13)	6	0.76	(0.08)	6	0.41	(0.01)
Fr	8	0.31	(0.11)	6	0.90	(0.05)	6	0.85	(0.04)
gg	16	0.52	(0.11)	7	0.88	(0.11)	7	0.62	(0.06)
Gr	14	0.43	(0.10)	4	0.86	(0.06)	4	0.90	(0.05)
ma	22	0.60	(0.14)	11	0.90	(0.06)	11	0.83	(0.07)
me	12	0.27	(0.05)	8	0.76	(0.09)	8	0.67	(0.07)
mt	16	0.50	(0.17)	8	0.82	(0.07)	8	0.71	(0.19)
ne	4	0.31	(0.08)	3	0.85	(0.05)	3	0.63	(0.11)
rp	16	0.42	(0.07)	6	0.72	(0.12)	6	0.34	(0.08)
we	22	0.38	(0.21)	7	0.82	(0.12)	9	0.74	(0.15)
wp	16	0.43	(0.05)	8	0.78	(0.05)	8	0.88	(0.05)
ye	18	0.59	(0.13)	8	0.87	(0.11)	8	0.70	(0.12)
wild-type:									
PENT	12	0.75	(0.05)	4	0.88	(0.10)	4	0.94	(0.03)
SAKA	12	0.72	(0.10)	4	0.85	(0.02)	4	0.86	(0.01)
TEST	22	0.49	(0.08)	8	0.88	(0.06)	8	0.85	(0.04)

[a] 100 eggs per replicate.
[b] 100 first instar larvae per replicate.
[c] Given as the ratio of adults:pupae.
All values are shown with standard deviation (SD) in parentheses.

Table 2.
One-way analysis of variance tables for hatch (A), larval viability (B), and eclosion (C) of described strains of the oriental fruit fly, *Bactrocera dorsalis*

A. One-way analysis of variance table for hatch by strain

Source of variation	df	SS	MS	Fs [a]
Among strains	18	4.061	0.2256	14.130***
Within strains	275	4.391	0.016	
Total	293	8.452		

B. One-way analysis of variance table for larval viability by strain

Source of variation	df	SS	MS	Fs [a]
Among strains	18	0.469	0.0261	3.366***
Within strains	117	0.906	0.0077	
Total	135	1.375		

C. One-way analysis of variance table for eclosion by strain

Source of variation	df	SS	MS	Fs [a]
Among strains	18	2.797	0.155	14.676***
Within strains	110	1.165	0.011	
Total	128	3.962		

[a] Significant at the 0.000 level

Table 3.
Fitness measurements for described strains of
the oriental fruit fly, *Bactrocera dorsalis*

Strain	Overall fitness [a]	Post-hatch fitness [b]
am	0.34	0.69
Az	0.09	0.47
ch	0.25	0.51
cu	0.36	0.74
ep	0.16	0.31
Fr	0.24	0.77
gg	0.28	0.55
Gr	0.33	0.77
ma	0.45	0.75
me	0.14	0.51
mt	0.29	0.58
ne	0.17	0.54
rp	0.10	0.24
we	0.23	0.61
wp	0.30	0.69
ye	0.36	0.61
wild-type:		
PENT	0.62	0.83
SAKA	0.53	0.73
TEST	0.37	0.75

[a] Overall fitness = (hatch rate) × (larval viability) × (adult eclosion); values shown in Table 1.
[b] Post-hatch fitness = (larval viability) × (adult eclosion); values shown in Table 1.

Table 4.
One-way analysis of variance tables for puparium length (A) and width (B) of the *ep*, *rp*, and wild-type strains of the oriental fruit fly, *Bactrocera dorsalis*

A. One-way analysis of variance table for puparium length by strain

Source of variation	df	SS	MS	Fs [a]
Among strains	2	37.497	18.740	548.737***
Within strains	147	5.023	0.034	
Total	149	42.520		

B. One-way analysis of variance table for puparium width by strain

Source of variation	df	SS	MS	Fs [a]
Among strains	2	4.614	2.307	225.462***
Within strains	147	1.504	0.010	
Total	149	6.118		

[a] Significant at the 0.000 level.

Table 5.
Mean length and width of puparia from the *ep*, *rp* and wild-type strains of the oriental fruit fly, *Bactrocera dorsalis*

Strain	N	Mean length (mm)	(S.D.) [a]		Mean width (mm)	(S.D.) [b]	
elongate puparium	50	5.79	(0.23)	a	2.20	(0.01)	a
robust puparium	50	4.57	(0.13)	b	2.66	(0.01)	b
wild-type puparium	50	5.06	(0.18)	c	2.36	(0.01)	c

[a] Scheffe's multiple range analysis indicates that mean length values designated by different letters are significantly different ($P = 0.05$).
[b] Scheffe's multiple range analysis indicates that mean width values designated by different letters are significantly different ($P = 0.05$).

Table 6.
One-way analysis of variance tables for wing length (A.) and width (B.) of the *sw* and wild-type strains of the oriental fruit fly, *Bactrocera dorsalis*

A. One-way analysis of variance table for wing length by strain

Source of variation	df	SS	MS	Fs [a]
Among strains	1	14957.29	14957.29	960.04***
Within strains	98	1526.82	15.58	
Total	99	16484.11		

B. One-way analysis of variance table for wing width by strain

Source of variation	df	SS	MS	Fs [a]
Among strains	1	84.64	84.64	29.69***
Within strains	98	279.36	2.85	
Total	99	364.0		

[a] Significant at the 0.000 level.

Table 7.
Mean length and width of wings from the *sw* and wild-type strains of the oriental fruit fly, *Bactrocera dorsalis*

Strain	N	Mean length (mm)	(S.D.) [a]		Mean width (mm)	(S.D.) [b]	
small wing	50	69.94	(4.51)	a	33.48	(1.98)	a
wild-type	50	94.40	(3.29)	b	35.32	(1.33)	b

[a] Scheffe's multiple range analysis indicates that mean length values designated by different letters are significantly different ($P = 0.05$).
[b] Scheffe's multiple range analysis indicates that mean width values designated by different letters are significantly different ($P = 0.05$).

Table 8.
Progeny numbers from crosses involving wild-type, *ch*, *gg*, *ma*, *we*, and *ye* alleles in the oriental fruit fly, *Bactrocera dorsalis*

Mating type (male x female)	No. pairs	No. progeny				X^2 (3:1) [b]
		wild-type		aa [a]		
		males	females	males	females	
1) wild-type x *ch*	9					
2) *ch* x wild-type	10	286	239	0	0	
F_1 from cross 1	mass-mated	235	247	93	79	0.587 NS
F_1 from cross 2	mass-mated	474	477	116	136	10.53***
4) wild-type x *gg*	13	146	146	0	0	
5) *gg* x wild-type	12	210	192	0	0	
F_1 from cross 5	mass-mated	1704	1533	569	579	3.20 NS
F_1 from cross 6	mass-mated	893	800	288	249	1.0 NS
6) wild-type x *ma*	13	260	280	0	0	
7) *ma* x wild-type	10	102	100	0	0	
F_1 from cross 7	mass-mated	848	881	289	277	0.14 NS
F_1 from cross 8	mass-mated	360	360	130	129	1.107 NS
8) wild-type x *we*	5	111	102	0	0	
9) *we* x wild-type	4	187	173	0	0	
F_1 from cross 9	mass-mated	299	340	111	110	0.223 NS
F_1 from cross 10	mass-mated	724	793	229	246	1.414 NS
10) wild-type x *ye*	8	76	67	0	0	
11) *ye* x wild-type	8	177	145	0	0	
F_1 from cross 11	mass-mated	428	437	153	150	0.55 NS
F_1 from cross 12	mass-mated	477	470	144	161	0.27 NS

[a] aa is used as a symbol for the phenotype of the mutant allele genotype used in each cross.
[b] NS, Not Significant; ***, $P \leq 0.001$.

Table 9.
Progeny numbers from crosses involving the wild-type, *cu*, and *ye* alleles of the oriental fruit fly, *Bactrocera dorsalis*

Mating types (male x female)	F_1 phenotypes	Phenotypes of the F_2 progeny [a]			X^2	(ratio) [b]
		wild-type	yellow eye	copper		
1) wild-type x *cu*	wild-type	468	134	42	2.178 NS	(12:3:1)
2) *cu* x wild-type	wild-type	157	40	16	0.595 NS	(12:3:1)
3) *cu* x *ye*	yellow eye	0	1777	616	0.703 NS	(3:1)
4) *ye* x *cu*	yellow eye	0	757	250	0.016 NS	(3:1)

[a] Ten pairs of flies were used for each parental cross. The F_1 generations were mass-mated to form the F_2. Counts from male and female flies did not show any significant differences and are pooled in this table.
[b] NS, Not Significant.

Table 10.
Progeny numbers from crosses involving the wild-type, *Az*, *Fr* and *Gr* alleles of the oriental fruit fly, *Bactrocera dorsalis*

Mating types (male x female)	F_1 phenotypes (aa) [a]	Phenotypes of the F_2 progeny [b]			X^2	(ratio) [c]
		wild-type	aa / wild-type	aa		
1) wild-type x *Az*	aa / wild-type	660	1148	348		(1:2:0.5)
2) *Az* x wild-type	aa / wild-type	422	901	280		(1:2:0.5)
3) wild-type x *Fr*	aa / wild-type	89		174	10.96 ***	(1:3)
4) *Fr* x wild-type	aa / wild-type	429		708	98.42 ***	(1:3)
5) wild-type x *Gr*	aa / wild-type	140	430	212	21.04 ***	(1:2:1)
combined [d]		570		212	1.86 NS	(3:1)
6) *Gr* x wild-type	aa / wild-type	236	413	259	8.57 **	(1:2:1)
combined [d]		649		259	6.02 *	(3:1)

[a] aa is used as a symbol for the phenotype of the mutant allele genotype used in each cross. aa/wild-type is used as a symbol for the heterozygote phenotype which is intermediate between the wild-type and aa.

[b] Ten pairs of flies were used for each parental cross. The F_1 phenotype was wild-type in all crosses and the F_1 generations were mass-mated to form the F_2. Counts from male and female flies did not show any significant differences and are pooled in this table.

[c] *, $P \leq 0.05$; **, $P \leq 0.01$, ***, $P \leq 0.001$.

[d] Combined progeny numbers with wild-type and aa/wild-type phenotypes.

Table 11.
Progeny numbers from crosses involving the wild-type, *am*, *mt*, and *ne* alleles in the oriental fruit fly, *Bactrocera dorsalis*

Mating type (male x female)	No. pairs	No. progeny				X^2 (3:1) [b]
		wild-type		aa [a]		
		males	females	males	females	
1) wild-type x *am*	15	198	219	15	16	[c]
2) *am* x wild-type	5	92	107	7	10	[d]
F1 from cross 1	mass-mated	430	440	111	131	5.69 *
F1 from cross 2	mass-mated	201	210	58	73	0.20 NS
3) wild-type x *mt*	8	37	55	0	0	
4) *mt* x wild-type	8	40	36	0	0	
F1 from cross 3	mass-mated	338	313	99	99	1.28 NS
F1 from cross 4	mass-mated	247	235	82	58	2.06 NS
5) wild-type x *ne*	7	58	57	0	0	
6) *ne* x wild-type	5	99	95	0	0	
F1 from cross 5	mass-mated	253	213	61	76	1.672 NS
F1 from cross 6	mass-mated	173	174	56	60	0.005 NS

[a] aa is used as a symbol for the phenotype of the mutant allele genotype used in each cross.
[b] NS, Not Significant; *, $P \leq 0.05$.
[c] *am*/*am* appeared in the F_1 of three of the single pairs.
[d] *am*/*am* appeared in the F_1 of one of the single pairs.

Table 12.
Progeny numbers from crosses involving wild-type, *ep*, *me*, *rp*, and *wp* alleles of the oriental fruit fly, *Bactrocera dorsalis*

Mating type (male x female)	No. pairs	No. progeny				X^2 (3:1) [b]
		wild-type		aa [a]		
		male	female	male	female	
1) wild-type x *ep*	7	69	74	0	0	
2) *ep* x wild-type	9	168	166	0	0	
F_1 adults from cross 1	mass-mated	695	693	216	210	2.22 NS
F_1 adults from cross 2	mass-mated	651	630	189	177	6.77 *
F_1 pupae from cross 1	mass-mated	2058		663		0.583 NS
F_1 pupae from cross 2	mass-mated	1724		584		0.113 NS
3) wild-type x *me*	5	58	52	0	0	
4) *me* x wild-type	5	56	57	0	0	
F_1 adults from cross 3	mass-mated	371	382	112	132	0.148 NS
F_1 adults from cross 4	mass-mated	168	217	87	82	8.9 *
F_1 pupae from cross 3	mass-mated	274		81		0.91 NS
F_1 pupae from cross 4	mass-mated	296		96		0.06 NS
5) wild-type x *rp*	9	208	243	0	0	
6) *rp* x wild-type	7	40	54	0	0	
F_1 adults from cross 5	mass-mated	311	299	50	61	35.47 ***
F_1 adults from cross 6	mass-mated	115	138	35	42	0.489 NS
F_1 pupae from cross 5	mass-mated	838		253		1.907 NS
F_1 pupae from cross 6	mass-mated	442		172		2.973 NS
7) wild-type x *wp*	4	35	24	0	0	
8) *wp* x wild-type	4	73	75	0	0	
F_1 adults from cross 7	mass-mated	133	143	53	45	0.29 NS
F_1 adults from cross 8	mass-mated	158	167	65	71	4.99 *

[a] aa is used as a symbol for the phenotype of the mutant allele genotype used in each cross.
[b] NS, Not Significant; *, $P \leq 0.05$, ***, $P \leq 0.001$.

Table 13.
Progeny numbers from crosses involving wild-type, *cd, cv, nw,* and *sw* alleles in the oriental fruit fly, *Bactrocera dorsalis*

Mating type (male x female)	No. pairs	No. progeny				X^2 (3:1) [b]
		wild-type		aa [a]		
		males	females	males	females	
1) wild-type x *cd*	4	108	114	0	0	
2) *cd* x wild-type	4	87	92	0	0	
F1 from cross 1	mass-mated	214	250	81	82	0.332 NS
F1 from cross 2	mass-mated	265	259	68	75	4.511 *
3) wild-type x *cv*	9	293	289	0	0	
4) *cv* x wild-type	9	281	270	0	0	
F1 from cross 3	mass-mated	575	459	107	178	8.097**
F1 from cross 4	mass-mated	564	433	136	199	0.123 NS
5) wild-type x *nw*	3					
6) *nw* x wild-type	4					
F1 from cross 5	mass-mated	197	185	29	33	28.84***
F1 from cross 6	mass-mated	360	321	21	27	131.8***
7) wild-type x *sw*	5	65	64	0	0	
8) *sw* x wild-type	3	42	38	0	0	
F1 from cross 7	mass-mated	623	696	197	185	5.87 *
F1 from cross 8	mass-mated	153	150	48	58	0.184 NS

[a] aa is used as a symbol for the phenotype of the mutant allele genotype used in each cross.
[b] NS, Not Significant; *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

Table 14.
Linkage data from crosses in the repulsion configuration involving the *am*, *ma*, *mt*, *we*, *wp*, and *ye* alleles in the oriental fruit fly, *Bactrocera dorsalis*

Parental mating (aa x bb) [a]	No. of F_2 progeny (phenotypes) [b] aa (b)	bb (c)	wild-type (a)	aa-bb (d)	X^2 [c]	(ratio)	L=ad/bc [d]	p [e]
1) *am* x *ma*	311	474	1322	98	51.13***	(3:3:9:1)	0.8789	0.480
2) *am* x *mt*	172	77	328	32	42.97***	(3:3:9:1)	0.7925	0.470
3) *am* x *we*	750	721	1488	0	0.67 NS	(1:1:2)		
4) *am* x *wp*	339	510	1046	0	51.34***	(1:1:2)		
5) *am* x *ye*	355	491	1104	0	0.38 NS	(3:4:9)		
6) *ma* x *mt*	421	317	900	0	29.23***	(1:1:2)		
7) *ma* x *we*	530	674	1684	0	5.61 NS	(3:4:9)		
8) *ma* x *wp*	433	450	1200	151	6.27 NS	(3:3:9:1)	0.9299	0.490
9) *ma* x *ye*	177	221	535	0	0.87 NS	(3:4:9)		
10) *mt* x *we*	526	593	1906	177	18.08***	(3:3:9:1)	1.0816	0.510
11) *mt* x *wp*	179	253	655	62	14.17***	(3:3:9:1)	0.8967	0.485
12) *we* x *wp*	812	813	1840	0	13.34***	(1:1:2)		
13) *we* x *ye*	310	250	659	0	3.14 NS	(4:3:9)		
14) *wp* x *ye*	98	119	279	40	6.41 NS	(3:3:9:1)	0.9570	0.495

[a] aa and bb are used as symbols for the phenotypes of the male and female parental genotypes used in each cross. aa-bb is used as the symbol for the phenotype of the double homozygote from each cross if it has a distinct appearance.

[b] Ten pairs of flies were used for each parental cross. The F_1 phenotype was wild-type in all crosses and the F_1 generations were mass-mated to form the F_2. Data from reciprocal crosses and counts from male and female flies did not show any significant differences and are pooled in this table.

[c] NS, Not Significant; ***, $P \leq 0.001$.

[d] L is the linkage intensity.

[e] p is the crossover value, expressed as a decimal fraction.

Table 15.
Linkage data from crosses in the coupling configuration involving the wild-type, *am*, *ep*, *ma*, *me*, *mt*, *rp*, *we*, *wp*, and *ye* alleles in the oriental fruit fly, *Bactrocera dorsalis*

Parental mating (wild-type x aa bb) [a]	No. F_2 progeny (phenotypes) [b] aa (b)	bb (c)	wild-type (a)	aa-bb (d)	X^2 [c]	(ratio)	L=bc/ad [d]	p [e]
1) wild-type x *am/am;ma/ma*	488	615	1709	209	19.33***	(3:3:9:1)	0.8022	0.470
2) wild-type x *am/am;mt/mt*	189	148	694	32	47.03***	(3:3:9:1)	1.2595	0.530
3) wild-type x *ep/ep;wp/wp*	948	965	2817	299	1.364 NS	(3:3:9:1)	1.0861	0.510
4) wild-type x *me/me;wp/wp*	623	707	2135	245	9.621 NS	(3:3:9:1)	0.8421	0.475
5) wild-type x *rp/rp;wp/wp*	677	767	2204	210	10.79*	(3:3:9:1)	1.1219	0.515
6) wild-type x *ye/ye;mt/mt*	404	240	1144	78	64.15***	(3:3:9:1)	1.0866	0.510

[a] aa and bb are used as symbols for the phenotypes of parental genotypes used in each cross. aa-bb is used as the symbol for the phenotype of the double homozygote from each cross if it has a distinct appearance.
[b] Ten pairs of flies were used for each parental cross. The F_1 phenotype was wild-type in all crosses and the F_1 generations were mass-mated to form the F_2. Data from reciprocal crosses and counts from male and female flies did not show any significant differences and are pooled in this table.
[c] NS, Not Significant; *, $P \leq 0.05$; ***, $P \leq 0.001$.
[d] L is the linakge intensity.
[e] p is the crossover value, expressed as a decimal fraction.

Table 16.
Linkage data from crosses in the coupling configuration involving the wild-type, *am, ep, ma, me, mt, rp, we,* and *wp* alleles in the oriental fruit fly, *Bactrocera dorsalis*

Parental mating (aa x bb) [a]	No. F_2 progeny (phenotypes) [b] aa (b)	bb (c)	wild-type (a)	aa-bb (d)	Map distance	(SD)	L=bc/ad [d]	p [e]
1) wild-type x *am/am wp/wp*	217	179	2406	518	23.9 m.u.	(8.32)	0.0312	0.135
2) wild-type x *ep/ep ma/ma*	155	299	2071	399	30.2 m.u.	(7.11)	0.0561	0.175
3) wild-type x *ep/ep me/me*	87	76	1363	413	15.5 m.u.	(6.83)	0.0117	0.085
4) wild-type x *ep/ep rp/rp*	112	196	2504	754	14.0 m.u.	(8.53)	0.0116	0.085
5) wild-type x *ma/ma me/me*	128	118	2491	710	14.1 m.u.	(5.16)	0.0085	0.075
6) wild-type x *ma/ma mt/mt*	299	73	2011	333	27.4 m.u.	(2.42)	0.0326	0.125
7) wild-type x *me/me mt/mt*	93	49	1718	485	11.02	(3.67)	0.0055	0.06
8) wild-type x *me/me rp/rp*	216	200	7870	2445	8.7 m.u.	(0.61)	0.0022	0.040
9) wild-type x *mt/mt rp/rp*	102	154	2416	384	19.6 m.u.	(4.76)	0.0169	0.105
10) wild-type x *we/we wp/wp*	160	153	1355	334	31.3 m.u.	(8.65)	0.0541	0.17

[a] aa and bb are used as symbols for the phenotypes of parental genotypes used in each cross. aa-bb is used as the symbol for the phenotype of the double homozygote from each cross if it has a distinct appearance.
[b] Ten pairs of flies were used for each parental cross. The F_1 phenotype was wild-type in all crosses and the F_1 generations were mass-mated to form the F_2. Data from reciprocal crosses and counts from male and female flies did not show any significant differences and are pooled in this table.
[c] L is the linkage intensity.
[d] p is the crossover percentage, expressed as a decimal fraction. The recombination frequency is equal to 2p.

Table 17.
Linkage data from backcrosses involving the wild-type, *ep*, *ma*, *me*, *we*, and *wp* alleles in the oriental fruit fly, *Bactrocera dorsalis*

Backcross mating [a]	No. pairs	No. of progeny (phenotypes) [b] aa	bb	wild-type	aa-bb	(map distance)
1) *me/me ma/ma* male x (wild-type male x *me/me ma/ma* female) female	3	13	25	159	144	11.1 m.u.
2) *me/me ma/ma* male x (*me/me ma/ma* male x wild-type female) female	3	12	17	120	77	12.8 m.u.
3) *ep/ep ma/ma* male x (wild-type male x *ep/ep ma/ma* female) female	2	45	52	157	132	24.7 mu.u
4) *ep/ep ma/ma* male x (*ep/ep ma/ma* male x wild-type female) female	5	152	211	588	475	25.4 m.u.
5) *we/we wp/wp* male x (wild-type male x *we/we wp/wp* female) female	8	322	304	885	808	27.0 m.u.
6) *we/we wp/wp* male x (*we/we wp/wp* male x wild-type female) female	7	81	83	186	189	30.4 m.u.

[a] aa and bb are used as symbols for the phenotypes of the genotypes used in each cross. aa-bb is used as a symbol for the double homozygote from each cross if it has a distinct appearance.
[b] NS, Not Significant.

Table 18.
Linkage data from crosses in the repulsion configuration involving the *ep*, *ma*, *me*, *mt*, *rp*, and *ye* alleles of the oriental fruit fly, *Bactrocera dorsalis*

Parental mating (aa x bb) [a]	No. of F_2 progeny (phenotypes) [b] aa (b)	bb (c)	wild-type (a)	aa-bb (d)	X^2 [c]	(ratio)	L=ad/bc [d]	p [e]
1) *ep* x *ma*	161	250	563	0	17.45***	(1:1:2)		
2) *ma* x *ep*	176	123	379	0	39.98***	(1:1:2)		
3) *ep* x *me*	278	213	518	0	13.66***	(1:1:2)		
4) *me* x *ep*	841	882	1939	0	9.10**	(1:1:2)		
5) *ep* x *mt*	79	64	196	0	9.61**	(1:1:2)		
6) *mt* x *ep*	173	241	691	0	77.81***	(1:1:2)		
7) *ep* x *rp*	164	139	353	0	5.12 NS	(1:1:2)		
8) *rp* x *ep*	313	310	655	0	0.81 NS	(1:1:2)		
9) *ep* x *ye*	161	251	834	67	44.65***	(3:3:9:1)	1.3827	0.545
10) *ye* x *ep*	198	141	591	32	28.07***	(3:3:9:1)	0.6774	0.445
11) *ma* x *rp*	46	31	98	0	5.09 NS	(1:1:2)		
12) *rp* x *ma*	132	174	331	0	6.52 *	(1:1:2)		
13) *me* x *mt*	876	761	2274	0	110.5***	(1:1:2)		
14) *mt* x *me*	386	469	1284	0	92.48***	(1:1:2)		
15) *me* x *rp*	552	446	1111	0	16.87***	(1:1:2)		
16) *mt* x *rp*	349	416	1103	0	65.97***	(1:1:2)		
17) *rp* x *mt*	360	386	955	0	26.48***	(1:1:2)		
18) *rp* x *ye*	300	476	1404	101	67.01***	(3:3:9:1)	0.993	0.500
10) *ye* x *ep*	141	122	510	42	14.49***	(3:3:9:1)	1.245	0.530

[a] aa and bb are used as symbols for the phenotypes of the genotypes used in each cross. aa-bb is used as the symbol for the phenotype of the double homozygote from each cross if it has a distinct appearance.

[b] Ten pairs of flies were used for each parental cross. The F1 phenotype was wild-type in all crosses and the F1 generations were mass-mated to form the F2. Counts from male and female flies did not show any significant differences and are pooled in this table.

[c] NS, Not Significant.; **, $P \leq 0.01$; ***, $P \leq 0.001$.

[d] L is the linkage intensity.

[e] p is the crossover value.

Table 19.
Linkage data from backcrosses involving the wild-type, *Gr*, *we*, *wp*, and *ye* alleles in the oriental fruit fly, *Bactrocera dorsalis*

Backcross mating [a]	No. pairs	No. of progeny (phenotypes) [b] aa	bb	wild-type	aa-bb	X^2	(ratio)
1) *ep/ep;wp/wp* male x (wild-type male x *ep/ep;wp/wp* female) female	9	52	53	53	62	1.19 NS	(1:1:1:1)
2) *ep/ep;wp/wp* male x (*ep/ep;wp/wp* female x wild-type male) female	5	122	124	114	110	1.12 NS	(1:1:1:1)
3) *Gr/Gr;we/we* male x (wild-type male x *Gr/Gr;we/we* female) female	8	246	256	243	233	1.1 NS	(1:1:1:1)
4) *Gr/Gr;we/we* male x (*Gr/Gr;we/we* male x wild-type female) female	7	659	704	696	686	1.68 NS	(1:1:1:1)
5) *Gr/Gr;ye/ye* male x (wild-type male x *Gr/Gr;ye/ye* female) female	12	114	111	98	104	1.45 NS	(1:1:1:1)

[a] aa and bb are used as symbols for the phenotypes of the genotypes used in each cross. aa-bb is used as a symbol for the double homozygote from each cross if it has a distinct appearance.
[b] NS, Not Significant.

Table 20.
Linkage data from crosses in the coupling configuration involving the wild-type, *Gr*, *ma*, and *ne* alleles of *Bactrocera dorsalis*

Parental mating	No. of F_2 progeny (phenotypes)[a, b]				X^2 [c]	(ratio)
	aa	bb	wild-type	aa-bb		
1) *Gr/Gr;ma/ma* male x wild-type female	415	385	1174	141	1.99 NS	(3:3:9:1)
2) Wild-type male x *Gr/Gr;ma/ma* female	50	69	196	24	3.87 NS	(3:3:9:1)
3) wild-type x *ne/ne;ma/ma*	388	412	1271	108	8.57 NS	(3:3:9:1)

[a] aa and bb are used as symbols for the phenotypes of the male and female parental genotypes used in each cross. aa-bb is used as the symbol for the phenotype of the double homozygote from each cross if it has a distinct appearance.
[b] Ten pairs of flies were used for each parental cross. The F_1 phenotype was intermediate in crosses 1 and 2 and wild-type in cross 3. The F_1 generations were mass-mated to form the F_2. Counts from male and female flies did not show any significant differences and are pooled in this table.
[c] NS, Not Significant.

Table 21.

Linkage data from crosses involving the *Az, am, Fr, Gr, ma,* and *wp* alleles of the oriental fruit fly, *Bactrocera dorsalis.*

Parental mating (AA x bb) [a]	No. F_2 progeny (phenotypes) [b]								
	$A^+A^+ b^+ -$	$AA^+ b^+-$	AA^+ bb	A^+A^+ bb	AA $b^+ -$	AA bb	X^2		(ratio)
1) *Gr* male x *wp* female	185	285	109	64	174	47	11.61	*	(3:6:2:1:3:1)
2) *Gr* female x *wp* male	475	749	233	121	441	129	26.77	***	(3:6:2:1:3:1)
3) *Gr* male x *am* female	117	902	0	301	472	54	>200	***	(3:7:0:2:3:1)

Table 21. (Continued)
Linkage data from crosses involving the *Az, am, Fr, Gr, ma,* and *wp* alleles of the oriental fruit fly, *Bactrocera dorsalis*.

Parental mating (AA x bb) [a]	No. F_2 progeny (phenotypes) [b]								
	$A^+A^+ b^+ -$	$AA^+ b^+ -$	AA^+ bb	A^+A^+ bb	AA $b^+ -$	AA bb	X^2		(ratio)
4) *Gr* female x *am* male	174	722	0	200	315	29	98.62	***	(3:7:2:3:1)
5) *Az* female x *ma* male	85	197	73	28	77	25	7.42	NS	(3:6:2:1:3:1)
6) *Az* male x *ma* female	221	532	196	71	163	73	78.6	***	(3:6:2:1:3:1)

[a] AA and bb are used as symbols for the phenotypes of the parental genotypes used in each cross.
[b] Ten pairs of flies were used for each parental cross. The F_1 phenotype was A/wild-type in all crosses and the F_1 generations were mass-mated to form the F_2. Counts from male and female flies did not show any significant differences and are pooled in this table.

Table 22.
Linkage data from crosses in the repulsion configuration involving the *Fr*, *Gr*, *ne*, and *sw* alleles of the oriental fruit fly, *Bactrocera dorsalis*

Parental mating (aa x bb) [a]	No. of F_2 progeny (phenotypes) [b] aa	bb	wild-type	aa-bb	X^2 [c]	(ratio)
1) *Fr* x *ne*	331	45	109	183	34.59***	(9:1:3:3)
2) *ne* x *Fr*	60	540	137	141	12.81***	(1:9:3:3)
3) *Gr* x *ne*	475	416	1472	36	101.7***	(3:3:9:1)
4) *ne* x *Gr*	222	210	624	53	4.81 NS	(3:3:9:1)
5) *Gr* x *sw*	336	306	628	0	6.31*	(1:1:2)
6) *sw* x *Gr*	520	654	1287	0	19.78***	(1:1:2)

[a] aa and bb are used as symbols for the phenotypes of the male and female parental genotypes used in each cross. aa-bb is used as the symbol for the phenotype of the double homozygote from each cross if it has a distinct appearance.
[b] Ten pairs of flies were used for each parental cross. The F_1 phenotype was Fr in crosses 1 and 2 and was Grape/intermediate in all other crosses. The F_1 generations were mass-mated to form the F_2. Counts from male and female flies did not show any significant differences and are pooled in this table.
[c] NS, Not Significant; *, $P \leq 0.05$; ***, $P \leq 0.001$.

Table 23.
Linkage data from crosses in the coupling configuration involving the wild-type, *gg*, *Gr*, *sw*, and *wp* alleles in the oriental fruit fly, *Bactrocera dorsalis*

Parental mating (aa x bb) [a]	No. F_2 progeny (phenotypes) [b] aa (b)	bb (c)	wild-type (a)	aa-bb (d)	Map distance	(SD)	L=bc/ad [d]	p [e]
1) wild-type x *gg/gg wp/wp*	32	47	1654	493	6.7 m.u.	(3.31)	0.0018	0.035
2) wild-type x *Gr/Gr sw/sw*	796	765	3994	795	43.7 m.u.	(13.6)	0.1918	0.29

[a] aa and bb are used as symbols for the phenotypes of parental genotypes used in each cross. aa-bb is used as the symbol for the phenotype of the double homozygote from each cross if it has a distinct appearance.
[b] Ten pairs of flies were used for each parental cross. The F_1 phenotype was wild-type in cross 1 and Grape/intermediate in cross 2. The F_1 generations were mass-mated to form the F_2. Data from reciprocal crosses and counts from male and female flies did not show any significant differences and are pooled in this table.
[c] L is the linkage intensity.
[d] p is the crossover percentage, expressed as a decimal fraction. The recombination frequency is equal to 2p.

Table 24.
Linkage data from crosses involving the *Fr* and *Gr* alleles of the oriental fruit fly, *Bactrocera dorsalis*.

Parental mating (AA x BB) [a]	No. F_2 progeny (phenotypes) [b]							
	$A^+A^+B^+B^+$	AA B_	A^+A^+B-	A^+A B-	$AA^+B^+B^+$	AA B^+B^+	X^2	(ratio)
Gr male x *Fr* female	98	203	184	551	194	143	77.75 ***	(1:3:3:6:2:1)
Gr female x *Fr* male	84	76	185	222	90	52	88.92 ***	(1:3:3:6:2:1)

[a] AA and BB are used as symbols for the phenotypes of the parental genotypes used in each cross.
[b] Ten pairs of flies were used for each parental cross. The F_1 phenotype was intermediate for the Grape trait and Furrow in all crosses and the F_1 generations were mass-mated to form the F_2. Counts from male and female flies did not show any significant differneces and are pooled in this table.

Table 25.
Linkage data from crosses involving the multiple marker strains *ep/ep;wp/wp;ye/ye* or *rp/rp;wp/wp;ye/ye* and the *ne*, *nw*, and *sw* alleles of the oriental fruit fly, *Bactrocera dorsalis*.

Parental mating (aa x bb;cc;dd) [a]	No. F_2 progeny (phenotypes) [b]															
	wild-type	aa	bb	cc	dd	aabb	aacc	aadd	bbcc	bbdd	ccdd	aabbcc	bbccdd	aabbdd	aaccdd	aabbccdd
nw x *ep;wp;ye*	156	38	17	48	63	5	9	4	6	5	23	2	1	0	4	1
sw x *ep;wp;ye*	519	185	126	230	194	43	84	62	39	54	54	16	18	10	18	3
ne x *rp;wp;ye*	521	102	105	120	167	22	19	34	38	23	31	2	6	2	8	0

[a] aa and bb;cc;dd are used as symbols for the phenotypes of the parental genotypes used in each cross.

[b] Ten pairs of flies were used for each parental cross. The F_1 phenotype was wild-type in all crosses and the F_1 generations were mass-mated to form the F_2. Data from reciprocal crosses and counts from male and female flies did not show any significant differneces and are pooled in this table.

Table 26.
Linkage data from crosses in the repulsion configuration involving the *cu*, *gg*, and *we* alleles of the oriental fruit fly, *Bactrocera dorsalis*

Parental mating (aa x bb) [a]	No. of F_2 progeny (phenotypes) [b]				X^2 [c]	(ratio)
	aa	bb	wild-type	*ye/ye*		
1) *cu* x *gg*	35	115	279	76	4.89 NS	(9:1:4:2)
2) *gg* x *cu*	79	36	263	53	12.46**	(9:1:4:2)
3) *cu* x *we*	82	271	647	126	4.15 NS	(9:1:4:2)
4) *we* x *cu*	168	33	322	90	6.0 NS	(9:1:4:2)

[a] aa and bb are used as symbols for the phenotypes of the male and female parental genotypes used in each cross. aa-bb is used as the symbol for the phenotype of the double homozygote from each cross if it has a distinct appearance.
[b] Ten pairs of flies were used for each parental cross. The F_1 phenotype was wild-type in all crosses and the F_1 generations were mass-mated to form the F_2. Counts from male and female flies did not show any significant differences and are pooled in this table.
[c] NS, Not Significant; *, $P \leq 0.05$; **, $P \leq 0.01$.

Table 27.
Linkage data from crosses involving the *cu* and *wp* alleles of the oriental fruit fly, *Bactrocera dorsalis*.

Parental mating (aa x bb) [a]	No. F_2 progeny (phenotypes) [b]								
	wild-type	wp	yellow eye	yellow, wp	copper	copper, wp	X^2		(ratio)
wp x *cu*	128	29	29	11	16	0	4.7	NS	(9:3:2:1:1:0)
cu x *wp*	349	119	84	50	42	0	3.74	NS	(9:3:2:1:1:0)

[a] aa and bb are used as symbols for the phenotypes of the male and female parental genotypes, respectively, used in each cross.
[b] Ten pairs of flies were used for each parental cross. The F_1 phenotype was wild-type in all crosses and the F_1 generations were mass-mated to form the F_2. Counts from male and female flies did not show any significant differences and are pooled in this table.

Table 28.
Linkage data from crosses in the repulsion configuration involving the *ch*, *cv*, *gg*, *ma*, *we*, *wp* and *ye* alleles of the oriental fruit fly, *Bactrocera dorsalis*

Parental mating (aa x bb) [a]	No. of F_2 progeny (phenotypes) [b] aa	bb	wild-type	aa-bb	X^2 [c]	(ratio)	
1) *ch* x *ma*	233	787	0	0	0.781 NS	(1:3)	[d]
2) *ma* x *ch*	857	272	0	0	0.496 NS	(3:1)	[d]
3) *gg* x *we*	54	53	94	0	0.85 NS	(1:1:2)	
4) *we* x *gg*	115	114	226	0	0.024 NS	(1:1:2)	
5) *gg* x *wp*	171	169	363	0	0.763 NS	(1:1:2)	
6) *wp* x *gg*	164	163	326	0	0.003 NS	(1:1:2)	
7) *cv* x *wp*	109	143	449	24	19.89***	(3:3:9:1)	
8) *cv* x *ma*	186	355	871	59	8.66*	(3:3:9:1)	
9) *ma* x *cv*	210	155	531	59	66.1***	(3:3:9:1)	
10) *cv* x *ye*	144	130	492	28	16.58***	(3:3:9:1)	
11) *ye* x *cv*	225	244	731	69	2.61 NS	(3:3:9:1)	

[a] aa and bb are used as symbols for the phenotypes of the male and female parental genotypes used in each cross. aa-bb is used as the symbol for the phenotype of the double homozygote from each cross if it has a distinct appearance.
[b] Ten pairs of flies were used for each parental cross. The F_1 phenotype was wild-type in all crosses and the F_1 generations were mass-mated to form the F_2. Counts from male and female flies did not show any significant differences and are pooled in this table.
[c] NS, Not Significant; *, $P \leq 0.05$; ***, $P \leq 0.001$.
[d] F1 phenotype was mandarin red.

Table 29.
Linkage data from crosses involving the wild-type, *Az*, and *ye* alleles of the oriental fruit fly, *Bactrocera dorsalis*.

Parental mating (AA x bb) [a]	No. F_2 progeny (phenotypes) [b]								
	$A^+A^+ b^+ -$	$AA^+ b^{+-}$	AA^+ bb	A^+A^+ bb	AA $b^+ -$	AA bb	X^2		(ratio)
Az/Az;ye/ye male x wild-type female	280	604	188	107	223	58	56.66	***	(3:6:3:1:2:1)
Wild-type female x *Az/Az;ye/ye* male	97	171	69	31	45	19	9.9	NS	(3:6:3:1:2:1)

[a] AA and bb are used as symbols for the phenotypes of the parental genotypes used in each cross.
[b] Ten pairs of flies were used for each parental cross. The F_1 phenotype was A/wild-type in all crosses and the F_1 generations were mass-mated to form the F_2. Counts from male and female flies did not show any significant differences and are pooled in this table.

Table 30.
Autosomal linkage groups of the oriental fruit fly, *Bactrocera dorsalis*

A	B	C	D	E
ch	*ye*	*am*	*Gr*	*ne*
ep		*cu*	*sw*	
ma		*gg*		
me		*we*		
mt		*wp*		
rp				

Table 31.
Summary of map distance determinations for linkage group A
in the oriental fruit fly, *Bactrocera dorsalis*

Genotypes (aa to bb) [a]	Type of cross	Map distance (SD) [b]	Map function (SD) [c]	X^2 aa	X^2 bb	X^2 Linkage	(df)
ep to *ma*	coupling	30.24 (7.11)	50.23 (22.01)	24.72	153.09	806.12	(12)
ep to *me*	coupling	15.46 (6.86)	19.34 (9.52)	21.18	26.69	1247.13	(11)
ep to *rp*	coupling	14.04 (8.53)	19.25 (11.07)	33.85	22.93	2195.99	(12)
ma to *me*	coupling	14.09 (5.14)	17.02 (7.06)	44.95	41.53	2196.20	(13)
ma to *mt*	coupling	26.72 (2.15)	38.43 (5.14)	169.86	14.99	635.69	(5)
me to *mt*	coupling	11.02 (3.67)	12.62 (4.66)	8.42	9.58	1519.9	(3)
me to *rp*	coupling	8.71 (0.61)	9.58 (0.74)	5.07	13.01	1646.33	(10)
mt to *rp*	coupling	19.59 (4.76)	25.48 (8.54)	100.29	150.81	966.41	(9)

[a] aa and bb are used as symbols for the phenotypes of the genotypes used in each class. aa-bb is used as a symbol for the double homozygote from each cross if it has a distinct appearance.
[b] Map distance is equal to the recombinant frequency times 100, one map unit is equal to 1% crossover.
[c] Map function is calculated from the formula: recombinant frequency = $0.5 (1 - e^{-m})$, where m is the mean recombination frequency, and e is the base of natural logarithms.

Table 32.
Summary of map distance determinations for the autosomal linkage groups C and D in the oriental fruit fly, *Bactrocera dorsalis*

Genotypes (aa to bb)[a]	Type of cross	Map distance (SD) [b]	Map function (SD) [c]	X^2 aa	X^2 bb	X^2 Linkage	(df)
LINKAGE GROUP C							
am to *wp*	coupling	23.35 (8.32)	34.33 (20.24)	44.43	52.04	975.21	(6)
gg to *wp*	coupling	6.66 (3.31)	7.27 (3.76)	17.29	27.27	1738.77	(6)
we to *wp*	coupling	31.2 (8.65)	54.63 (26.85)	41.67	27.29	772.09	(10)
LINKAGE GROUP D							
Gr to *sw*	coupling	43.17 (13.64)		9.01	8.62	739.17	(12)

[a] aa and bb are used as symbols for the phenotypes of the genotypes used in each class. aa-bb is used as a symbol for the double homozygote from each cross if it has a distinct appearance.
[b] Map distance is equal to the recombinant frequency times 100, one map unit is equal to 1% crossover.
[c] Map function is calculated from the formula: recombinant frequency = $0.5 (1 - e^{-m})$, where m is the mean recombination frequency, and e is the base of natural logarithms.

APPENDIX B. PLATES

Plate I. Scanning electron micrograph of the compound eye of the wild-type strain in the oriental fruit fly, *Bactrocera dorsalis*. (a) Frontal aspect of the head, (b) typical hexagonal corneal lenses in the dorsal region of eye, and (c) corneal lenses with a square appearance in the equatorial region of the eye.

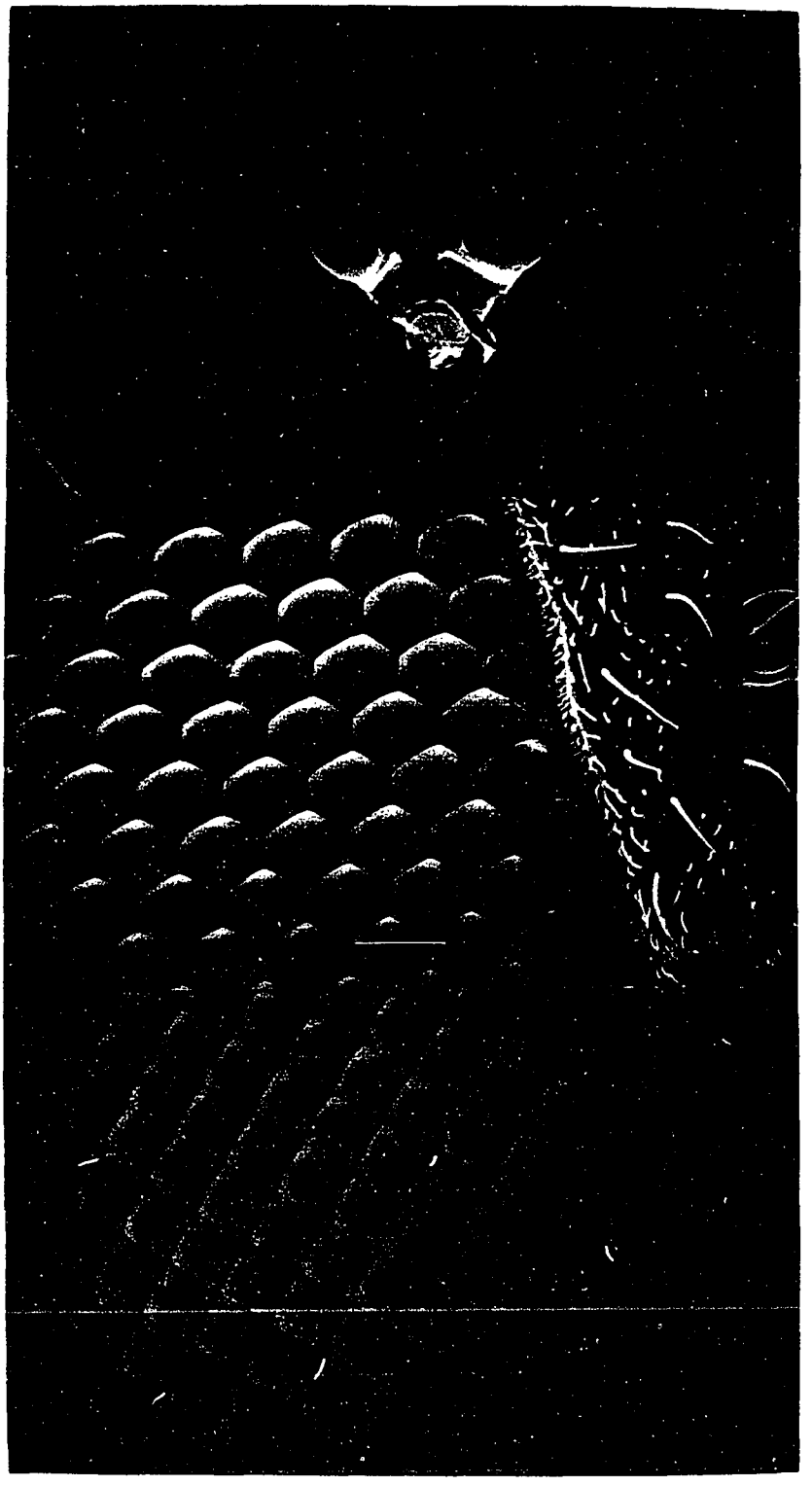


Plate II. Histological sections of the compound eye of the wild-type strain in the oriental fruit fly, *Bactrocera dorsalis*. (a) Unstained and (b) stained longitudinal sections of the peripheral retina, (c) primary pigment cells, (d) cross section of the longitudinal pigment cells at the level of retinula, and (e) longitudinal section at the level of the basement membrane. **lpc**, longitudinal pigment cell; **lpcn**, longitudinal pigment cell nuclei; **n8**, retinula cell 8 nucleus; **ppc**, primary pigment cell; **sc**, Semper cell; **scbpb**, Semper cell basal pigment body; **rc**, retinula cell; **rca**, retinula cell axons; **rcn**, retinula cell nuclei; and **rhc**, rhabdomere cap

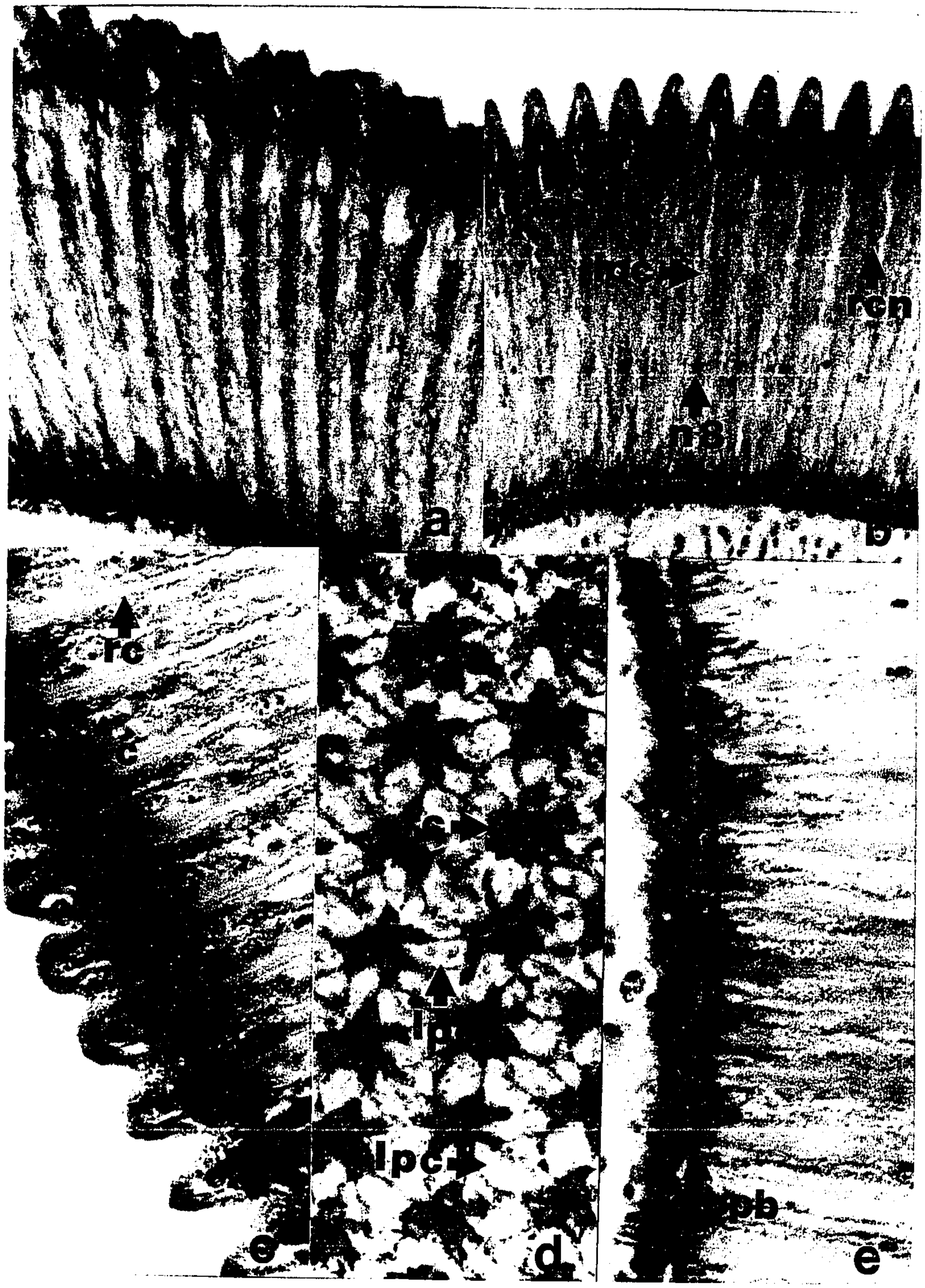

rcn
ns
a
b
rc
c
lp
lpc
pb
e
d
e

Plate III. Compound eye of (a) newly eclosed and (b) 7 d old adults of the wild-type strain in the oriental fruit fly, *Bactrocera dorsalis*.

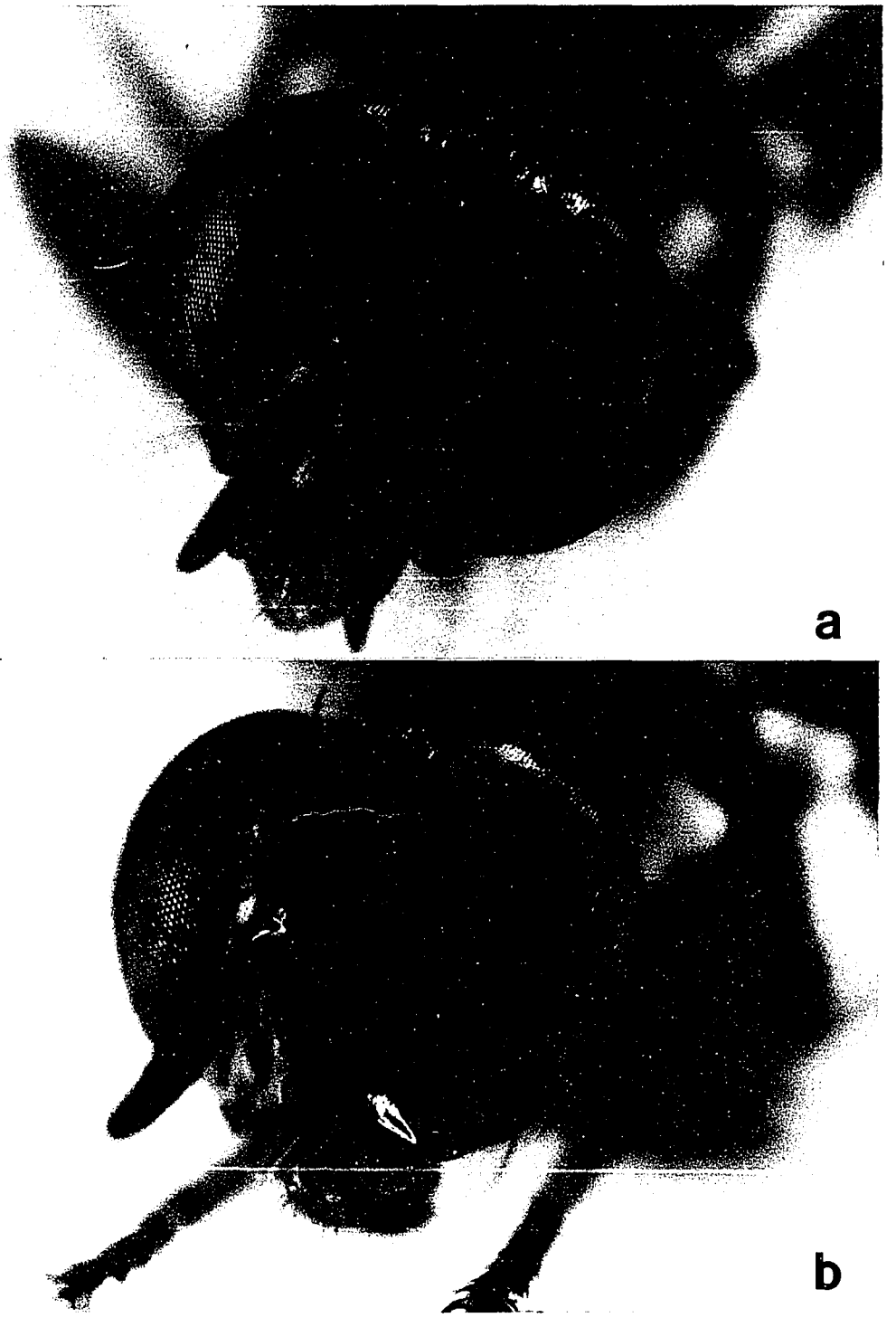

a
b

Plate IV. Compound eye of (a) newly eclosed and (b) 7 d old adults of the cherry strain in the oriental fruit fly, *Bactrocera dorsalis*.


a
b

Plate V. Compound eye of (a) newly eclosed and (b) 7 d old adults of the copper strain in the oriental fruit fly, *Bactrocera dorsalis*.

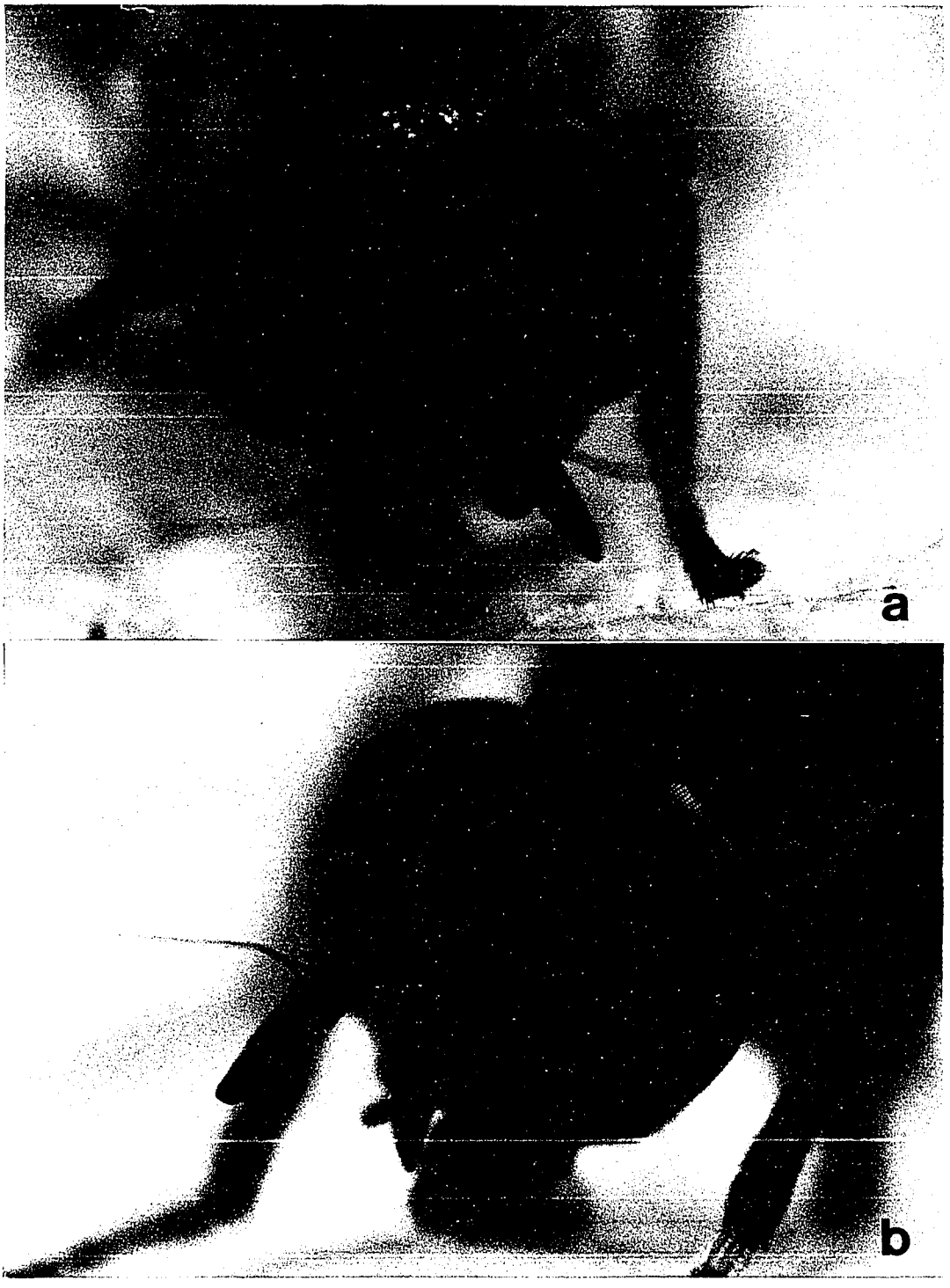

a
b

Plate VI. Histological sections of the compound eye of the copper strain in the oriental fruit fly, *Bactrocera dorsalis*. (a) Unstained and (b) stained longitudinal sections of the peripheral retina, (c) primary pigment cells, (d) cross section of the longitudinal pigment cells at the level of retinula, and (e) longitudinal section at the level of the basement membrane.

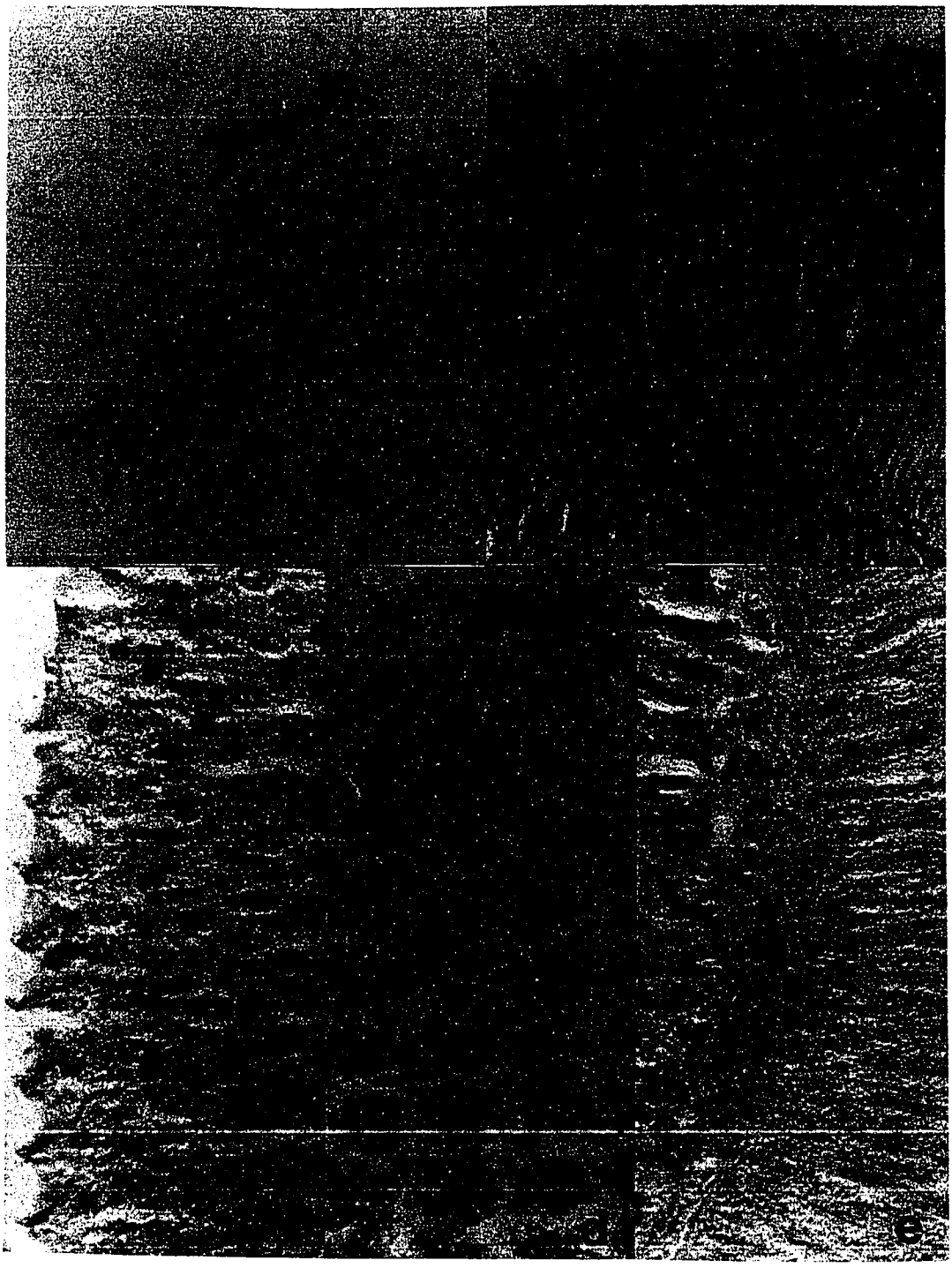


Plate VII. Compound eye of the gold-grey strain in the oriental fruit fly, *Bactrocera dorsalis*.

Plate VIII. Scanning electron micrographs of the compound eye of the gold-grey strain in the oriental fruit fly, *Bactrocera dorsalis*. (a) Ommatidia at frontomedial and (b) dorsal areas of the compound eye.

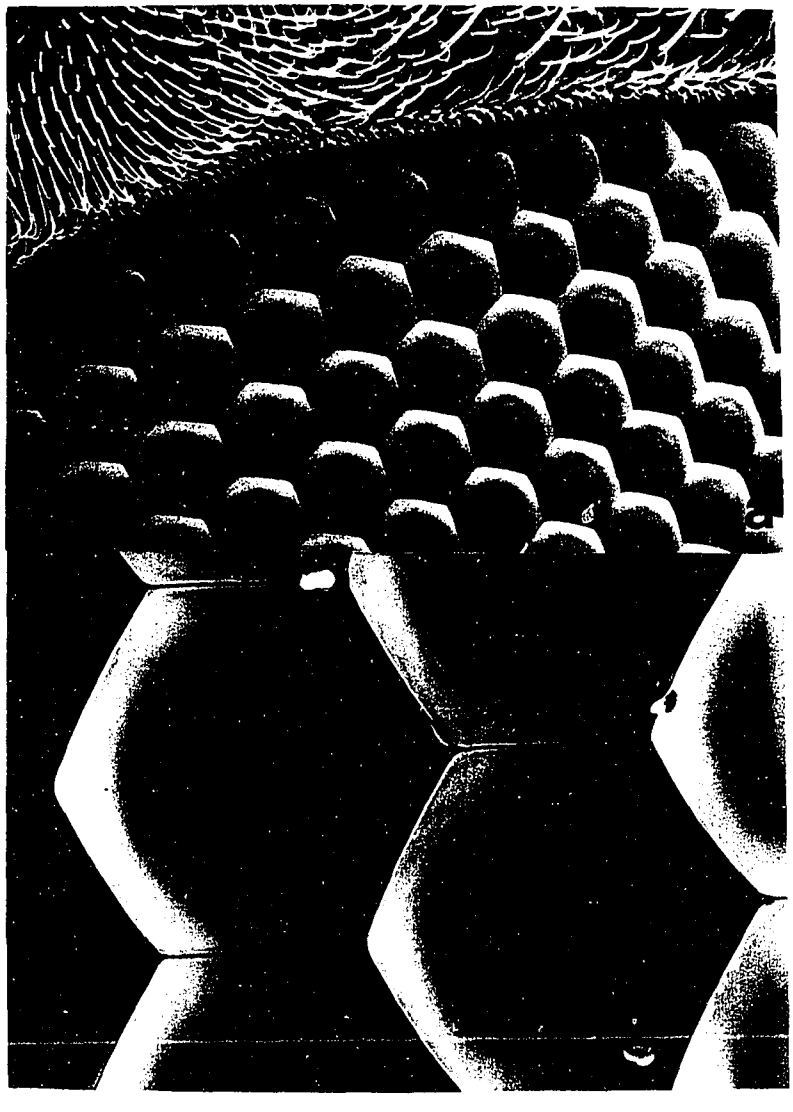


Plate IX. Histological sections of the compound eye of the gold-grey strain in the oriental fruit fly, *Bactrocera dorsalis*. (a) Unstained and (b) stained longitudinal sections of the peripheral retina, (c) primary pigment cells, (d) cross section of the longitudinal pigment cells at the level of retinula, and (e) longitudinal section at the level of the basement membrane.

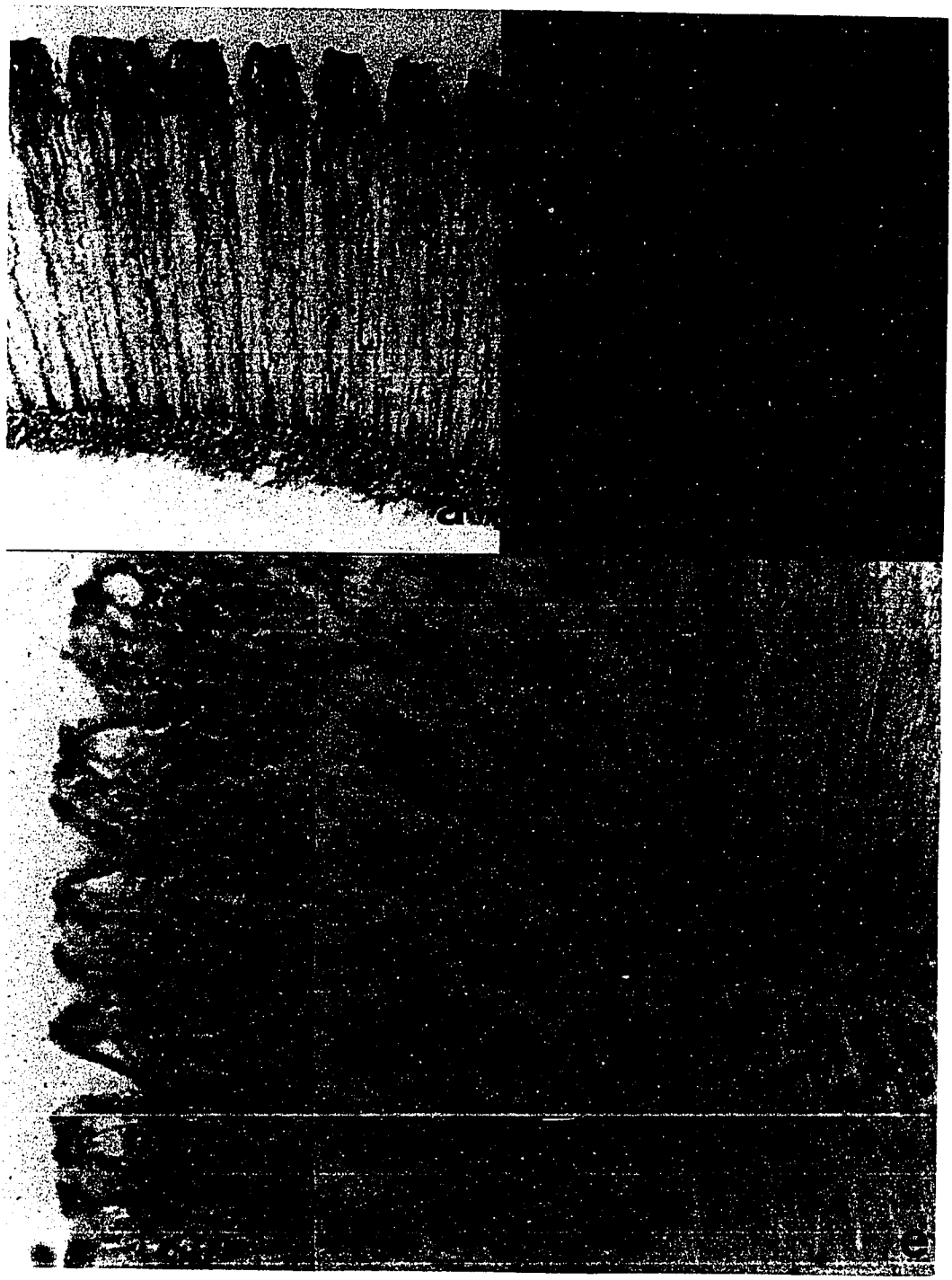


Plate X. The compound eye of the Grape strain in the oriental fruit fly, *Bactrocera dorsalis*.

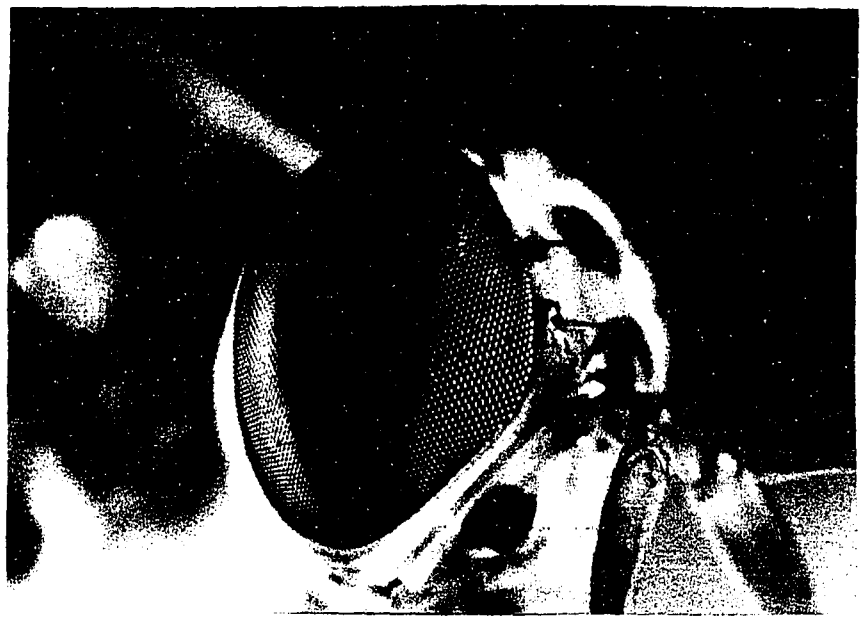


Plate XI. Histological sections of the compound eye of the Grape strain in the oriental fruit fly, *Bactrocera dorsalis*. (a) Unstained and (b) stained longitudinal sections of the peripheral retina, (c) primary pigment cells, (d) cross section of the longitudinal pigment cells at the level of retinula, and (e) longitudinal section at the level of the basement membrane.


e

Plate XII. The compound eye of the mandarin red strain in the oriental fruit fly, *Bactrocera dorsalis*.

Plate XIII. Histological sections of the compound eye of the mandarin red strain in the oriental fruit fly, *Bactrocera dorsalis*. (a) Unstained and (b) stained longitudinal sections of the peripheral retina, (c) primary pigment cells, (d) cross section of the longitudinal pigment cells at the level of retinula, and (e) longitudinal section at the level of the basement membrane.

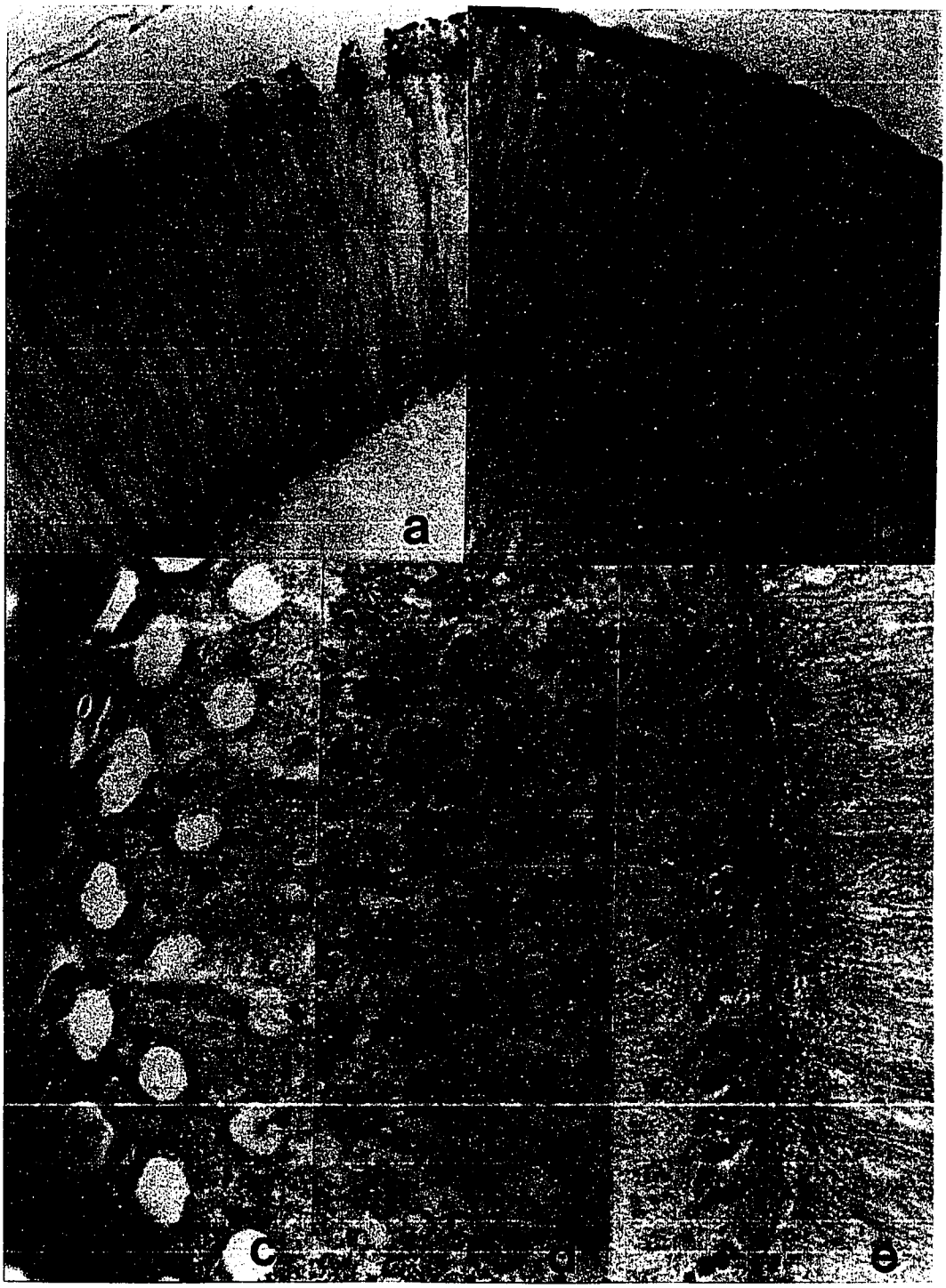

a
c
e

Plate XIV. The compound eye of the white eye strain in the oriental fruit fly, *Bactrocera dorsalis*.

Plate XV. Histological sections of the compound eye of the white eye strain in the oriental fruit fly, *Bactrocera dorsalis*. (a) Stained longitudinal sections of the peripheral retina, (b) primary pigment cells, (c) cross section of the longitudinal pigment cells at the level of retinula, and (d) longitudinal section at the level of the basement membrane.


a
b
c

Plate XVI. The compound eye of the yellow eye strain in the oriental fruit fly, *Bactrocera dorsalis*.

Plate XVII. Histological sections of the compound eye of the yellow eye strain in the oriental fruit fly, *Bactrocera dorsalis*. (a) Unstained and (b) stained longitudinal sections of the peripheral retina, (c) primary pigment cells, (d) cross section of the longitudinal pigment cells at the level of retinula, and (e) longitudinal section at the level of the basement membrane.


b
c
d
e

Plate XVIII. Scanning electron micrographs of the compound eye of (a) wild-type, (b) amethyst, and (c) matte strains in the oriental fruit fly, *Bactrocera dorsalis*.

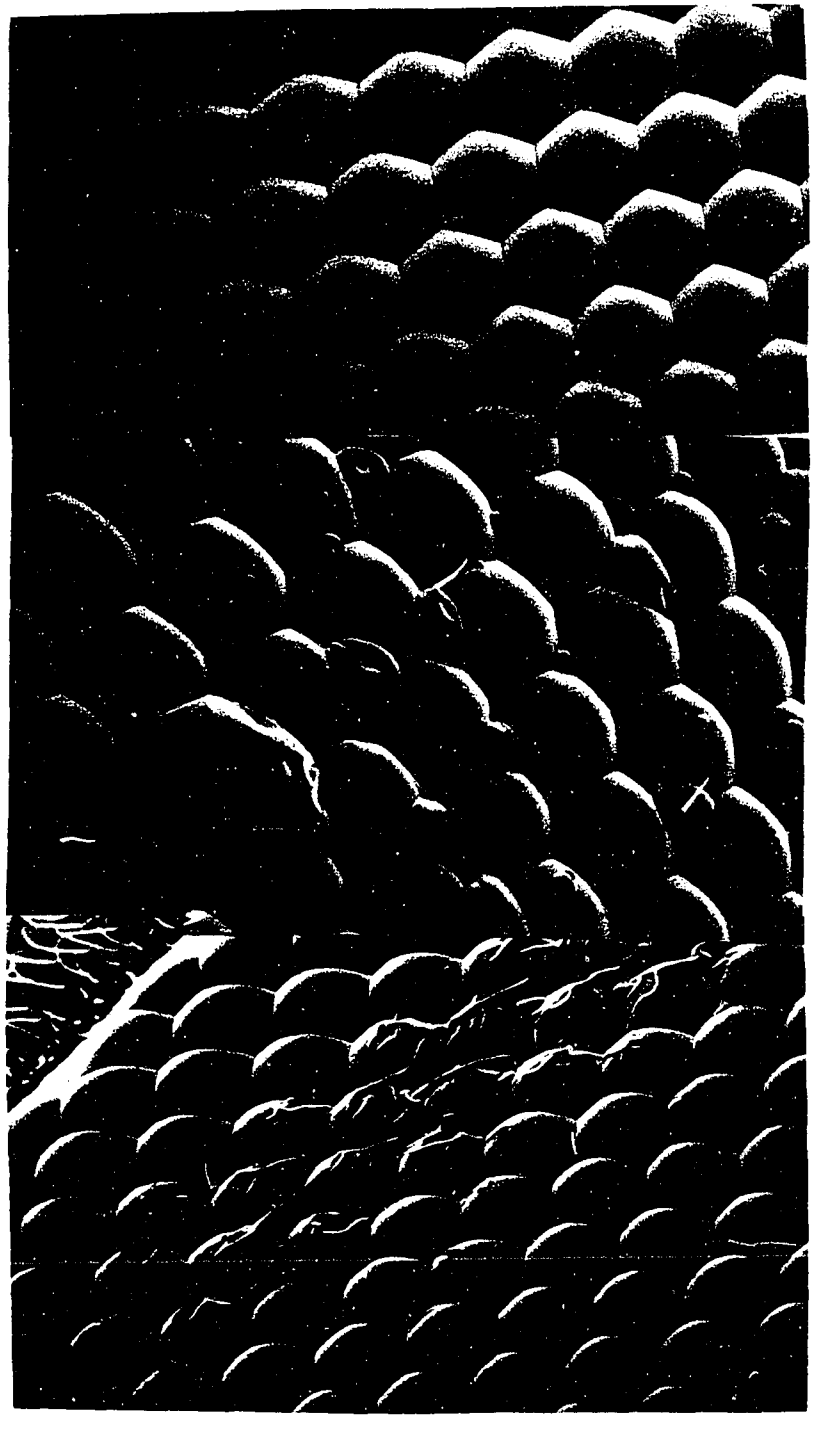


Plate XIX. The compound eye of the amethyst strain in the oriental fruit fly, *Bactrocera dorsalis*.

Plate XX. The compound eye of the Azure strain in the oriental fruit fly, *Bactrocera dorsalis*.

Plate XXI. Scanning electron micrographs of the compound eye of the (a) Azure and (b) Furrow strains in the oriental fruit fly, *Bactrocera dorsalis*.

Plate XXII. The compound eye of the Furrow strain in the oriental fruit fly, *Bactrocera dorsalis*.

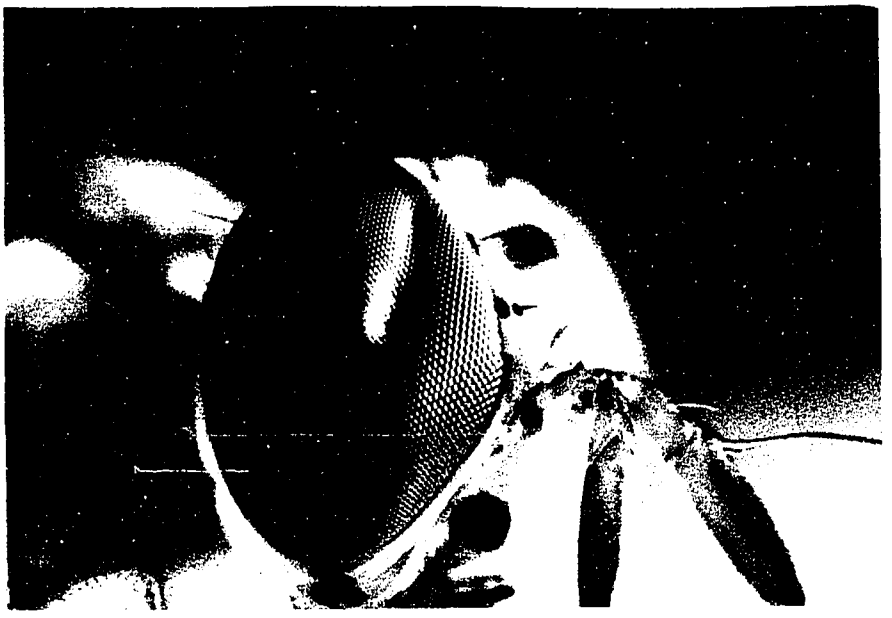


Plate XXIII. Longitudinal sections of the peripheral retina of the (a) wild-type, (b) Furrow, and (c) notched eye strains in the oriental fruit fly, *Bactrocera dorsalis*.

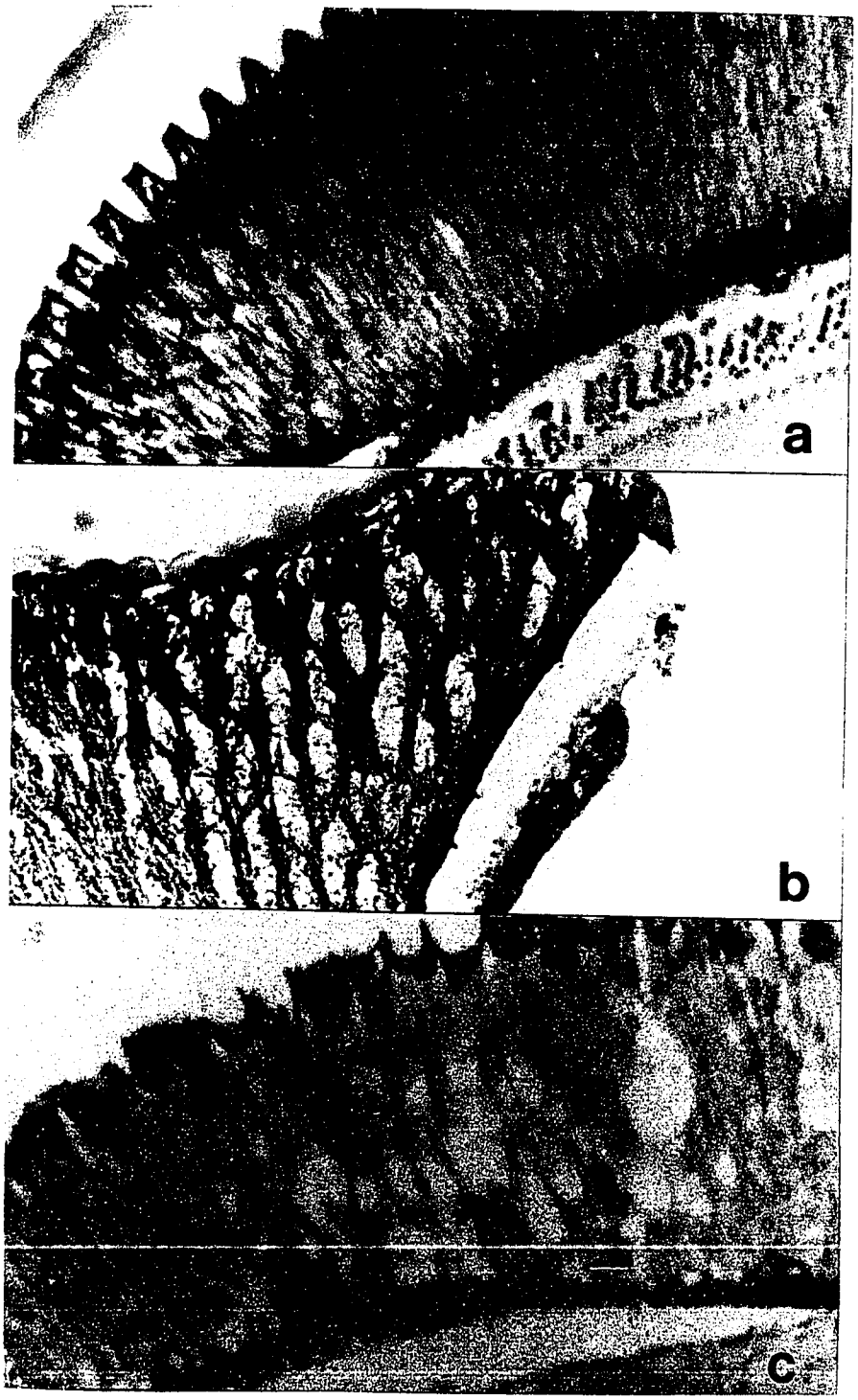

a
b
c

Plate XXIV. (a) moderate and (b) extreme expression of the matte trait in the oriental fruit fly, *Bactrocera dorsalis* (matte area at arrow).


b

Plate XXV. The compound eye of the notched eye strain in the oriental fruit fly, *Bactrocera dorsalis*.

Plate XXVI. Scanning electron micrographs of (a) moderate and (b) extreme expression of the notched eye trait in the oriental fruit fly, *Bactrocera dorsalis*.

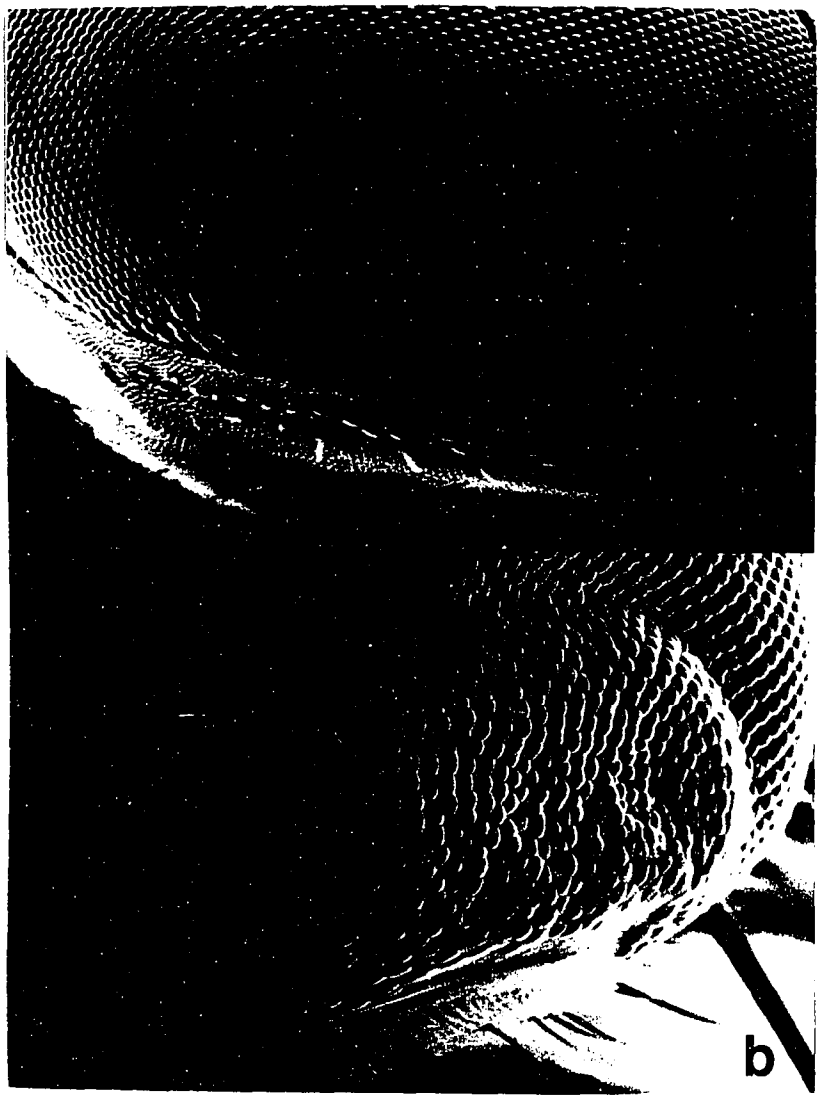

b

Plate XXVII. Puparia of the melanistic, white puparium, and wild-type strains (left to right) in the oriental fruit fly, *Bactrocera dorsalis*.

Plate XXVIII. Puparia of the elongate puparium, elongate puparium/robust puparium double homozygote, wild-type, and robust puparium strains (left to right) in the oriental fruit fly, *Bactrocera dorsalis*.

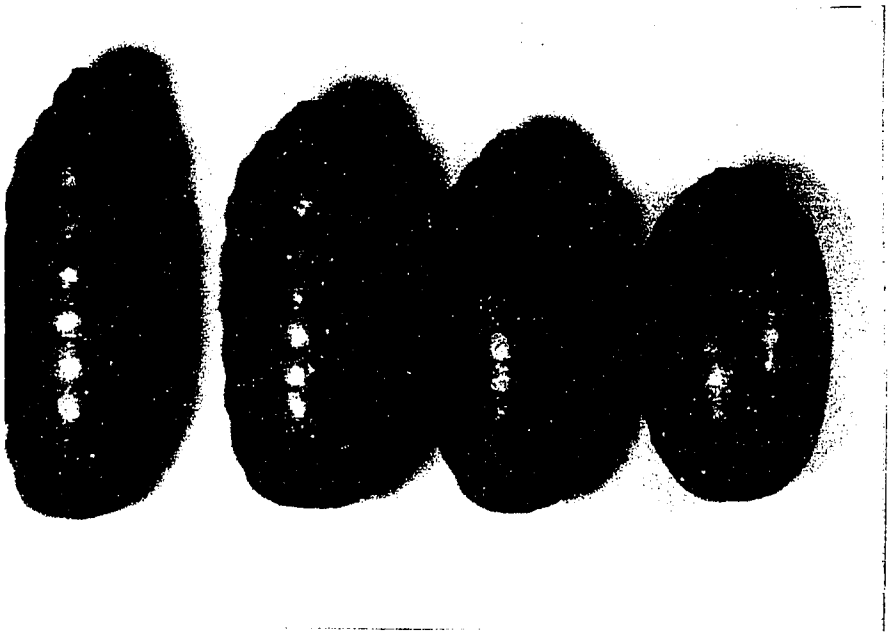


Plate XXIX. Wing of the wild-type strain in the oriental fruit fly, *Bactrocera dorsalis*. **c**, costal vein; **bc**, basal crossvein; and **M**, media cell

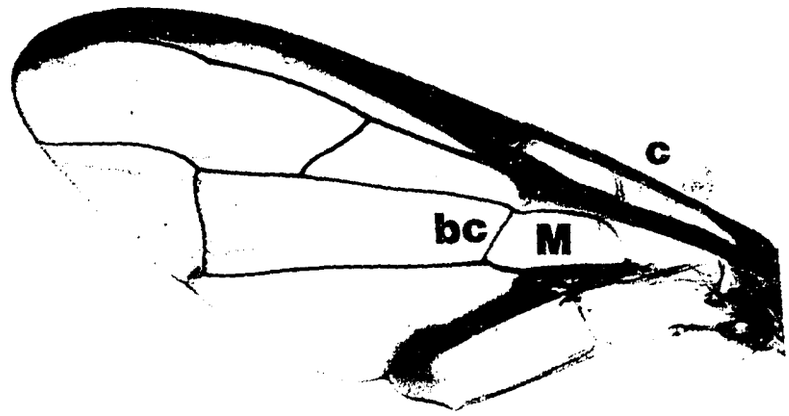

C
bc
M

Plate XXX. (a) moderate and (b) extreme expression of the crossveinless trait in the oriental fruit fly, *Bactrocera dorsalis*. **bc**, basal crossvein

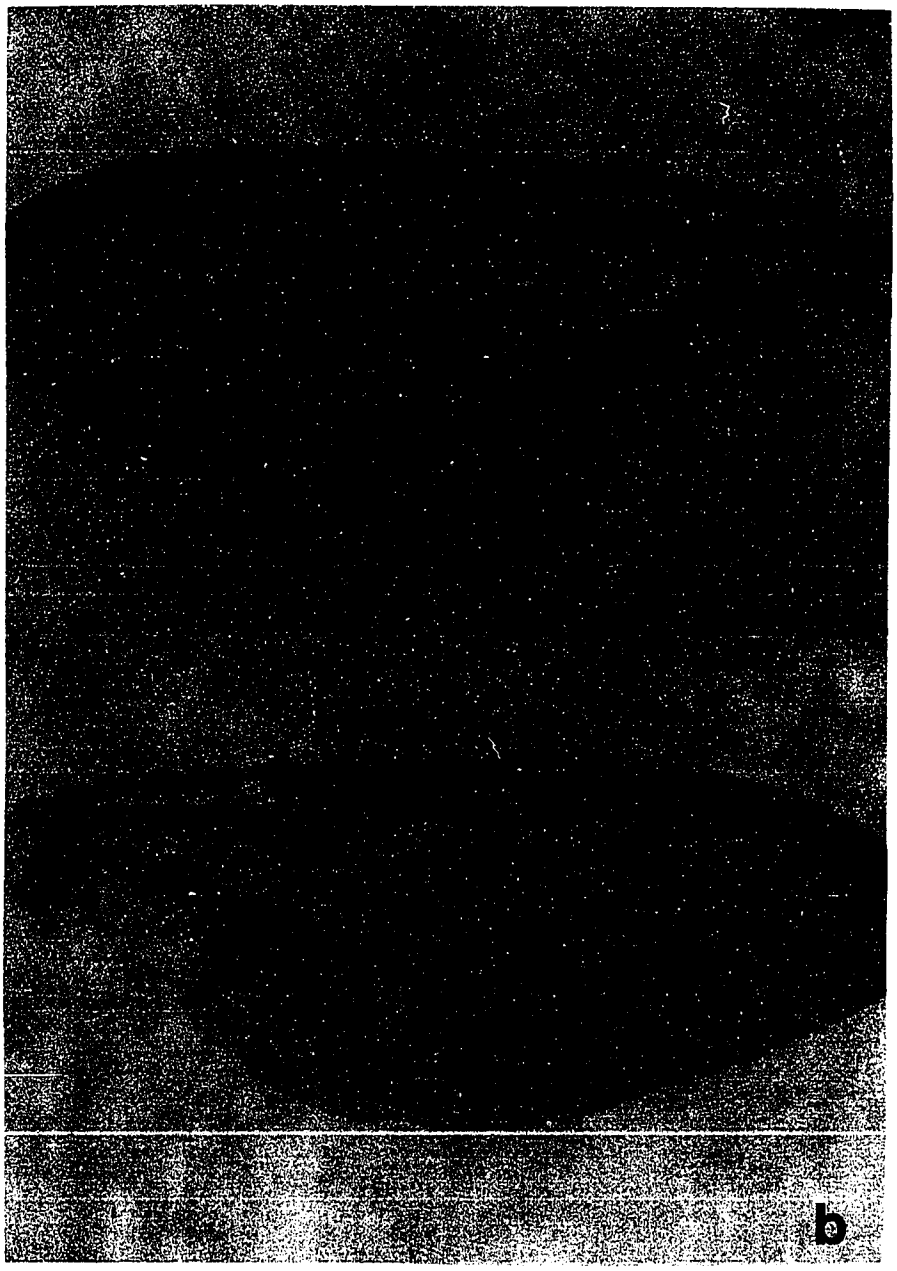

b

Plate XXXI. Wing of the curled strain in the oriental fruit fly, *Bactrocera dorsalis*.

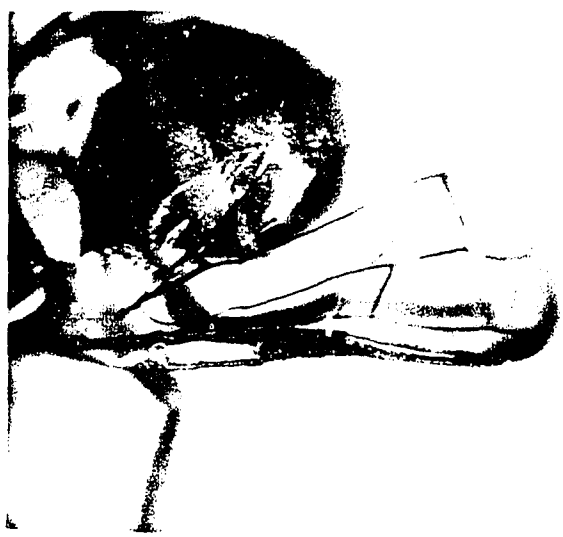


Plate XXXII. Scanning electron micrographs of the compound eye of the curled strain in the oriental fruit fly, *Bactrocera dorsalis*. (a) low magnification of the dorsal surface of the eye and (b) high magnification of the disrupted facets in the dorsal area.

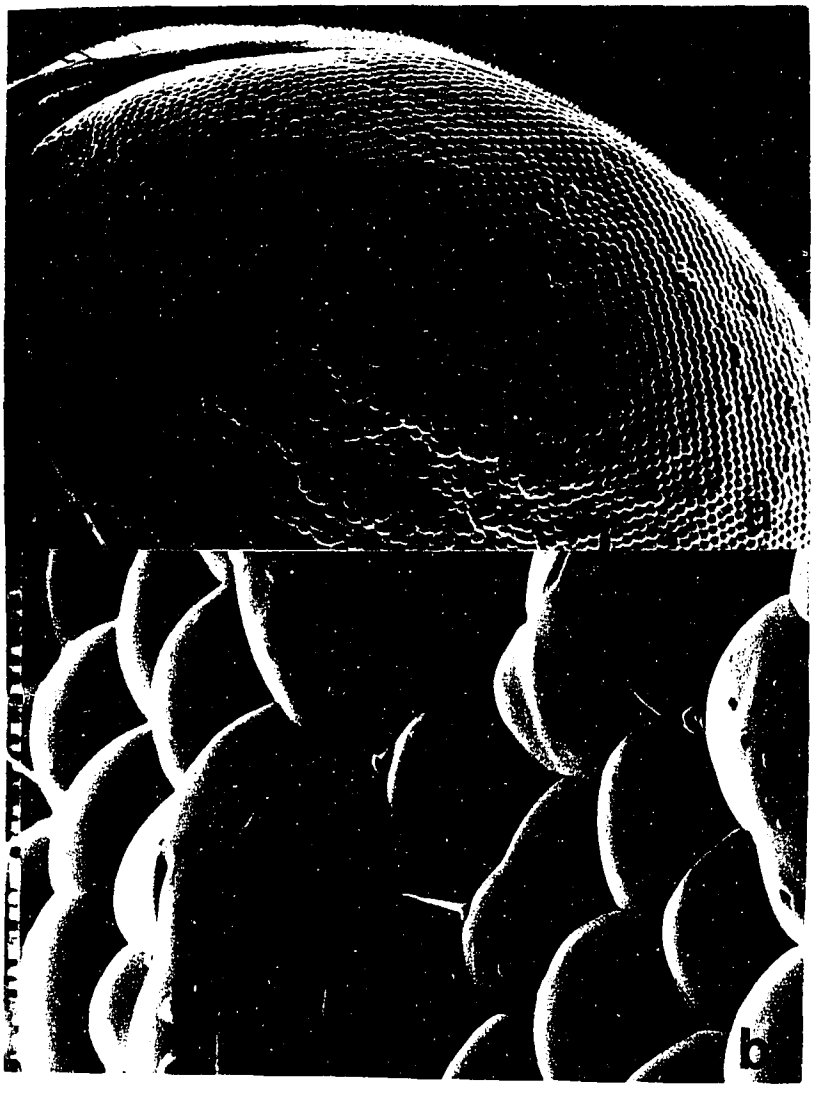
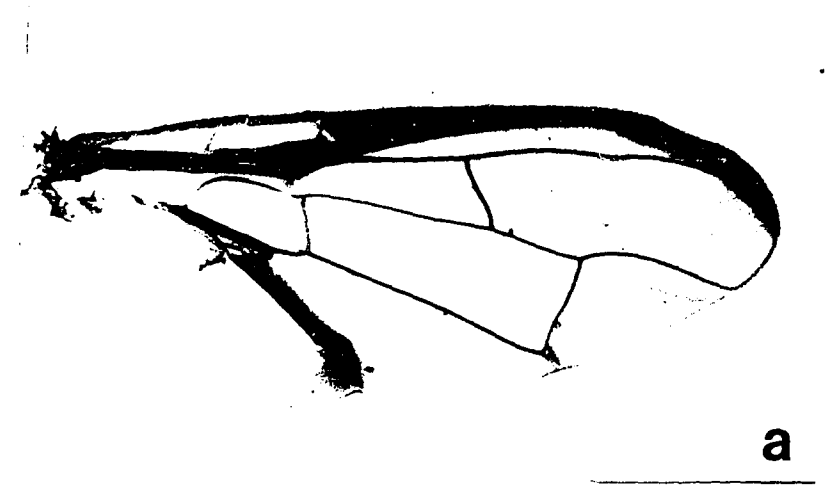
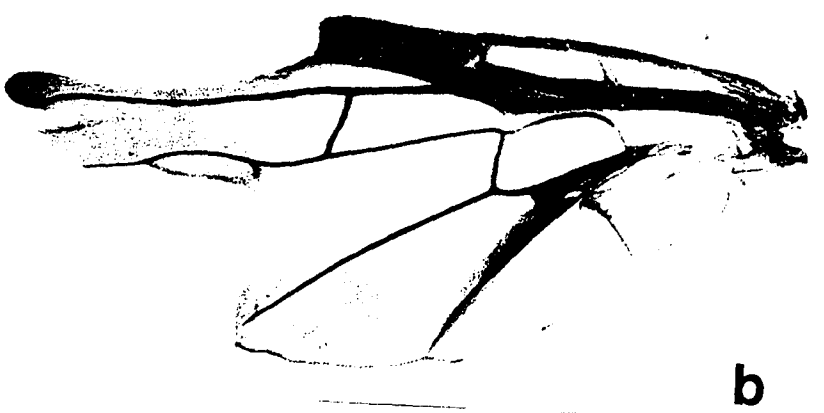


Plate XXXIII. (a) low and (b) extreme expression of the notch wing trait in the oriental fruit fly, *Bactrocera dorsalis*.



a



b

Plate XXXIV. Wing of the small wing strain in the oriental fruit fly, *Bactrocera dorsalis*. (a) entire wing and (b) indented costal margin at the first costal cell. **c**, costal vein and **M**, media cell

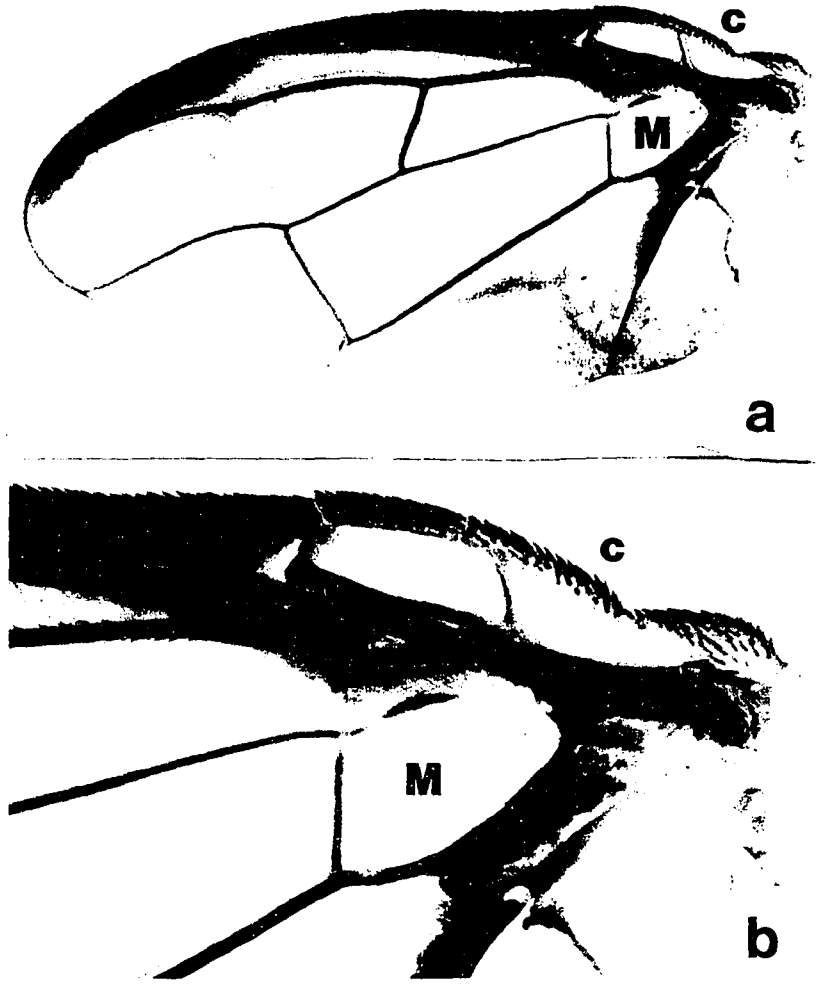
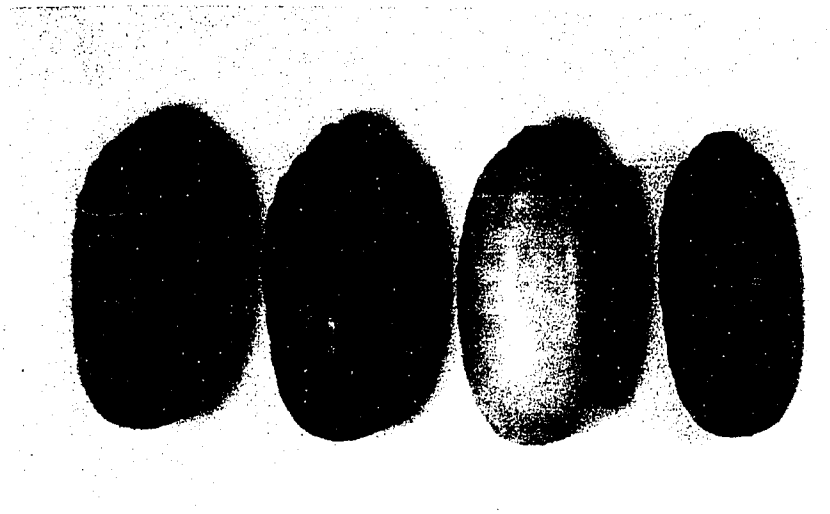

c
M
a
c
M
b

Plate XXXV. Puparia of the melanistic, melanistic/white puparium double homozygote, white puparium, and wild-type strains (left to right) in the oriental fruit fly, *Bactrocera dorsalis*.



APPENDIX C: HISTOLOGICAL METHODS

Fixation

Carnoy's solution

100% ethanol	60 ml
chloroform	30 ml
acetic acid	10 ml

Fix tissues for 2 hr in Carnoy's solution.

Dehydration

Step	1	2	3	4	5	6	7	8
dH_2O	30	15	30	15	5	0	0	0
Ethanol	70	85	50	50	40	25	0	0
n-butanol	0	0	20	35	55	75	100	100
Time (hr)	1	12	1	12	1	1	1	

Infiltration

At Step 11, transfer the n-butanol and the dehydrated tissue to a glass vial containing a approximately 2 ml of solid Paraplast Plus. Place in a vaccum oven at 56°C for 16 hr. Change tissues to liquid Paraplast Plus and place in oven at 56°C for 4 hr.

Imbedding

Pour liquid Paraplast Plus (56°C) into molds to a depth of 2 cm and quickly arrange tissues in mold with heated forceps. Allow a film of solidified paraffin to form over the surface and then submerge molded paraffin in cold water to ensure even cooling. Remove from molds and store in sealed plastic bags until sectioning.

Sectioning

Trim molded paraffin to approximately 0.5 cm^2 with a scalpel so that the sides are straight and flat. Affix block to stub with paraffin and attach to Histocut (Reichert) rotary microtome. Section tissue at 7 μm, collecting paraffin ribbons on a clean sheet of paper.

Spread and mount sections

Cut paraffin ribbons into 3-4 cm sections. Place onto the surface of water (37°C) for 1-2 minutes. Pick up the flattened section on a glass microscope slide subbed with albumin. Carefully remove excess water with a Kimwipe and place slide on slide warmer at 37°C. Hold for 12-18 hr.

Staining

Step	Solution	Time (minutes)
1.	xylene	3
2.	100% ethanol	3
3.	50% ethanol	3
4.	Running water	5
5.	Hematoxylin	2
6.	Running water	5
7.	Acid alcohol	3
8.	50% ethanol	3
9.	95% ethanol	3
10.	Eosin	3
11.	95% ethanol	3
12.	95 % ethanol	3
13.	xylene	3
14.	Mount coverslips with Permount	

APPENDIX D: GENETIC MODELS

Model 1. Autosomal recessive gene

Generation	Genotypes
P	a/a x a^+/a^+
F1	a/a^+ x a/a^+
F2	1 a/a : 2 a/a^+ : 1 a^+/a^+

If a is recessive to a^+, then the F1 progeny will all be wild-type and the F2 will have two phenotypic classes in the ratio of 0.75 wild-type : 0.25 aa.

Model 2. Autosomal dominant gene

Generation	Genotypes
P	A/A x A^+/A^+
F1	A/A^+ x A/A^+
F2	1 A^+/A^+ : 2 A^+/A : 1 A/A

If A is completely dominant to A^+, then the F1 phenotype will be AA and the F2 progeny will have two phenotypic classes in the ratio of 0.75 A/A : 0.25 wild-type.
If A is incompletely dominant to A^+, then the F1 progeny will all have an intermediate phenotype and the F2 progeny will have three phenotypic classes in the ratio of 0.25 wild-type : 0.5 intermediate : 0.25 AA.

Model 3. Non-linkage of recessive genes in the repulsion configuration

Generation	Genotypes
P	a/a ; b^+/b^+ x a^+/a^+ ; b/b
F1	a^+/a ; b^+/b x a^+/a ; b^+/b
F2	9 $a^+/\_$; $b^+/\_$: 3 $a^+/\_$; b/b : 3 a/a ; $b^+/\_$: 1 a/a ; b/b

The F2 progeny will have four phenotypic classes in the ratio of 9 wild-type : 3 bb: 3 aa : 1 double homozygote aa-bb.

Model 4. Linkage of recessive genes in the repulsion configuration

Specific for the case where no recombination occurs in the male.

Generation	Genotypes
P	a b^+/a b^+ x a^+ b/a $^+$b
F1	a b^+/a^+ b x a b^+/a^+ b
F2	1 a b^+ /a b^+ : 2 a^+ b/a b^+ : 1 a^+ b/a^+ b

The F1 phenotype will be wild-type and there will be three phenotypic classes in the F2 in the ratio of 0.25 aa : 0.5 wild-type : 0.25 bb. No recombinants will be observed because there is no crossing over in males.

Model 5. Recessive epistasis

If a^+ is dominant to a; b^+ is dominant to b, and aa is epistatic to b^+ then:

Generation	Genotypes
P	a/a ; b^+/b^+ x a^+/a^+ ; b/b
F1	a^+/a ; b^+/b x a^+/a ; b^+/b
F2	9 a^+/_ ; b^+/_ : 3 a^+/_ ; b/b : 4 a/a ; _/_

Model 6. Dominant epistasis

If a^+ is dominant to a; b^+ is dominant to b, and a^+ is epistatic to b^+ then:

Generation	Genotypes
P	a/a ; b^+/b^+ x a^+/a^+ ; b/b
F1	a^+/a ; b^+/b x a^+/a ; b^+/b
F2	12 a^+/_ ; _/_ : 3 a/a ; b^+/_ : 1 a/a ; b/b

Model 7. Non-linkage of two recessive genes in the coupling configuration

Generation	Genotypes
P	a/a ; b/b x a^+/a ; b^+/b^+
F1	a /a^+ ; b /b^+ x a /a^+ ; b /b^+
F2	9 $a^+/\_$; $b^+/\_$: 3 $a^+/\_$; b/b : 3 a/a ; $b^+/\_$: 1 a/a ; b/b

The F2 progeny will have four phenotypic classes in the ratio of

9 wild-type : 3 bb : 3 aa : 1 double homozygote aa-bb.

Model 8. Linkage of two recessive genes in the coupling configuration

Generation	Genotypes
P	a b / a b x a^+ b^+ / a $^+b^+$
F1	a b / a^+ b^+ x a b / a^+ b^+
F2	1 a b / a b : 2 a^+ b^+ / a b : 1 a^+ b^+ / a^+ b^+

r=recombinant fraction in females, ratios (0.25 -0.25r): (0.5-0.5r):(0.25-0.25r)

Model 9. Non-linkage of one dominant gene and one recessive gene in repulsion

Generation	Genotypes
P	A/A ; b/b x A^+/A^+ ; b^+/b^+
F1	A/A^+ ; b /b^+ x A /A^+ ; b /b^+
F2	9 A/_ ; $b^+/\_$: 3 A/_ ; b/b : 3 A^+/A^+ ; $b^+/\_$: 1 A^+/A^+ ; b/b

Model 10. Linkage of one dominant gene and one recessive gene in repulsion

Generation	Genotypes
P	A b^+ / A b^+ x A^+ b / A^+ b
F1	A b^+ / A^+ b x A b^+ / A^+ b
F2	1 A^+ b / A _ : 2 A b^+ / A^+ _ : 1 A^+b / A^+b

The F2 will have two phenotypic ratio of 3 dominant: 1 recessive.

Model 11. Linkage of one incompletely dominant gene and one recessive gene in repulsion

Generation	Genotypes
P	$A\ b^+ / A\ b^+ \times A^+\ b / A^+\ b$
F1	$A\ b^+ / A^+\ b \times A\ b^+ / A^+\ b$
F2	$1\ A\ b^+ / A\ \_ : 2\ A\ b^+ / A^+\ \_ : 1\ A^+b / A^+b$

The F2 will have three phenotypic classes in the ratio of 1 of the incomplete dominant phenotype : 2 of the intermediate phenotype : 1 of the recessive phenotype.

Model 12. Non-linkage of one incompletely dominant gene and one recessive gene in repulsion.

Generation	Genotypes
P	$A/A\ ;\ b^+/b^+ \times A^+/A\ ;\ b/b$
F1	$A/A\ ;^+\ b/b^+ \times A/A^+\ ;\ b/b^+$
F2	$6\ A/A^+\ ;\ b^+/\_ : 3\ A/A\ ;\ b^+/\_ :\ 3\ A^+/A^+\ ;\ b^+/\_ :$ $2\ A/A^+\ ;\ b/b : 1\ A^+/A^+\ ;\ b/b : 1\ A/A\ ;\ b/b$

Six phenotypic classes in the F2, A/A^+ has a distinct intermediate phenotype.

Model 13. Linkage of one incompletely dominant gene and one recessive gene in coupling

Generation	Genotypes
P	$A\ b^+ / A\ b^+ \times A^+\ b / A^+\ b$
F1	$A\ b^+ / A^+\ b \times A\ b^+ / A^+\ b$
F2	$1\ A\ b^+ / A\ \_ : 2\ A^+\ b^+ / A\ \_ : 1\ A^+b / A^+b$

The F2 progeny have three phenotypic classes in the ratio of 0.25 AA : 0.5 intermediate : 0.25 bb.

Model 14. Non-linkage of one incompletely dominant gene and one recessive gene in coupling

Generation	Genotypes
P	A/A ; b/b x A^+/A^+ ; b^+/b^+
F1	A/A^+ ; b /b^+ x A/A^+ ; b /b^+
F2	3 A^+/A^+ ; $b^+/\_$: 6 A/A^+ ; $b^+/\_$: 1 A^+/A^+ ; b/b : 3 A/A^+ ; b/b : 2 A/A ; $b^+/\_$: 1 A/A ; b/b

Model 15. Non-linkage of one incompletely dominant gene and one dominant gene in repulsion

Generation	Genotypes
P	A/A ; B/B x A^+/A^+ ; B^+/B^+
F1	A /A^+ ; B /B^+ x A /A^+ ; B /B^+
F2	1 A^+/A^+ ; B^+/B^+ : 2 A^+/A^+ ; B^+/B : 3 A/ _ ; B^+/B^+ : 6 A/ _ ; B^+/B : 1 A^+/A^+ ; B/B: 3 A/A ; b/b:

Model 16. Linkage of one incompletely dominant gene and one dominant gene in repulsion

Generation	Genotypes
P	A B^+ / A B^+ x A^+ B / A^+ B
F1	A B^+ / A^+ B x A B^+ / A^+ B
F2	1 A B^+ / A B^+ : 2 A^+ B / A B^+ : 1 A^+B / A^+B

Model 17. Backcrosses involving two non-linked recessive genes

Generation	Genotypes
P	a/a ; b/b x a^+/a^+ ; b^+/b^+
F1	a /a^+ ; b /b^+
Backcross	a /a^+ ; b /b^+ x a/a ; b/b
Backcross	1 a^+/a ; b^+/b : 1 a^+/a ; b/b : 1 a/a ; b^+/b: 1 a/a ; b/b

The backcross progeny will have four phenotypic classes in the ratio of 0.25 wild-type : 0.25 aa : 0.25 bb : 0.25 aa-bb.

Model 18. Backcrosses involving two linked recessive genes

Generation	Genotypes
P	a b / a b x a^+ b^+ / a $^+b^+$
F1	a b / a^+ b^+
Backcross	a b / a^+ b^+ x a b / a b
F2	1 a b / a b : 1 a^+ b^+ / a b

The F2 progeny will have two phenotypic classes if there is no recombination. If recombination occurs there will be four phenotypic classes in the ratio of (0.5-0.5r) ab : (0.5r) a b^+ : (0.5r) a^+ b : (0.5-0.5r) a^+ b^+ .

Model 19. Backcrosses involving two non-linked genes, one incompletely dominant and one recessive.

Generation	Genotypes
P	A/A ; b/b x A^+/A^+ ; b^+/b^+
F1	A /A^+ ; b /b^+
Backcross	A /A^+ ; b /b^+ x A/A ; b/b
Backcross	1 A^+/A ; b^+/b : 1 A^+/A ; b/b : 1 A/A ; b^+/b: 1 A/A ; b/b

The backcross progeny will have four phenotypic classes in the ratio of 0.25 wild-type : 0.25 aa : 0.25 bb : 0.25 aa-bb.

Model 20. Backcrosses involving two linked genes, one incompletely dominant and one recessive.

Generation	Genotypes
P	A b / A b x A^+ b^+ / A $^+b^+$
F1	A b / A^+ b^+
Backcross	A b / A^+ b^+ x A b / A b
F2	1 A b / A b : 1 A^+ b^+ / A b

The F2 progeny will have two phenotypic classes if there is no recombination. If recombination occurs there will be four phenotypic classes in the ratio of (0.5-0.5r) Ab : (0.5r) A b^+ : (0.5r) A^+ b : (0.5-0.5r) A^+ b^+ .

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