DROSOPHILA INFORMATION SERVICE

81

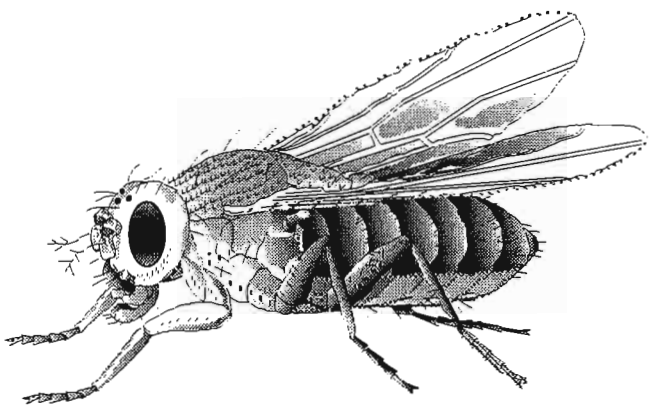
July 1998

Material contributed by
DROSOPHILA WORKERS

and arranged by
James N. Thompson jr.

prepared at
Department of Zoology
University of Oklahoma
Norman, Oklahoma 73019

Drosophila Information Service



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Preface

Drosophila Information Service was first printed in March, 1934. Material contributed by *Drosophila* workers was arranged by C.B. Bridges and M. Demerec. As noted in its preface, which is reprinted in DIS 75, Drosophila Information Service was undertaken because, "An appreciable share of credit for the fine accomplishments in *Drosophila* genetics is due to the broadmindedness of the original *Drosophila* workers who established the policy of a free exchange of material and information among all actively interested in *Drosophila* research. This policy has proved to be a great stimulus for the use of *Drosophila* material in genetic research and is directly responsible for many important contributions." During the nearly 65 years since that first issue, DIS has continued to promote open communication.

The production of DIS 81 could not have been completed without the generous efforts of many people. Diane Jackson, Stanton Gray, Russell Fletcher, and other volunteers helped with manuscripts; Gloria Stephens, Diane Jackson, and Shalia Newby maintained key records; and Coral McCallister advised on artwork and computer graphics.

For this issue, we owe a special acknowledgment to William Etges (University of Arkansas) and Hampton Carson (University of Hawaii) for facilitating the publication of the late Dr. Frances Elizabeth Clayton's compilation of the karyotypes of the Drosophilidae. We also thank Michael Ashburner who arranged the incorporation of these data into FlyBase. The research community is diminished by each loss of a person who, like Dr. Clayton, holds generous communication, rigor, and a respect for people and for knowledge as ideals sometimes taken for granted, but still valued above all else. Drosophila Information Service will continue as a resource for recording the contributions of those who have helped shaped our field.

We are grateful for the continued support of the DIS Advisory Group: Michael Ashburner (Cambridge University), Daniel Hartl (Harvard University), Kathleen Matthews (Indiana University), and R.C. Woodruff (Bowling Green State University). We hope that you find a lot of useful information here, and we invite you to let us know what can be done to improve DIS as a source of communication among *Drosophila* researchers.

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Drosophila Information Service

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Obituary

Frances Elizabeth Clayton, 1922-1998

Frances Clayton was born in Texarkana, Texas, to Carl C. and Louise Heath Morriss Clayton. After a degree from Texarkana College, she attended Texas State College for Women at Denton, receiving a B.A. degree in 1944. The following year she began graduate work at the University of Texas in Austin with Professor Wilson S. Stone as supervisor. She finished a Ph.D. in Zoology-Genetics in 1951, with minors in physiology and bacteriology. She then stayed on at Austin, working as a postdoctoral fellow in the Genetics Group.

Those of us who were present in the laboratory at the same time remember her as a person who quietly and diligently went about her work with a minimum of ostentation and a maximum of productivity. From 1951 to 1954, she was involved in a wide spectrum of research activities ranging from the expression of the alleles at the lozenge locus in *Drosophila melanogaster* to the effects of ionizing radiation on the production of chromosomal aberrations in *Drosophila virilis*. The latter studies resulted in the publication of a series of nine important papers in refereed journals, written alone or in collaboration with Professor Stone and then graduate students Felix Haas, Calvin Ward, Edna Dudgeon, and Mary Alexander. She continued with these areas of research after she left to join the faculty at the University of Arkansas, much of it in collaboration with Professor Marshall Wheeler and then student Marvin Wasserman at the University of Texas.

During her years at the University of Texas, and indeed throughout her career at the University of Arkansas since 1951, Frances did an extensive amount of teaching at both the graduate and undergraduate levels while at the same time doing the significant cytological research that formed the basis for her subsequent active life in science. All the while she maintained the same unpretentious demeanor, wry sense of humor and perceptive appreciation of those she worked with, both as a graduate student and in later professional life.

The last project of Wilson Stone, who died in 1968, centered on Hawaiian species of *Drosophila*. In June of 1963, he and Elmo Hardy of the University of Hawaii, launched "The Hawaiian *Drosophila* project", by bringing to Hawaii a group of seven scientists for the summer. These were picked by Stone and Hardy as having certain specialized skills that might contribute to the development of an interdisciplinary study of the evolutionary biology and genetics of the amazingly diverse and complex fauna endemic to the islands. Frances Clayton was "charter member" of this group. For the next 25 years, before failing eyesight ruled otherwise, she took on the job of characterizing the metaphase karyotypes of hundreds of closely related species of drosophilids. Such a project was never "assigned" to her. She saw the usefulness of the data for unraveling patterns of evolution in the family and slowly and unselfishly took over this job, not only for the Hawaiian species, but the rest of the family as well. Meticulous metaphase study of *Drosophila* has always been a frustrating undertaking that most cytologists have studiously avoided. About fourteen references in the bibliography and her final metaphase catalog summary for the family Drosophilidae that follows in this issue are a major scientific legacy of Frances Clayton.

But to equate Frances' contributions to the Hawaiian project simply with her metaphase chromosome counts is to oversimplify. Clayton sought out, recorded, and documented with great accuracy the numerous exceptions, supernumeraries, intra-specific polymorphisms, and heterochromatin variations within and among these many closely related species. She was also involved in a discovery that made possible the laboratory rearing of these species. Many of the species have unusual nutritional requirements; these were met by the invention of the Wheeler/Clayton (1965) method of rearing *Drosophila*. This success did much to convince granting agencies in the 1960's that the Hawaiian drosophilids were worthy material for study and could be handled routinely in the laboratory. The technique is still used today with little modification.

Clayton was not satisfied with laboratory contributions alone and never missed an opportunity to go along on field expeditions in Hawaii, as usual, developing her own particular style of work. For example, during one week-long expedition to a rain forest a mile above sea level on the island of Hawaii, she chose to intensively work one small area in great detail, rather than range widely in the mud and rain. At the bottom of one deep forested pit, she found a convenient

perch in the chilling rain on a low horizontal tree-limb. Here she would sit under her poncho between rounds of inspecting a series of sponges baited with banana mash. When rounded up at days' end, she would silently hand over her catch of flies. She often had more than the other collectors, a fact she would accept with amusement and minimum of unnecessary conversation.

Janet Gallman has written the following:

> "I had the privilege as an under-grad and then grad student to take Human Genetics and Experimental Genetics from Dr. Clayton. Never spoon feeding, but inspiring you to inquire and intuitively assimilate knowledge . . . she had a very sly (& acute) sense of humor . . . she was a neat lady and an inspiration to me. . . ."

Publications of Frances E. Clayton

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1998. Metaphase Chromosomes of Species in the Family Drosophilidae. *Drosophila* Information Service 81: 5-119 (posthumously published).

Hampton L. Carson[a], Robert P. Wagner[b] and William K. Baker[c]

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Published Karyotypes of the Drosophilidae

Frances E. Clayton
University of Arkansas

(The karyotypes listed are not comprehensive for the species but include the earliest published reports. Where different configurations have been reported all variations may be given; the category "Listings" refers to the references of 1975 and 1986 in which metaphase configurations were tabulated.)

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"A Catalog of Drosophila Metaphase Configurations".
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Chromosomal Evolution in the Family Drosophilidae".
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Academic Press: London. Pages 1 - 38.

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anomala
hamata
palmae
varifrons
xanthopleura
Subgenus: Dentiscaptomyza
denticauda
Subgenus: Elmomyza
argentifrons
melancholica
waialeale
Subgenus: Parascaptomyza
adusta
pallida
Subgenus: Scaptomyza
graminum
noei
Subgenus: Tantalia
albovittata

(Uncertain affinity)
parva
Genus: Titanochaeta
bryani
Genus: Zaprionus
Subgenus: Aprionus
argentostriatus
obscuricornis
silvistriatus
Subgenus: Zaprionus

bogoriensis
ghesquierei
indianus
inermis
ornatus
sepsoides
tuberculatus
vittiger

(Species of uncertain classification)
alexandrei
florae
pagliolii

Family Drosophilidae

Subfamily: Steganinae

Genus: Acletoxenus

Species: formosus (Loew) 1864 (Gitona)
Karyotype:
4R, 1V, 1D
Ashburner (in Clayton and Guest)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Genus: Cacoxenus

Species: indagator Loew 1858
Karyotype:
2R, 1V, 1J
Ashburner (in Clayton and Guest)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Genus: Leucophenga
Subgenus: Leucophenga

Species: maculata (Dufour) 1839 (Drosophila)
Karyotype:
5V, 1D
Kang et al. (1964)
X and Y are V-shaped
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: varia (Walker) 1849 (Drosophila)
Karyotype:
5V, 1D
Clayton (in Clayton and Guest)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Subfamily: Drosophilinae

Genus: Chymomyza

Species: aldrichi Sturtevant 1916
Karyotype:
1R, 2V, lD
Clayton and Ward (1954)
X and Y are rods
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: amoena (Loew) 1862 (Drosophila)
Karyotype:
1R, 2V, lD
Metz (1914)
Reported as D. amoena
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: costata (Zetterstedt) 1838 (Drosophila)
Karyotype:
1R, 2V, lD
Hackman, et al. (1970)
Acrocentric X; submeta-centric Y
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: coxata Wheeler 1952
Karyotype:
1R, 2V, lD
Clayton and Ward (1954)
X and Y are rods
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: procnemis (Williston) 1896 (Drosophila)
Karyotype:
1R, 2V, lD
Metz (1916a)
Reported as D. procnemis
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: wirthii Wheeler 1954a
Karyotype:
1R, 2V, lD
Clayton and Ward (1954)
X and Y are rods
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Genus: Cladochaeta

Species: nebulosa Coquillett 1900
Karyotype:
3V, lD
Metz (1916a)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (l981)

Genus: Dettopsomyia

Species: nigrovittata (Malloch) 1924c (Drosophila)
Karyotype:
3R, lV, lD
Prevosti (1976)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Genus: Drosophila
Subgenus: Antopocerus

Species: adunca (Hardy) 1965 (Antopocerus)
Karyotype:
6R
Clayton (1968)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: arcuata (Hardy) 1965 (Antopocerus)
Karyotype:
5R, lD
Clayton (1968)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: cognata Grimshaw 1901
Karyotype:
5R, lD
Clayton (1971)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: diamphidiopoda (Hardy) 1968 (Antopocerus)
Karyotype:
6R
Clayton (1968)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (l981)

Species: entrichocnema (Hardy) 1968 (Antopocerus)
Karyotype:
6R
Clayton (1968)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: longiseta Grimshaw 1901
Karyotype:
5R, lD
Yoon and Richardson (1976b)
X and Y are rods
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (l981)

Species: tanythrix (Hardy) 1965 (Antopocerus)
Karyotype:
5R, lD
Yoon and Richardson (1976)
X and Y are rods
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Genus: Drosophila
Subgenus: Dorsilopha

Species: busckii Coquillet 1901b (as buskii)
Karyotype:
1R, 2V, lD
Metz (1916a)
1R, 2V
Wharton (1943)
Y may appear J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Genus: Drosophila
Subgenus: Drosophila

Species: acanthoptera Wheeler 1949a
Karyotype:
2R, lV 2v
Ward (1949)
X is large; Y is J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: acutilabella Stalker 1953
Karyotype:

1R, 2V, 1D
Stalker (1953)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: adiastola Hardy 1965
Karyotype:
5R, 1D
Clayton (1966)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: after Tan, Hsu, and Sheng 1949
Karyotype:
1R, 2V, 1J, 1D
Tan et al. (1949)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: affinidisjuncta Hardy 1978b
Karyotype:
3V, 2J, 1D
Baimai and Ahearn (1978)
Printing error in karyotype description
5R, 1D
Ahearn and Baimai (1987)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: aglaia Hardy 1965
Karyotype:
5R, 1D
Clayton et al. (1972)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: albirostris Sturtevant 1921
Karyotype:
5R, 1D
Clayton and Wasserman (1957)
Y is rod shorter than X
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: albomicans Duda 1923
Karyotype:
1R, 1V, 1J
Wilson et al. (1969)
3R, 1V

Kikkawa and Peng (1938)
Y is a small v; reported as komaii
2V, 1D
Rajasekarasetty et al. (1979)
as subspecies of nasuta
One arm of V is X chromosome
Listings:
Cytological: Clayton and Wheeler; Clayton and Guest
Taxonomic: Wheeler (1981)

Species: aldrichi Patterson in Patterson and Crow 1940
Karyotype:
5R, lD
Patterson and Crow (1940)
Y is short rod; 1/3 length of X
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (l981)

Species: alsophila Hardy and Kaneshiro 1971
Karyotype:
5R, lD
Clayton (1971)
Metaphase omitted in Clayton and Wheeler (1975)
Listings:
Cytological: Clayton and Wheeler;
Clayton and Guest
Taxonomic: Wheeler (1981)

Species: altiplanica Brncic and Santibanez 1957
Karyotype:
3R, 1V, 1D
Brncic and Santibanez (1957)
One pair rods bent in middle giving appearance of small v's; elongated dots
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: americana Spencer 1938
Karyotype:
1R, 2V, 1D
Hughes (1939); Wharton (1943)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (l918)
ssp. texana Patterson in Patterson, Stone, and Griffen 1940
Karyotype:
3R, 1V, 1D
Patterson (1940)
Reported as species not subspecies
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: anceps Patterson and Mainland 1944
Karyotype:
6R
Patterson and Mainland (1944)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: andina Dobzhansky and Pavan 1943
Karyotype:
4R, 1V
Dobzhansky and Pavan (1943)
Reported as species of uncertain classification
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: angularis Okada 1956
Karyotype:
5R, 1D
Tokumitsu et al. (1967)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: angustibucca Duda 1925
Karyotype:
5R, 1D
Franck et al. (1984)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: annulimana Duda 1927
Karyotype:
1R, 1V, 3v
Dobzhansky and Pavan (1943)
XO male; X is rod- or J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: annulipes Duda 1924a
Karyotype:
1R, 1J, 1V
Lin et al. (1974)
X is V-shaped; Y is J-shaped
1R, 2V
Moriwaki et al. (1956)
Reported as virgata
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

syn. virgata Tan, Hsu and Sheng 1949

Species: anomalipes Grimshaw 1901
Karyotype:
5R, lD
Clayton (1976)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: anoplastoma Hardy and Kaneshiro 1968
Karyotype:
5R, lD
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (l981)

Species: antillea Heed 1962
Karyotype:
1R, 2V, 1D
Heed and Krishnamurthy (1959)
Reported as stock SL
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: aracea Heed and Wheeler 1957
Karyotype:
1R, 1V, 1J
Clayton and Wasserman (1957)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: araicas Pavan and Nacrur 1950
Karyotype:
1V, 3v, 1D
Pavan and Nacrur (1950)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: arapuan Cunha and Pavan in Pavan and Cunha 1947
Karyotype:
1R, 2V, 1D
Pavan and Cunha (1947)
Y is J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: ararama Pavan and Cunha 1947
Karyotype:
4R, lJ
Pavan and Cunha (1947)

Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: arassari Cunha and Frota-Pessoa in Pavan and Cunha 1947
Karyotype:
5R, 1D
Pavan and Cunha (1947)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: araucana Brncic 1957a
Karyotype:
5R, lD
Brncic (1957)
One pair rods double-length
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: arauna Pavan and Nacrur 1950
Karyotype:
1R, 1V, 3v, 1Dr
Pavan and Nacrur (1950)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: arawakana Heed 1962
Karyotype:
1R, 2V, 1D
Heed and Krishnamurthy (1959)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
ssp. kittensis Heed 1962
Karyotype:
1R, 2V, 1D
Heed and Krishnamurthy (1959)
Reported as stock SK
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler 1981

(Species argentostriata = argentostriatus to Zaprionus)

(Species arizonensis = mojavensis)

Species: asketostoma Hardy 1965
Karyotype:
R
Clayton (1966)
Listings:
Cytological: Clayton and Wheeler

Taxonomic: Wheeler (1981)

Species: asper Lin and Tseng 1971
Karyotype:
2R, 1V, 2J, 1D
Lin and Tseng (1971)
X is telocentric; Y is metacentric
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: assita Hardy and Kaneshiro 1969
Karyotype:
5R, 1D
Clayton (1971)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: atalaia Vilela and Sene 1982
Karyotype:
3V
Vilela and Sene (1982)
Y is J-shaped
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1986)

Species: atrata Burla and Pavan 1953
Karyotype:
5R, 1D
Dobzhansky and Pavan (1943)
Reported as calloptera
Clayton and Ward (1954)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: atrimentum Hardy and Kaneshiro 1971
Karyotype:
5R, 1D
Clayton (1971)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: atroscutellata Hardy 1966
Karyotype:
5R, 1D
Clayton (1966)
Reported as "dark scutellum" sp
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: attigua Hardy and Kaneshiro 1969
Karyotype:
5R, lD
Clayton (1969)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: aureata Wheeler 1957
Karyotype:
5R, lD
Clayton and Wasserman (1957)
Y is J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: balioptera Hardy 1965
Karyotype:
5R, lD
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: bandeirantorum Dobzhansky and Pavan 1943
Karyotype:
4R, 1V, 1D
Dobzhansky and Pavan (1943)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: basimacula Hardy 1965
Karyotype:
5R, lD
Clayton (1966)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: basisetae Hardy and Kaneshiro 1968
Karyotype:
5R, 1D
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: bedicheki Heed and Russell 1971
Karyotype:
1R, 2V, 1D
Heed and Russell (1971)
Listings:
Cytological: Clayton and Wheeler

Taxonomic: Wheeler (1981)

Species: belladunni Heed and Krishnamurthy 1959
Karyotype:
2R, 2V
Heed and Krishnamurthy (1959)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

(Species: betari = repleta)

Species: bifurca Patterson and Wheeler 1942
Karyotype:
5R, 1D
Patterson and Wheeler (1943)
Wharton (1943): Y is a rod
Ward (1949): Y is small, V-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: bipunctata Patterson and Mainland in Patterson 1943
Karyotype:
5R, 1D
Clayton and Wasserman (1957)
Y is rod shorter than X
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: biseriata Hardy 1965
Karyotype:
3R, 1V, 1D
Clayton et al. (1972)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: bizonata Kikkawa and Peng 1938
Karyotype:
3V, 1D
Kikkawa and Peng (1938)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: blumelae Pipkin and Heed 1964
Karyotype:
5R, 1D
Pipkin and Heed (1964)
Y is J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: borborema Vilela and Sene 1977
Karyotype:
5R, lD
Wasserman (1982)
Baimai et al. (1983)
Telocentric X; acrocentric Y
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: borealis Patterson 1952a
Karyotype:
4R, 1v, 1D
Patterson (1952)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: bostrycha Hardy 1965
Karyotype:
5R, lD
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: brachynephros Okada 1956
Karyotype:
5R, lD
Tokumitsu et al. (1967)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: brevicarinata Patterson and Wheeler 1942
Karyotype:
5R, lD
Wharton (1943)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: briegeri Pavan and Breuer 1954
Karyotype:
3V, 1D
Pavan and Breuer (1954)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: brncici Hunter and Hunter 1964
Karyotype:
3R, 1V, 1D
Hunter and Hunter (1964)

Dots usually appear as small rods

Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: bromeliae Sturtevant 1921
Karyotype:
1R, 2V, 1D
Metz (1916b)
Y is J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: bromelioides Pavan and Cunha 1947
Karyotype:
1R, 3V
Pavan and Cunha (1947)
4V
Clayton and Wasserman (1957)
Species identity uncertain
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

(Species brunneipalpa = repleta)

Species: buzzatii Patterson and Wheeler 1942
Karyotype:
5R, 1D
Patterson and Wheeler (1942)
Y is short rod; half length of X
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
syn. versicolor Mather 1955

Species: californica Sturtevant 1923
Karyotype:
1R, 3V
Patterson and Wheeler (1942)
Reported as fuliginea
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
syn. fuliginea Patterson and Wheeler 1942

Species: calloptera Schiner 1868
Karyotype:
3R, 1V, 1D
Metz (1916a,b)
4R, 1J
Clayton and Ward (1954)
Listings:
Cytological: Clayton and Wheeler

Taxonomic: Wheeler (1981)

Species: camargoi Dobzhansky and Pavan in Pavan 1950
Karyotype:
1R, 2V, 1J
Pavan (1950)
Stock from Brazil
3V, 1J
Clayton and Wasserman (1957)
Stock from Honduras
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: camaronensis Brncic 1957a
Karyotype:
3R, 1V, 1D
Brncic (1957)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: canalinea Patterson and Mainland 1944
Karyotype:
1R, 1V, 1v, 1D
Patterson and Mainland (1944)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: canalinioides Wheeler 1957
Karyotype:
6R
Clayton and Wasserman (1957)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

(Species: canapalpa = repleta, neorepleta)

Species: caponei Pavan and Cunha 1947
Karyotype:
3V, 1D
Pavan and Cunha (1947)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: carbonaria Patterson and Wheeler 1942
Karyotype:
2R, 2J, 1v, 1D
Ward (1949)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: carcinophila Wheeler 1960b
Karyotype:
3R, 1V, 1D
Carson (1967)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: cardini Sturtevant 1916
Karyotype:
5R, 1D
Metz (1916a,b)
1R, 2V, 1D
Wharton (1943) (Species uncertain)
6R
Ward (1949); Clayton and Wasserman (1957); Heed and Russell (1971)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: cardinoides Dobzhansky and Pavan 1943
Karyotype:
1R, 2V, 1D
Dobzhansky and Pavan (1943)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: caribiana Heed 1962
Karyotype:
1R, 2V, 1D
Heed and Krishnamurthy (1959)
Listed as Stock MA
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: carsoni Wheeler 1957
Karyotype:
2R, 1V, 2J, 1D
Clayton and Wasserman (1957)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: castanea Patterson and Mainland 1944
Karyotype:
1R, 2V, 1D
Clayton and Wasserman (1957)
X is V-shaped; Y is J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: chaetopeza Hardy 1965
Karyotype:
5R, lD
Clayton (1966)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (l981)

Species: chamundiensis Sajjan and Krishnamurthy 1972
Karyotype:
2R, 1V, 1D
Sajjan and Krishnamurthy (1975)
X is rod-shaped; Y is J-shaped
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: cheda Tan, Hsu and Sheng 1949
Karyotype:
1R, 1V, 1v, 1D
Tan et al. (1949)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (198l)

(Species: chinoi = repletoides)

Species: ciliaticrus Hardy 1965
Karyotype:
5R, lD
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (l981)

Species: cilifemorata Hardy 1965
Karyotype:
5R, lD
Clayton (1976)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: cilifera Hardy and Kaneshiro 1968
Karyotype:
5R, lD
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: circumdata Duda 1926a
Karyotype:
5R, lD
Clyde and Hasnah (1983)

Y is a rod; half length of X

Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: clavisetae (Hardy) 1966 (Idiomyia)
Karyotype:
5R, lD
Carson et al. (1967)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: claytonae Hardy and Kaneshiro 1969
Karyotype:
5R, lD
Clayton (1969)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: colorata Walker 1849
Karyotype:
2R, 2V, 1v, 1J
Wharton (1943)
X is V-shaped; Y is a rod
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
syn. sulcata Sturtevant 1916

Species: comatifemora Hardy 1965
Karyotype:
5R, lD
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (198l)

Species: conspicua Grimshaw 1901
Karyotype:
5R, lD
Carson et al. (1967)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: converga Heed and Wheeler 1957
Karyotype:
5R, lD
Heed and Wheeler (1957)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: coroica Wasserman 1962c
Karyotype:
5R, 1D
Wasserman (1960)
Reported as species L
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: crocina Patterson and Mainland 1944
Karyotype:
5R, 1D
Patterson and Mainland (1944)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: crucigera Grimshaw 1902
Karyotype:
5R, 1D
Clayton (1966)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: curviceps Okada and Kurokawa 1957
Karyotype:
2R, 1V, 1J
Okada and Kurokawa (1957)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: cyrtoloma Hardy 1969
Karyotype:
5V, 1J
Clayton (1968)
Reported as perkinsi (?)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: deltaneuron Bryan 1938
Karyotype:
1R, 2V, 1D
Clayton et al. (1972)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: desertorum Wasserman 1962b
Karyotype:
5R, 1D
Wasserman (1982)
Listings:

Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: differens Hardy and Kaneshiro 1975
Karyotype:
5R, lD
Clayton (1976)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (l981)

Species: digressa Hardy and Kaneshiro 1968
Karyotype:
5R, lD
Clayton (1976)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: diminuens Hardy 1965
Karyotype:
5R, lD
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: discreta Hardy and Kaneshiro 1968
Karyotype:
5R, lD
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: disjuncta Hardy 1965
Karyotype:
5R, lD
Carson et al. (1967)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: dissita Hardy 1965
Karyotype:
n = 6
Yoon and Wheeler (1973)
Listings:
Cytological: Clayton and Guest (1986)
Taxonomic: Wheeler (1981)

Species: distinguenda Hardy 1965
Karyotype:
5R, lD
Clayton (1969)

Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: divaricata Hardy and Kaneshiro 1971
Karyotype:
5R, lD
Clayton (1971)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: dreyfusi Dobzhansky and Pavan 1943
Karyotype:
2V, 1J
Dobzhansky and Pavan (1943)
X and Y are J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: dunni Townsend and Wheeler 1955
Karyotype:
1R, 2V, 1r
Townsend and Wheeler (1955)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
ssp. thomasensis Heed 1962
Karyotype:
1V, 2J, 1r
Heed and Krishnamurthy (1959)
Reported as stock ST
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: endobranchia Carson and Wheeler 1968
Karyotype:
1R, 2V, 1D
Carson and Wheeler (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: engyochracea Hardy 1965
Karyotype:
5R, lD
Clayton (1966)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

(Species: enigma to Scaptodrosophila)

Species: eohydei Wasserman 1962a
Karyotype:
5R, 1D
Wasserman (1962a)
Y is a short rod
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
syn. pseudoneohydei Hennig, Hennig and Stein 1970

Species: eremophila Wasserman 1962b
Karyotype:
5R, 1Dv
Wasserman (1960)
Reported as Species F
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: euronotus Patterson and Ward 1952
Karyotype:
2R, 1V, 1v, 1D
Patterson and Ward (1952)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: eurypeza Hardy 1965
Karyotype:
5R, 1D
Clayton (1966)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: ezoana Takada and Okada 1957
Karyotype:
4R, 1v, 1D
Stone et al. (1960)
Y is a small v
Listings:
Cytological: Clayton and Wheeler; Clayton and Guest
Taxonomic: Wheeler (1981)
syn. ezoana Takada and Okada 1958

Species: facialba Heed and Wheeler 1957
Karyotype:
3R, 1V, 1D
Mettler in Heed and Wheeler (1957)
Stock (H67.5) from El Salvador
5R, 1D
Clayton in Heed and Wheeler (1957)
Stock (H26.2a) from El Salvador
Listings:
Cytological: Clayton and Wheeler

Taxonomic: Wheeler (1981)

Species: fairchildi Pipkin and Heed 1964
Karyotype:
5R
Pipkin and Heed (1964)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: falleni Wheeler 1960c
Karyotype:
5R, lD
Wharton (1943)
Reported as transversa
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: fasciculisetae Hardy 1965
Karyotype:
5R, lD
Carson et al. (1967)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: fasciola Williston 1896
Karyotype:
5R, lD
Wasserman (1962c)
X is rod- or J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: fascioloides Dobzhansky and Pavan 1943
Karyotype:
3V, 1D
Dobzhansky and Pavan (1943)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: flavibasis Hardy 1965
Karyotype:
5R, lD
Clayton (1966)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: flavomontana Patterson 1952
Karyotype:
4R, 1v, 1D

Patterson (1952)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: flavopilosa Frey 1918
Karyotype:
3R, 2V, 1J
Wheeler et al. (1962)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: flavopinicola Wheeler 1954
Karyotype:
5R, 1D
Clayton and Ward (1954)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: flexipes Hardy and Kaneshiro 1968
Karyotype:
5R, 1D
Clayton (1971)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: formella Hardy and Kaneshiro 1972
Karyotype:
5R, lD
Clayton et al. (1972)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: formosana Duda 1926a
Karyotype:
3R, 1V
Lin et al. (1974)
X and Y are rod-shaped
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: fragilis Wheeler 1949a
Karyotype:
3R, 1V, 3D
Clayton and Ward (1954)
3R, 1V, 1D
Clayton and Wasserman (1957)
Stock from El Salvador
Listings:
Cytological: Clayton and Wheeler

Taxonomic: Wheeler (1981)

Species: freycinetiae Hardy 1965
Karyotype:
5R, lD
Clayton (1966)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: fulvilineata Patterson and Wheeler 1942
Karyotype:
5R, lV
Patterson and Wheeler (1942); Wharton (1943)
X is a rod; Y is a short rod
Clayton and Wasserman (1957)
Stock from New Mexico
3R, 1V, 1v (Arizona stock)
Clayton and Wasserman (1957)
X is large V; Y is J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: fulvimacula Patterson and Mainland 1944
Karyotype:
5R, lD
Clayton and Ward (1954)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
ssp. flavorepleta Patterson and Pavan in Patterson 1952b
Karyotype:
5R, lD
Clayton and Ward (1954)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: fulvimaculoides Wasserman and Wilson 1957
Karyotype:
5R, lD
Clayton and Wasserman (1957)
Y is J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: fumosa Pavan and Cunha 1947
Karyotype:
1R, 2V, lD
Pavan and Cunha (1947)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: funebris (Fabricius) 1787 (Musca)
Karyotype:
5R, lD
Metz (1914)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: fuscoamoeba Bryan 1934
Karyotype:
5R, lD
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: gasici Brncic 1957a
Karyotype:
3R, 1V, 1D
Brncic (1957)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: gaucha Jaeger and Salzano 1953
Karyotype:
3R, 1V, 1D
Jaeger and Salzano (1953)
Y has subterminal constriction
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: gibberosa Patterson and Mainland in Patterson 1943
Karyotype:
5R
Wharton (1943)
X is long rod with proximal constriction; Y is J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: glabriapex Hardy and Kaneshiro 1968
Karyotype:
5R, lD
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: goureaui Hardy in Hardy and Kaneshiro1972
Karyotype:
5R, lD
Clayton (1966)

Reported as mycetophila Hardy

Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

syn. mycetophila Hardy 1965 (preocc.)

Species: gradata Hardy and Kaneshiro 1968
Karyotype:
5R, lD
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: greerae Pipkin and Heed 1964
Karyotype:
5R, lD
Pipkin and Heed (1964)
X is rod; Y is shorter rod
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: grimshawi Oldenberg 1914
Karyotype:
5R, lD
Clayton (1966)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: griseolineata Duda 1927
Karyotype:
5R, lD
Dobzhansky and Pavan (1943)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: guaraja King 1947
Karyotype:
3R, 1V, 1D
King (1947)
Y is rod with large satellite; X is a short rod
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: guaramunu Dobzhansky and Pavan 1943
Karyotype:
5R, lD
Dobzhansky and Pavan (1943)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: guarani Dobzhansky and Pavan 1943
Karyotype:
5R, 1V
Dobzhansky and Pavan (1943)
X and Y are V-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: guaru Dobzhansky and Pavan 1943
Karyotype:
4R, 1V, 1D
Dobzhansky and Pavan (1943)
Y is J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: guttifera Walker 1849
Karyotype:
5R, lD
Wharton (1943)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: gymnobasis Hardy and Kaneshiro 1971
Karyotype:
5R, lD
Clayton (1971)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: gymnophallus Hardy and Kaneshiro 1975
Karyotype:
5R, lD
Clayton (1976)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: hamatofila Patterson and Wheeler 1942
Karyotype:
5R, lD
Patterson and Wheeler (1942)
Y is small, V-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: hamifera Hardy and Kaneshiro 1968
Karyotype:
5R, lD

Clayton (1971)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: hanaulae Hardy 1969
Karyotype:
5R, lD
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: hawaiiensis Grimshaw 1901
Karyotype:
5R, lD
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: heedi Hardy and Kaneshiro 1971
Karyotype:
6R
Clayton et al. (1972)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: hemipeza (Hardy) 1965 (Idiomyia)
Karyotype:
5R, lD
Carson et al. (1967)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: heterobristalis Tan, Hsu and Sheng 1949
Karyotype:
1R, 2V, lD
Tan et al. (1949)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: heteroneura (Perkins) 1910 (Idiomyia)
Karyotype:
5R, lD
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: hexachaetae Hardy 1965
Karyotype:
5R, lD

Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: hexastigma Patterson and Mainland 1944
Karyotype:
5R, 1D
Patterson and Mainland (1944)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: hexastriata Tan, Hsu and Sheng 1949
Karyotype:
2R, 1V, 1D
Tan et al. (1949)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: hirtipalpus Hardy and Kaneshiro 1968
Karyotype:
5R, 1D
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: histrio Meigen 1830
Karyotype:
5R, 1D
Frolova (1926)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: hydei Sturtevant 1921
Karyotype:
4R, 1V, 1D
Kikkawa (1935); Wharton (1943)
Kikkawa and Peng (1938); Wasserman (1960):
Y is a rod
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

(Species: hydeiodes = nigrohydei)

Species: hypocausta Osten-Sacken 1812
Karyotype:
2R, 1V, 1D
Pipkin (1956)
syn. pararubida Mather 1961
Listings:

Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: hystricosa Hardy and Kaneshiro 1969
Karyotype:
3R, 1V, 1D
Clayton (1968)
Reported as "n. sp. near caccabata"

Species: immigrans Sturtevant 1921
Karyotype:
2R, 1V, 1J
Emmens (1937); Wharton (1943)
3R, 1V
Metz and Moses (1923)
Y is a small v
Ward (1949); Clayton and Wasser-man (1957); Mather (1962)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
syn. flexipilosa Pipkin 1964

Species: imparisetae Hardy 1965
Karyotype:
5R, 1D
Clayton et al. (1972)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: inca Dobzhansky and Pavan 1943
Karyotype:
5R, 1J
Dobzhansky and Pavan (1943)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: inedita Hardy 1965
Karyotype:
5R, 1D
Clayton (1969)
Dots are extremely large
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: infuscata Grimshaw 1901
Karyotype:
5R, 1D
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: ingens Hardy and Kaneshiro 1971
Karyotype:
5R, 1D
Clayton (1969)
Reported as melanocephala
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: innubila Spencer in Patterson 1943
Karyotype:
5R, 1D
Wharton (1943)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: ischnotrix Hardy 1965
Karyotype:
1R, 2V, 1D
Clayton (1966)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: johnstonae Pipkin and Heed 1964
Karyotype:
5R, 1D
Pipkin and Heed (1964)
Y is J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: kambysellisi Hardy and Kaneshiro 1969
Karyotype:
6R
Clayton et al. (1972)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: kanekoi Watabe and Higuchi 1979
Karyotype:
4R, 1v, 1D
DeSalle et al. (1980)
X is a rod; Y is J-shaped
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: kauluai Bryan 1934
Karyotype:
5R, 1D
Clayton (1966)

Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: kepulauana Wheeler in Wilson et al. 1969
Karyotype:
2R, 1V, 1D
Wilson et al. (1969)
X is a rod; Y is rod- or J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: kohkoa Wheeler in Wilson et al. 1969
Karyotype:
2R, 1V, 1D
Wilson et al. (1969)
X is a rod; Y is rod- or J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

(Species: komaii = albomicans)

Species: lacertosa Okada 1956
Karyotype:
1V, 4J, 1D
Momma (1956)
Determined from oogonial metaphases
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: lacicola Patterson 1944
Karyotype:
4R, 1v, 1D
Patterson (1944)
Listings:
Cytological: Clayton and Wheeler; Clayton and Guest
Taxonomic: Wheeler (1981)

Species: lasiopoda Hardy and Kaneshiro 1975
Karyotype:
6R
Clayton (1976)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: leonis Patterson and Wheeler 1942
Karyotype:
6R
Wharton (1943)
Listings:
Cytological: Clayton and Wheeler

Taxonomic: Wheeler (1981)

Species: limbinervis Duda 1925
Karyotype:
5R, lD
Clayton and Wasserman (1957)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: limensis Pavan and Patterson in Pavan and Cunha 1947
Karyotype:
6R
Pavan and Cunha (1947)
Y is a very short rod
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: limitata Hardy and Kaneshiro 1968
Karyotype:
6R
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: linearepleta Patterson and Wheeler 1942
Karyotype:
5R, lD
Patterson and Wheeler (1975)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: lineosetae Hardy and Kaneshiro 1968
Karyotype:
5R, 1D
Clayton (1969)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: liophallus Hardy and kaneshiro 1968
Karyotype:
5R, lD
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: littoralis Meigen 1830
Karyotype:
2R, 1V, 1J, 1D
Clayton and Ward (1954)

X is a rod; Y is J-shaped

Listings:
Cytological: Clayton and Wheeler; Clayton and Guest
Taxonomic: Wheeler (1981)

syn. imeretensis Sokolov 1948

Species: longicornis Patterson and Wheeler 1942
Karyotype:
5R, lD
Patterson and Wheeler (1942)
Y is a short rod
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: lummei Hackman 1972
Karyotype:
5R, lD
Sokolov (1959)
Described as chromosomes for littoralis
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: macroptera Patterson and Wheeler 1942
Karyotype:
5R, lD
Patterson and Wheeler (1942)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: macrospina Stalker and Spencer 1939
Karyotype:
5R, lD
Wharton (1943)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

ssp. limpiensis Mainland 1941
Karyotype:
5R, lD
Patterson and Wheeler (1942)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: macrothrix Hardy and Kaneshiro 1968
Karyotype:
5R, lD
Clayton (1971)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: magnaquinaria Wheeler 1954
Karyotype:
6R
Clayton and Ward (1954)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: mainlandi Patterson 1943
Karyotype:
5R, 1D
Wasserman (1982)
Y is J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: martensis Wasserman and Wilson 1957
Karyotype:
5R, 1D
Clayton and Wasserman (1957)
Y is small, v-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: mathisi Vilela 1983
Karyotype:
5R, lD
Vilela (1983)
X is rod; Y is J-shaped
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1986)

Species: mcclintockae Pipkin 1964
Karyotype:
3R, 1V, 1D
Pipkin (1964)
Y is a rod
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (198l)

Species: mediodiffusa Heed and Wheeler 1957
Karyotype:
5R, lD
Heed and Wheeler (1957)
X is double-length rod; Y is short rod
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: medionotata Frota-Pessoa l954
Karyotype:

5R, lD
Clayton and Wasserman (1957)
Species identity uncertain
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: mediopictoides Heed and Wheeler 1957
Karyotype:
4R, 1r, 1V
Heed and Wheeler (1957)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: mediopunctata Dobzhansky and Pavan 1943
Karyotype:
5R, 1D
Dobzhansky and Pavan (1943)
Stock from Brazil
2R, 1V, 1J, 1D
Clayton and Wasserman (1957)
Stock from El Salvador
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: mediosignata Dobzhansky and Pavan 1943
Karyotype:
5R, 1D
Dobzhansky and Pavan (1943)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: mediostriata Duda 1925
Karyotype:
5R, lD
Dobzhansky and Pavan (1943)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: meitanensis Tan, Hsu and Sheng 1949
Karyotype:
2V, 1J, 1D
Tan et al. (1949)
X and Y are J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (l981)

Species: melanica Sturtevant 1916
Karyotype:
2R, 1V, 1v, 1D

Metz (1916a)
Ward (1949)
Y is J-shaped; X is V-shaped

Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: melanocephala (Hardy) 1966 (Idiomyia)
Karyotype:
5R, 1V
Clayton (1969)
Reported as melanocephala ?

Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

(Species: melanopalpa = repleta)

Species: melanosoma Grimshaw 1901
Karyotype:
5R, lD
Clayton (1966)

Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: melanura Miller 1944
Karyotype:
2R, 1V, 1v, 1Dv
Miller (1944)
X is large V; Y is J-shaped

1R, 1V, 1v, 1Dr
Ward (1949)
X is large V; Y is V-shaped

Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: mercatorum Patterson and Wheeler 1942
Karyotype:
3R, 1V, 1Dv
Patterson and Wheeler (1942);
Wharton (1943)
Male is XO
Ward (1949); Clayton and Wasser-man (1957)
X is a rod; Y is short rod

Listing:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

ssp. pararepleta Dobzhansky and Pavan 1943
Karyotype:
3R, 1V, 1D
Dobzhansky and Pavan (1943)
Y is a short rod

Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: meridiana Patterson and Wheeler 1942
Karyotype:
5R, 1D
Patterson and Wheeler (1942)
Y is a short rod
5R, 1V (Error in table of Clayton and Wheeler, 1975)
Listings:
Cytological: Clayton and Wheeler; Clayton and Guest
Taxonomic: Wheeler (1981)
ssp. rioensis Patterson 1943
Karyotype:
3R, 1V, 1D
Wharton (1943)
Y is a short rod
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: meridionalis Wasserman 1962b
Karyotype:
3R, 1V, 1D
Wasserman (1962b)
X is a rod; Y is short rod
Listings:
Cytological: Clayton and Wheeler; Clayton and Guest
Taxonomic: Wheeler (1981)

Species: mesophragmatica Duda 1927
Karyotype:
3R, 1V, 1D
Pavan and Cunha (1947)
3R, 1V, 1r
Brncic and Santibanez (1957)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: mettleri Heed 1977
Karyotype:
5R, 1r
Heed (1977)
Y is rod-shaped
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: metzii Sturtevant 1921
Karyotype:
5R, 1D
Clayton and Wasserman (1957)
Listings:

Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: micromelanica Patterson in Sturtevant and Novitski 1941
Karyotype:
5R, 1D
Patterson (1941a)
2R, 1V, 1v, 1D
Patterson and Wheeler (1942); Wharton (1943)
4R, 1v, 1D
Ward (1949); Clayton and Ward (1954)
Stalker (1965)
4R, 1v, 1Dr
Ward (1949); Stalker (1965)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: micromyia Hardy and Kaneshiro 1975
Karyotype:
5R, lD
Carson and Yoon (1982)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: mimica Hardy 1965
Karyotype:
6R
Clayton (1966)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: mitchelli Hardy 1965
Karyotype:
5R, 1D
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: mojavensis Patterson and Crow 1944
Karyotype:
5R, lD
Patterson and Crow (1940)
Y is short rod
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
syn. arizonensis
Karyotype:
5R, lD
Patterson and Wheeler (1942)
Y is short, 1/3 length of X

Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
ssp. baja Mettler 1963
Karyotype:
5R, 1D
Mettler (1961)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: moju Pavan 1950
Karyotype:
5R, 1Dr
Wasserman (1962c)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: mojuoides Wasserman 1962c
Karyotype:
5R, 1D
Wasserman (1960)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: montana Patterson and Wheeler 1942
Karyotype:
4R, 1v, 1D
Stone et al. (1942)
Listings:
Cytological: Clayton and Wheeler; Clayton and Guest
Taxonomic: Wheeler (1981)
syn. ovivovorum Lakovaara and Hackman 1973

Species: montgomeryi Hardy and Kaneshiro 1971
Karyotype:
6R
Clayton et al. (1972)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: moriwakii Okada and Kurokawa 1957
Karyotype:
3R, 1V, 1J, 1r
Tokumitsu et al. (1967)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: mulleri Sturtevant 1921
Karyotype:
5R, lD

Patterson and Crow (1940);
Wharton (1943)
4R, 1V, 1D
Metz (1916b)
Reported as repleta. variety b
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: munda Spencer 1942
Karyotype:
1R, 2V, 1D
Spencer (1942)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: murphyi Hardy and Kaneshiro 1969
Karyotype:
5R, 1D
Clayton (1969)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: musaphilia Hardy 1965
Karyotype:
5R, lD
Clayton (1969)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: mutandis Tan, Hsu and Sheng 1949
Karyotype:
5R, lD
Tan et al. (1949)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: nannoptera Wheeler 1949a
Karyotype:
3V, 1J, 1v
Ward (1949)
X is large V; Y is a rod
Ward and Heed (1970) Y is J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: nasuta Lamb 1914
Karyotype:
2R, 1V, 1D
Wakahama and Kitagawa (1972)

X is a rod; Y is J-shaped
(as nasuta nasuta)
2R, 1V, 1D
Rajasekarasetty et al. (1979)
X is rod-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: nasutoides Okada 1964
Karyotype:
2R, 1V, 1v
Wheeler et al. (1973)
X is rod-shaped; Y is J-shaped
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: neocardini Streisinger 1946
Karyotype:
1R, 2V, 1D
Streisinger (1946)
Ward (1949)
Y is a small v
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: neogrimshawi Hardy and Kaneshiro 1968
Karyotype:
5R, lD
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
syn. grimshawi Bryan 1934 (Idiomyia)

Species: neoguaramunu Frydenberg 1956
Karyotype:
3V
Frydenberg (1956)
X is V-shaped; Y is a rod
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: neohydei Wasserman 1962a
Karyotype:
5R, lD
Wasserman (1962a)
X is J-shaped; Y is a rod
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: neohypocausta Lin and Wheeler in
Karyotype: Lin and Tseng 1973
2R, 1V
Wakahama et al. (1983)
Y chromosome is a rod
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: neomorpha Heed and Wheeler 1957
Karyotype:
1R, 2V, 1D
Heed and Wheeler (1957)
Y is a rod, shorter than X
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: neonasuta Sajjan and Krishnamurthy 1972
Karyotype:
2R, 1V, 1D
Sajjan and Krishnamurthy (1972)
X is rod-shaped; Y is J-shaped
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: neoperkinsi Hardy and Kaneshiro 1968
Karyotype:
5R, 1D
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
syn. perkinsi (Grimshaw) 1901 (Idiomyia)

Species: neopicta Hardy and Kaneshiro 1968
Karyotype:
5R, 1D
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
syn. picta (Grimshaw) 1901 (Idiomyia)

Species: neorepleta Patterson and Wheeler 1942
Karyotype:
4R, 2J
Wharton (1943)
X is J-shaped; Y is short rod
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
syn. canapalpa = neorepleta
Karyotype:

5R, 1V
Patterson and Mainland (1944)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: nigra Grimshaw 1901
Karyotype:
5R, lD
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: nigribasis Hardy 1969
Karyotype:
5R, 1D
Clayton (1968)
Reported as bruneipennis
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
syn. brunneipennis (Hardy) 1965 (Idiomyia)

Species: nigricruria Patterson and Mainland in Patterson 1943
Karyotype:
5R, lD
Wharton (1943)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: nigrilineata Angus 1967
Karyotype:
5R, 1D
Angus (1967)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: nigrodunni Heed and Wheeler 1957
Karyotype:
1R, 2V, 1D
Heed and Wheeler (1957)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: nigrohydei Patterson and Wheeler 1942
Karyotype:
6R
Patterson and Wheeler (1942)
Y is a very short rod
Listings:
Cytological: Clayton and Wheeler

Taxonomic: Wheeler (1981)
syn. hydeoides Patterson and Wheeler 1942

Species: nigromaculata Kikkawa and Peng 1938
Karyotype:
5R, 1D
Momma (1954); Tokumitsu et al. (1967)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: nigromelanica Patterson and Wheeler 1942
Karyotype:
1V, 1v, 2R, 1D
Patterson and Wheeler (1942)
X is a large V
1V, 1v, 2R, 1Dv
Wharton (1943)
1V, 1v, 2R, 1Dr
Stalker (1964)
1V, 1v, 3R
Ward (1949)
X is a large V; Y is V-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: nigrospiracula Patterson and Wheeler 1942
Karyotype:
5R, 1D
Patterson and Wheeler (1942)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: niveifrons Okada and Carson 1982a
Karyotype:
2R, 1V, 1D
Wakahama et al. (1983)
X is a rod; Y is J-shaped; dot is elongated
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1986)

Species: nixifrons Tan, Hsu and Sheng 1949
Karyotype:
3R, 1V, 1D
Tan et al. (1949)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: novamexicana Patterson 1941
Karyotype:
5R, 1D

Patterson (1941b)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: novemaristata Dobzhansky and Pavan 1943
Karyotype:
6R
Dobzhansky and Pavan (1943)
Microchromosome is a large dot (or short rod)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: oahuensis (Grimshaw) 1901 (Idiomyia)
Karyotype:
5R, lD
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: obatai Hardy and Kaneshiro 1972
Karyotype:
5R, lD
Clayton et al. (1972)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (l981)

Species: obscuripes (Grimshaw) 1901 (Idiomyia)
Karyotype:
5R, lD
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: occidentalis Spencer 1942
Karyotype:
5R. lD
Spencer (1942)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: ocellata Hardy and Kaneshiro 1969
Karyotype:
5R, lD
Clayton (1969)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: ochracea Grimshaw 1901

Karyotype:
5R, lD
Carson et al. (1967)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: ochrobasis Hardy and Kaneshiro 1968
Karyotype:
5R, lD
Clayton (1969)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (l981)

Species: odontophallus Hardy and Kaneshiro 1968
Karyotype:
5R, lD
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: olaae Grimshaw 1901
Karyotype:
n = 6 (Yoon and Wheeler, 1973)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: onca Dobzhansky and Pavan 1943
Karyotype:
1R, 1V, 1J, 1D
Dobzhansky and Pavan (1943)
X is a rod; Y is J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: orkui Brncic and Santibanez 1957
Karyotype:
4R, 1V
Brncic and Santibanez (1957)
One rod 1/2 length of others
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (l981)

Species: ornata Hardy and Kaneshiro 1969
Karyotype:
5R, lD
Clayton (1969)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: ornatipennis Williston 1896
Karyotype:
3R, lV, 1D
Metz (1916a, 1916b)
Reported as calloptera
3R, 1V, 3D
Clayton and Wasserman (1957)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: orphnopeza Hardy and Kaneshiro 1968
Karyotype:
5R, lD
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: orthofascia Hardy and kaneshiro 1968
Karyotype:
5R, lD
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler; Clayton and Guest
Taxonomic: Wheeler (1981)

Species: pachea Patterson and Wheeler 1942
Karyotype:
2R, 1V, 2J
Ward and Heed (1970)
Y is short rod; X is large V
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: pachuca Wasserman 1962b
Karyotype:
5R, lD
Wasserman (1962b)
Y is J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (198l)

Species: paenehamifera Hardy and Kaneshiro 1969
Karyotype:
5R, lD
Clayton (1969)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: pallidifrons Wheeler 1969

Karyotype:
2R, 1V, 1D
Wilson et al. (1969)
X and Y are rods
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (9181)

Species: pallidipennis Dobzhansky and Pavan 1943
Karyotype:
4R, 1V, 1D
Dobzhansky and Pavan (1943)
X and Y are V-shaped

Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
ssp. centralis Patterson and Mainland 1944
Karyotype:
4R, 1V, 1D
Patterson and Mainland (1944)
X and Y are V-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: palustris Spencer 1942
Karyotype:
5R, lD
Wharton (1943)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeleer (1981)

Species: paracanalinea Wheeler 1957
Karyotype:
1R, 1V, 1J, 1D
Clayton and Wasserman (1957)
J has a satellite
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: parachrogaster Patterson and Mainland, in Patterson 1943
Karyotype:
4R, lV, 1D
Wharton (1943)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: paraguttata Thompson in Wheeler 1957
Karyotype:
5R, lD
Clayton and Wasserman (1957)

Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: paramediostriata Townsend and Wheeler 1955
Karyotype:
5R, 1D
Townsend and Wheeler (1955)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: paramelanica Patterson 1942 (as ssp. of melanica)
Karyotype:
2R, 2V, 1D
Griffen (1942); Wharton (1943)
3R, 2V
Ward (1949)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: paranaensis Barros 1950
Karyotype:
3R, 1V, 1D
Barros (1950); Dreyfus and Barros (1949)
3R, 1V, 1v
Clayton and Ward (1954); Clayton and Wasserman (1957)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

(Species: pararubida = hypocausta)

Species: parthenogenetica Stalker 1953
Karyotype:
1R, 2V, 1D
Stalker (1953)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: paucicilia Hardy and Kaneshiro 1971
Karyotype:
5R, 1D
Clayton et al. (1972)
Listings:
Cytological: Clayton and wheeler
Taxonomic: Wheeler (1981)

Species: paucipuncta Grimshaw 1901
Karyotype:
5R, 1D
Carson et al. (1967)
Listings:

Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: pavani Brncic 1957a
Karyotype:
3R, 1V, 1D
Brncic and Santibanez (1951)
Y has subterminal constriction
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: pectinitarsus Hardy 1965
Karyotype:
5R, lD
Clayton (1966)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: pegasa Wasserman 1962b
Karyotype:
5R, 1v
Wasserman (1982)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: pellewae Pipkin and Heed 1964
Karyotype:
5R, lD
Pipkin and Heed (1964)
Y is J-shaped
Listings:
Cytological: Clayton and Wheeleer
Taxonomic: Wheeler (1981)

Species: pengi Okada and Kurokawa 1957
Karyotype:
2R, 1V, 1v, 1D
Kikkawa and Peng (1938); Okada and Kurokawa (1957)
(Reported as melanissima)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: peniculipedis Hardy 1965
Karyotype:
5R, lD
Clayton (1969)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: peninsularis Patterson and Wheeler 1942

Karyotype:
5R, lD
Patterson and Wheeler (1942)
Y is small, v-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: percnosoma Hardy 1965
Karyotype:
5R, lD
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: phalerata Meigen 1830
Karyotype:
5R, lD
Frolova (1926)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: picta Zetterstedt 1847
Karyotype:
2n = 12
Tsacas (1970) (as Hirtodrosophila)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: picticornis Grimshaw 1901
Karyotype:
5R, lD
Clayton (1966)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: pictilis Wasserman 1962c
Karyotype:
6R
Wasserman (1960)
Y is short rod (Reported as species J)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: pictura Wasserman 1962c
Karyotype:
4R, 1V, 1J
Wasserman (1960)
X is J-shaped; Y is short rod
Listings:

Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: pilimana Grimshaw 1901
Karyotype:
5R, lD
Clayton (1966)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: pinicola Sturtevant 1942
Karyotype:
1R, 2V
Sturtevant (1942)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: planitibia (Hardy) 1966 (Idiomyia)
Karyotype:
5R, lD
Carson et al. (1967)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: polliciforma Hardy 1965
Karyotype:
5R, lD
Clayton et al. (1972)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: polychaeta Patterson and Wheeler 1942
Karyotype:
2R, 2J, 1V, 1D
Patterson and Wheeler (1942)
X and Y are rods
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: polymorpha Dobzhansky and Pavan 1943
Karyotype:
1R, 2V, 1D
Dobzhansky and Pavan (1943)
Y is a short rod
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: ponera Tsacas and David 1975
Karyotype:

5R, lD
Tsacas and David (1975)
X and Y are rod-shaped
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: primaeva Hardy and Kaneshiro 1968
Karyotype:
5R, lD
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: procardinoides Frydenberg 1956
Karyotype:
1R, 2V, lD
Frydenberg (1956)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: prolaticilia Hardy 1965
Karyotype:
5R, lD
Carson et al. (1967)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: promeridiana Wasserman 1962b
Karyotype:
3R, 1V, 1D
Wasserman (1962b)
Y is short rod; X is a rod
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: propachuca Wasserman 1962b
Karyotype:
3R, 1V, 1D
Wasserman (1962b)
Y is J-shaped
Listings:
Cytological: Clayton and Wheelere
Taxonomic: Wheeler (1981)

Species: prosimilis Duda 1927
Karyotype:
5R, lD
Dobzhansky and Pavan (1943)
Listings:
Cytological: Clayton and Wheeler

Taxonomic: Wheeler (1981)

Species: prostopalpis Hardy and Kaneshiro 1968
Karyotype:
4R, 1V, 1D
Clayton (1971)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: pseudosordidula Kaneko, Tokumitsu and Takada 1964
Karyotype:
3R, 1V, 1D
Kaneko et al. (1964)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: pseudotetrachaeta Angus 1967
Karyotype:
5R, 1D
Angus (1967)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: psilophallus Hardy and Kaneshiro 1971
Karyotype:
6R
Clayton (1971)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: psilotarsalis Hardy and Kaneshiro 1975
Karyotype:
5R, 1D
Clayton (1976)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: pulaua Wheeler in Wilson et al. 1969
Karyotype:
2R, 1V, 1D
Wilson et al. (1969)
X and Y are rods
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: pullata Tan, Hsu and Sheng 1949
Karyotype:
4V, lD
Tan et al. (1949)

Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: pullipes Hardy and Kaneshiro 1972
Karyotype:
5R, lD
Clayton et al. (1972)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: punalua Bryan 1934
Karyotype:
5R, lD
Clayton (1966)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: putrida Sturtevant 1916
Karyotype:
1R, 2V, 1D
Wharton (1943) (Stock from Texas)
1R, 2V, 2D
Wharton (1943) (Stock from Florida)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: pychnochaetae Hardy 1965
Karyotype:
5R, lD
Clayton (1966)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: quadrilineata Meijere 1911
Karyotype:
4R, 1V, 1D
Wakahama et al. (1983)
X is V-shaped; Y is a rod
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: quadrisetae Hardy 1965
Karyotype:
5R, lD
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: quasianomalipes Hardy 1965
Karyotype:
5R, lD
Clayton (1969)
Listings:
Cytological: Clayton and Wheeler
Taxonomic; Wheeler (1981)

Species: quinaria Loew 1866a
Karyotype:
1V, 1J, 1D
Metz (1914)
X is rod; Y is J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: racemova Patterson and Mainland 1944
Karyotype:
5R, lD
Patterson and Mainland (1944)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: ramsdeni Sturtevant 1916
Karyotype:
5R, lD
Metz 1916b
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: recticilia Hardy and Kaneshiro 1968
Karyotype:
5R, lD
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: repleta Wollaston 1858
Karyotype:
5R, lD
Metz (1914); Wharton (1943);
Clayton and Wasserman (1957)
4R, 1V, 1D
Metz (1916a)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
syn. austrorepleta Dobzhansky and Pavan 1943
syn. betari Dobzhansky and Pavan 1943
syn. brunneipalpa Dobzhansky and Pavan 1943
syn. melanopalpa Patterson and Wheeler 1942

Species: repletoides Hsu 1943
Karyotype:
3V, 1D
Moriwaki et al. (1956) (as chinoi)
2V, 1J, 1D
Hsiang (1949) (as tumiditarsus)
X is V-shaped; Y is shorter and J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
syn. chinoi Okada
syn. tumiditarsus Tan, Hsu and Sheng 1945

Species: reynoldsiae Hardy and Kaneshiro 1972
Karyotype:
5R, 1D
Clayton et al. (1972)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: ritae Patterson and Wheeler 1942
Karyotype:
5R, 1D
Patterson and Wheeler (1942)
X and Y are longest rods
5R, 1D
Wasserman (1962b) (as tira)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
syn. tira Wasserman 1962b

Species: robusta Sturtevant 1916
Karyotype:
1R, 2V, 1D
Metz (1916a)
3V, 1D
Carson and Stalker (1947)
X is largest V; Y is V-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: roehrae Pipkin and Heed 1964
Karyotype:
5R, 1D
Pipkin and Heed (1964)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: ruberrima Meijere 1911
Karyotype:

2R, 1V, 1D
Lin et al. (1974)
X and Y are rods
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: rubida Mather 1960
Karyotype:
2R, 1V, 1D
Mather (1960)
2R, 2V
Mather (1962)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: rubra Sturtevant 1927
Karyotype:
2R, 1V, 1D
Clyde (1980)
X is a rod; Y is J-shaped
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: rubrifrons Patterson and Wheeler 1942
Karyotype:
4R, 1J, 1r
Clayton and Ward (1954)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: sejuncta Hardy and Kaneshiro 1968
Karyotype:
5R, 1D
Clayton (1969)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: serenensis Brncic 1957
Karyotype:
5R, 1D
Brncic (1957)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: serido Vilela and Sene 1977
Karyotype:
5R, 1D
Wasserman and Koepfer (1979)
Y is submetacentric

Ruiz et al. (1982)
Y is metacentric
Baimai et al. (1983)
Six types with variations in dot chromosomes and Y
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: setosifrons Hardy and Kaneshiro 1968
Karyotype:
5R, 1D
Clayton (1969)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: setosimentum Hardy and Kaneshiro 1968
Karyotype:
5R, 1D
Clayton (1968)
Listings:
Cytological: Clayton and Wheelere
Taxonomic: Wheeler (1981)

Species: setula Heed and Wheeler 1957
Karyotype:
4R, 1V, 1D
Clayton and Wasserman (1957)
(Stock from Colombia)
3R, 1V, 1D
Clayton and Wasserman (1957)
(Stock from Panama Zone)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: silvarentis Hardy and Kaneshiro 1968
Karyotype:
5R, 1D
Clayton (1968)
Large dots
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: silvestris (Perkins) 1910 (Idiomyia)
Karyotype:
5R, 1D
Carson et al. (1967)
(Reported as nigrifacies)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
syn. nigrifacies (Hardy) 1965 (Idiomyia)

(Species: silvistriatus to Genus Zaprionus)

Species: similis Williston 1896
Karyotype:
1R, 2V, 1D
Metz (1916b)
Species identity uncertain
Heed and Krishnamurthy (1959)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
ssp. grenadiensis Heed 1962
Karyotype:
1R, 2V, 1D
Heed and Krishnamurthy (1959)
Reported as stock GR
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: sobrina Hardy and Kaneshiro 1971
Karyotype:
5R, 1D
Clayton et al. (1972)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: sodomae Hardy and Kaneshiro 1968
Karyotype:
5R, 1D
Clayton (1971)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: sordidula Kikkawa and Peng 1938
Karyotype:
2R, 2V, 1D
Kikkawa and Peng (1938)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: spaniothrix Hardy and Kaneshiro 1968
Karyotype:
5R, 1D
Clayton (1971)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: spectabilis Hardy 1965
Karyotype:
5R, 1D

Clayton (1966)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: spenceri Patterson 1943
Karyotype:
5R, 1D
Wasserman (1982)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: spinatermina Heed and Wheeler 1957
Karyotype:
3R, 1V, 1D
Heed and Wheeler (1957)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: sproati Hardy and Kaneshiro 1968
Karyotype:
5R, 1D
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: stalkeri Wheeler 1954
Karyotype:
5R, 1D
Clayton and Ward (1954)
Y is J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: starmeri Wasserman, Koepfer and Ward 1973
Karyotype:
5R, 1D
Wasserman and Koepfer (1979)
X has dotlike arm; Y is sub-metacentric
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: sternopleuralis Okada and Kurokawa 1957
Karyotype:
4R
Okada and Kurokawa (1957)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: sticta Wheeler 1957
Karyotype:
5R, 1D
Clayton and Wasserman (1957)
Rods identical-1 pair dots
Rods identical-1 dot, 1 rod
Rods identical-2 dots, 1 rod
(Three forms observed)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: subbadia Patterson and Mainland in Patterson 1943
Karyotype:
3R, 2V
Wharton (1943)
5R, 1V
King (1947)
X is V-shaped; Y is J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: subfunebris Stalker and Spencer 1939
Karyotype:
5R, 1D
Wharton (1943)
Y has proximal constriction
Listings:
Cytological: Clayton and Wasserman
Taxonomic: Wheeler (1981)

Species: submacroptera Patterson and Mainland in Patterson 1943
Karyotype:
1R, 1V, 2v, 1D
Wharton (1943)
X and Y are rods
(Guerrero, Mexico stock)
2V, 1J, 1D
Clayton and Wasserman (1957)
(Hidalgo, Puebla, and Vera Cruz, Mexico stocks)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: suboccidentalis Spencer 1942
Karyotype:
5R, 1D
Spencer (1942)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: subpalustris Spencer 1942
Karyotype:

5R, 1D
Spencer (1942)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

(Species: subquinaria = subspecies of transversa)

Species: substenoptera Hardy 1969
Karyotype:
5R, 1D
Clayton et al. (1972)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
syn. stenoptera (Hardy) 1965 (Idiomyia)

Species: suffusca Spencer in Patterson 1943
Karyotype:
1R, 1V, 1v, 1D
Spencer (1943)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: sulfurigaster (Duda) 1923 (Spinulophila)
Karyotype:
2R, 1V, 1D
Mather (1962) (as setifemur)
Wilson et al. (1969)
X is rod; Y is V- or J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
ssp. albostrigata Wheeler in Wilson et al. 1969
Karyotype:
2R, 1V, 1D
Wilson et al. (1969)
X is rod; Y is J- or V-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
ssp. bilimbata Bezzi 1928
Karyotype:
2R, 1V, 1D
Patterson and Wheeler (1942)
(as spinofemora)
Wilson et al. (1969)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
ssp. sulfurigaster
syn. setifemur Malloch 1924
syn. willowsi Curran 1936

Species: talamancana Wheeler 1968
Karyotype:
4R, 1V
Wheeler (1968)
X is rod-shaped; Y is J- or V-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic; Wheeler (1981)

Species: tarphytrichia Hardy 1965
Karyotype:
5R, 1D
Clayton et al. (1972)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: tendomentum Hardy 1965
Karyotype:
n = 6
Yoon and Wheeler (1973)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: tenebrosa Spencer in Patterson 1943
Karyotype:
3R, 1J, 1D
Wharton (1943)
Y is J-shaped
3R, 1V, 1D
Blumel (1949)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species; tenuicauda Okada 1956
Karyotype:
4R, 1V, 1D
Kang et al. (1964)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: testacea Roser 1840
Karyotype:
2R, 1V, 1D
Wharton (1943)
X is a rod
Listings:
Cytological: Clayton and Wheeler
Taxonomic; Wheeler (l981)
syn. setosa Villenueve 1921

(Species: texana = ssp. americana)

(Species: tira = ritae)

Species: tongpua Lin and Tseng 1973
Karyotype:
3R, 1V
Lin et al. (1974)
X and Y are rod-shaped
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: touchardiae Hardy and Kaneshiro 1972
Karyotype:
5R, 1D
Clayton et al. (1972)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: tranquilla Spencer in Patterson 1943
Karyotype:
2R, 1V
Wharton (1943)
Chihuahua, Mexico stock
2R, 1V, 1D
Clayton and Ward (1954)
Puebla, Mexico stock
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: transversa Fallen 1823
Karyotype:
5R, 1D
Frolova (1926); Kim (1965)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
ssp. subquinaria Spencer 1942
Karyotype:
5R, 1D
Spencer (1942) (as species)
Y is small, V-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: trapeza Heed and Wheeler 1957
Karyotype:
5R, 1D
Clayton and Wasserman (1957)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: triangula Wheeler (1949a)
Karyotype:
4R, 1J, 1D
Clayton and Ward (1954)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: trifiloides Wheeler 1957
Karyotype:
5R
Clayton and Wasserman (1957)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: tripunctata Loew 1862
Karyotype:
3R, 1V
Metz (1914; 1916b)
5R, 1D
Metz and Moses (1923)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: trispina Wheeler 1949
Karyotype:
5R, 2D
Ward (1949)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: tristriata Heed and Wheeler 1957
Karyotype:
5R, 1D
Clayton and Wasserman (1957)
One rod is double-length
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: truncipenna Hardy 1965
Karyotype:
5R, 1D
Clayton (1969)
One rod extremely large
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: tsigana Burla and Gloor 1952
Karyotype:

2R, 1V, 1J, 1D
Burla and Gloor (1952)
X is rod-shaped; Y is J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

(Species: tumiditarsus = repletoides)

Species: turbata Hardy and Kaneshiro 1969
Karyotype:
5R, 1D
Clayton (1971)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: unipunctata Patterson and Mainland in Patterson 1943
Karyotype:
2R, 1V, 1J, 1D
Wharton (1943)
Listings:
Cytological; Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: uniseriata Hardy and Kaneshiro 1968
Karyotype:
6R
Clayton et al. (1972)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: uniseta Wasserman, Koepfer and Ward 1973
Karyotype:
5R, 1D
Wasserman and Koepfer (1979)
Y is submetacentric; median constriction in long arm
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: unispina Okada 1956
Karyotype:
5R, 1D
Kang et al. (1964)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: varipennis (Grimshaw) 1901 (Hypenomyia)
Karyotype:
5R, 1D
Clayton (1971)
Listings:

Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: vesciseta Hardy and Kaneshiro 1968
Karyotype:
5R, 1D
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: villosipedis Hardy 1965
Karyotype:
5R, 1D
Clayton (1966)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: viracochi Brncic and Santibanez 1957
Karyotype:
3R, 1V, 1D
Brncic and Santibanez (1957)
Y has subterminal constriction
Listings:
Cytological: Clayton and Wheeler
Taxonomic; Wheeler (1981)

Species: virgulata Hardy and Kaneshiro 1968
Karyotype:
5R, 1D
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: virilis Sturtevant 1916
Karyotype:
5R, 1D
Metz (1914)
(Reported as species B)
Listings:
Cytological: Clayton and Wheeler
Taxonomnic: Wheeler (1981)

Species: wheeleri Patterson and Alexander 1952
Karyotype:
5R, 1D
Patterson and Alexander (1952)
Y is a short rod
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: wingei Cordeiro 1964

Karyotype:
3V
Cordeiro (1964)
Largest V has satellite
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: xanthogaster Duda 1924
Karyotype:
2R, 2V, 1D
Lin et al. (1974)
X is metacentric; Y is sub-metacentric
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Genus: Drosophila
Subgenus: Engiscaptomyza

Species: amplilobus Hardy 1966
Karyotype:
3R, 1V, 1D
Clayton (1966)
(Reported as crassifemur)

Species: crassifemur Grimshaw 1901
Karyotype:
3R, 1V, 1D
Clayton (1968)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: inflatus Kaneshiro 1969
Karyotype:
3R, 1V, 1D
Yoon et al. (1975)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: nasalis Grimshaw 1901
Karyotype:
2R, 2V, lD
Clayton (1966)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: reducta Hardy 1965
Karyotype:
3R, 1V, 1D
Clayton (1968)
(Reported as crassifemur)

Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Subgenus: Hirtodrosophila

Species: alboralis Momma and Takada 1954
Karyotype:
5R, 1D
Momma (1954)
Y is shorter than X
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: confusa Staeger 1844
Karyotype:
5R, 1D
Burla (1950a) (as grischuna)
Okada and Kurokawa (1957)
(as histrioides)
3R, 1V, 1D
Kang et al. (1964) (as histrioides)
Listings:
Cytological: Clayton and Wheeler; Clayton and Guest
Taxonomic: Wheeler (1981)
syn. grischuna Burla 1954
syn. histrioides Okada and Kurokawa 1957
syn. vibrissima Duda 1924

Species: duncani Sturtevant 1918
Karyotype:
2V, 2v, 1Dv
Wharton (1943)
X is V-shaped; Y is a rod
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: grisea Patterson and Wheeler 1942
Karyotype:
5R, 1D
Clayton and Ward (1954)
Clayton in Patterson and Stone (1952); Male is XO
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: longala Patterson and Wheeler 1942
Karyotype:
5R, 1D
Patterson and Stone (1952)
Male is XO
Listings:
Cytological: Clayton and Wheeler

Taxonomic: Wheeler (1981)

Species: orbospiracula Patterson and Wheeler 1942
Karyotype:
5R, 1D
Patterson and Wheeler (1942)
Male is XO
Listings:
Cytological: Clayton and Wheeler
Taxonomic; Wheeler (1981)

Species: pictiventris Duda 1925
Karyotype:
1R, 1V, 1J, 1J
Clayton and Ward (1954)
Male is XO
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: subarctica Hackman 1969
Karyotype:
4R, 1J, 1D
Lakovaara and Sorsa (1970)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: thoracis Wiliston 1896
Karyotype:
5R, 1D
Clayton and Ward (1954)
Male is XO
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: trivittata Strobl 1893
Karyotype:
2R, 1V, 1v, 1D
Kikkawa and Peng (1938)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Subgenus: Scaptodrosophila

Species: brooksae Pipkin 1961 (as brooksi)
Karyotype:
1R, 2V, 1v
Pipkin (1961)
X is rod; Y is rod with constriction
Listings;
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (9181)

Species: bryani Malloch 1934
Karyotype:
1R, 2V
Mather (1956)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: cancellata Mather 1955
Karyotype:
3R, 1r, 1V
Mather (1956)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: coracina Kikkawa and Peng 1938
Karyotype:
1R, 2V, 1D
Kikkawa and Peng (1938)
Y is J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: enigma Malloch 1927
Karyotype:
4R, 2V
Mather (1956)
Listings:
Cytological: Clayton and Wheeler;
Clayton and Guest
Taxonomic: Wheeler (1981)

Species: fumida Mather 1960
Karyotype:
2R, 2V
Mather (1960)
Y is rod, half length of X
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: howensis Parsons and Bock 1979
Karyotype:
2R, 3V
Bock (1984)
X and Y are J-shaped; dot is a small v
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: latifasciaeformis Duda 1940
Karyotype:

1V, 2v
Dobzhansky and Pavan (1943)
(Reported as mirim)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
syn. mirim Dobzhansky and Pavan 1943
syn. baeomyia Wheeler 1949

Species: latifshahi Gupta and Ray-Chaudhuri 1970
Karyotype:
2R, 3V, 1D
Gupta (1973)
X and Y are rods
2R, 3J, 1D
Singh and Gupta (1979b)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: lativittata Malloch 1923
Karyotype:
4R, 1V, 1D
Bock (1984)
X and Y are rods
Listings:
Cytological: Clayton and Wheeler; Clayton and Guest
Taxonomic: Wheeler (1981)

Species: lebanonensis Wheeler 1949
Karyotype:
1R, 2V, 1v
Ward (1949)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

ssp. casteeli Pipkin 1961
Karyotype:
1R, 2V, 1v
Pipkin (1961)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: nitidithorax Malloch 1927
Karyotype:
4R, `V, 1D
Bock (1984)
X and Y are rods
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: novamaculosa Mather 1956

Karyotype:
6R
Mather (1956)
Listings:
Cytological: Clayton and Wheeler; Clayton and Guest
Taxonomic: Wheeler (1981)
syn. maculosa Mather 1955

(Species: novopaca = subnitida)

Species: paratriangulata Gupta and Ray-Chaudhuri 1970
Karyotype:
1R, 2V, 1D
Singh and Gupta (1979b)
X is a rod; Y is V-shaped
Listings:
Cytological; Clayton and Guest
Taxonomic: Wheeler (1981)

Species: pattersoni Pipkin 1956
Karyotype:
1V, 2v, 1D
Pipkin (1956)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: rufifrons Loew 1873
Karyotype:
2V, 2v
Buzzati-Traverso (1943)
(as nitens)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
syn. nitens Buzzati-Traverso 1943

Species: specensis Bock 1976
Karyotype:
4R, 1V, 1D
Bock (1984)
X and Y are rods
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: stonei Pipkin 1956
Karyotype:
1R, 1V, 1v, 1J
Pipkin (1956)
X is rod or J; Y is small, v-shaped; in some the large V's are J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler 1981

Species: subnitida Malloch 1927
Karyotype:
6R
Mather (1956)
One rod twice length of others
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
syn. novopaca Mather 1956

Species: subtilis Kikkawa and Peng 1938
Karyotype:
2R, 1V, 2J
Kikkawa and Peng (1938)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: victoria Sturtevant 1942
Karyotype:
1R, 1V, 1v, 1J
Wharton (1943)
Species identity uncertain; stock from Mexico
1V, 1v, 2J
Sturtevant (1942)
Stock from California
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (9181)

Subgenus: Siphlodora

Species: sigmoides Loew 1872
Karyotype:
5R, 1D
Butler and Mettler (1963)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Subgenus: Sophophora

Species: affinis Sturtevant 1916
Karyotype:
3R, 1V, 1D
Metz (1916a
1R, 2V, 1J, 1D
Kikkawa and Peng (1938)
1R, 1V, 2J, 1D
Miller and Stone (1962)
X is V-shaped; Y is J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: agumbensis Prakash and Reddy 1978
Karyotype:
1R, 2V, 1D
Prakash and Reddy (1978)
X is a rod; Y is J-shaped
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: algonquin Sturtevant and Dobzhansky 1936
Karyotype:
1R, 1V, 2J, 1D
Sturtevant and Dobzhansky (1936)
Miller and Stone (1962)
Pericentric inversion in V to form J in some
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: alpina Burla 1948
Karyotype:
1R, 2V
Moriwaki et al. (1956)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: ambigua Pomini 1940
Karyotype:
2V, 2J, 1D
Buzzati-Traverso (1941)
X is large V; Y is a rod
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: ananassae Doleschall 1858
Karyotype:
4V
Metz (1916b)
(Reported as caribea)
Kikkawa and Peng (1938)
X is large V; Y is J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: andamanensis Gupta and Ray-Chaudhuri 1970
Karyotype:
2V, 1J. 1D
Singh and Gupta (1979a)
X is J-shaped; Y is small rod
Listings:
Cytological: Clayton and Guest

Taxonomic: Wheeler (1981)

Species: anomelani Reddy and Krishnamurthy 1973
Karyotype:
1R, 2V, 1D
Reddy and Krishnamurthy (1973)
X is a rod; Y is V-shaped
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: athabasca Sturtevant and Dobzhansky 1936
Karyotype:
1R, 1V, 2J
Sturtevant and Dobzhansky (1936)
1R, 1V, 2J, 1D
Miller and Stone (1962)
X is V-shaped; rod has sub-terminal centromere
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: atripex Bock and Wheeler 1972
Karyotype:
4V
Kaneshiro and Wheeler (1970)
(Reported as "species 2")
Y is J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: auraria Peng 1937
Karyotype:
1R, 2V, 1D
Kikkawa and Peng (1938)
Y is a long rod
Listings:
Cytological: Clayton and Wheeler;
Clayton and Guest
Taxonomic: Wheeler (1981)

Species: austrosaltans Spassky 1957
Karyotype:
1R, 2V
Spassky (1957)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: azteca Sturtevant and Dobzhansky 1936
Karyotype:
1V, 3J, 1D
Sturtevant and Dobzhansky (1936)
1R, 2V, 1J, 1D

Kikkawa and Peng (1938)
1R, 1V, 2J, 1D
Miller and Stone (1962)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: baimaii Bock and Wheeler 1972
Karyotype:
1R, 2V
Bock and Wheeler (1972)
X is a rod; Y is a short rod
2V, 1J, 1D
Baimai (1980)
X and Y are J-shaped
Listings:
Cytological: Clayton and Wheeler; Clayton and Guest
Taxonomic: Wheeler (1981)

Species: bakoue Tsacas and Lachaise 1974
Karyotype:
1R, 2V, 1D
Lemeunier and Ashburner in Clayton and Guest (1986)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: barbarae Bock and Wheeler 1972
Karyotype:
1R, 2V
Bock and Wheeler (1972)
X is rod; Y is short rod
1J, 2V, 1D
Baimai (1980)
X and Y are J-shaped
Listings:
Cytological: Clayton and Wheeler; Clayton and Guest
Taxonomic: Wheeler (1981)

Species: biarmipes Malloch 1924
Karyotype:
1R, 2V, 1D
Singh and Gupta (1979b)
X is a rod; Y is a small rod
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: biauraria Bock and Wheeler 1972
Karyotype:
1R, 2V
Bock and Wheeler (1972)
X is rod; Y is short rod
Listings:
Cytological: Clayton and Wheeler

Taxonomic: Wheeler (1981)

Species: bicornuta Bock and Wheeler 1972
Karyotype:
1R, 2V, 1D
Bock and Wheeler (1972)
X is rod; Y is short rod
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: bifasciata Pomini 1940
Karyotype:
2V, 2J, 1D
Buzzatii-Traverso (1941)
X is large V; Y is rod; dots are large
Ashburner in Clayton and Guest(1986)
Listings:
Cytological: Clayton and Wheeler; Clayton and Guest
Taxonomic: Wheeler (1981)

Species: bipectinata Duda 1923
Karyotype:
4V
Kikkawa and Peng (1938)
X is medium V; Y is V-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
syn. szentivanii Mather and Dobzhansky 1962

Species: birchii Dobzhansky and Mather 1962
Karyotype:
2V, 1D, +1
Baimai (1969)
Pair of sex chromosomes of various types
Listings:
Cytological: Clayton and Wheeler; Clayton and Guest
Taxonomic: Wheeler (1981)

Species: bocainensis Pavan and Cunha 1947
Karyotype:
1R, 2V
Pavan and Cunha (1947)
Clayton and Wasserman (1957)
X is V-shaped; Y is J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: bocainoides Carson 1954
Karyotype:
1R, 2V
Carson (1954)
Listings:

Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: bocki Baimai 1979
Karyotype:
2V, 1J. 1D
Baimai (1979)
X is J-shaped; Y is small v
Baimai (1980)
X is J-shaped; Y is large V
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: bocqueti Tsacas and Lachaise 1974
Karyotype:
2V, 1J, 1D
Lemeunier and Ashburner in Clayton and Guest (1986)
X is J-shaped; Y is short rod
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: capricorni Dobzhansky and Pavan 1943
Karyotype:
1R, 2V
Dobzhansky and Pavan (1943
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: cordata Sturtevant 1942
Karyotype:
1R, 2V
Sturtevant 1942
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: cuauhtemoci Felix and Dobzhansky in Felix et al. 1976
Karyotype:
3R, 1V, 1D
Felix et al. (1976)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: davidi Tsacas 1975
Karyotype:
1R, 2V, 1D
Tsacas (1975)
X is a rod; y is J-shaped
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: denticulata Bock and Wheeler 1972
Karyotype:
1R, 2V
Bock and Wheeler (1972)
X is rod-shaped; Y is slightly longer rod with spherical expansion at end
Listings:
Cytological: Clayton and Wheeler
Taxonomic; Wheeler (1981)

Species: dominicana Ayala 1965
Karyotype:
2V, 1J, 1D
Baimai (1980)
X is J-shaped; Y is V-shaped
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: elegans Bock and Wheeler 1972
Karyotype:
5R
Bock and Wheeler (1972)
Y is short, J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: elliptica Sturtevant 1942
Karyotype:
4R, 1v, 1j
Sturtevant (1942)
1R, 2V
Clayton and Ward (1954); Clayton and Wasserman (1957)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: emarginata Sturtevant 1942
Karyotype:
1R, 2V
Sturtevant (1942)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: equinoxialis Dobzhansky 1946
Karyotype:
1R, 2V
Burla et al. (1949)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: ercepeae Tsacas and David 1975
Karyotype:
4V
Tsacas and David (1975)
X is V-shaped; Y is J-shaped
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: erecta Tsacas and Lachaise 1974
Karyotype:
1R, 2V, 1D
Lemeunier and Ashburner:
(1976) X is rod; Y is J-shaped
(1978) X and Y are acrocentric
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: eskoi Lakovaara and Lankinen 1974
Karyotype:
4V, 1D
Lakovaara and Saura (1982)
X is slightly metacentric; Y is a rod
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: eugracilis Bock and Wheeler 1972
Karyotype:
1R, 2V
Bock and Wheeler (1972)
X is short rod; Y is thick, heterochromatic rod
1R, 2V, 1D
Singh and Gupta (1979b)
X and Y are rods

Species: ficusphila Kikkawa and Peng 1938
Karyotype:
2V, 1J. 1D
Singh and Gupta (1979b)
X is J-shaped; Y is a rod
Listings:
Cytological: Clayton and Wheeler; Clayton and Guest
Taxonomic: Wheeler (1981)

Species: fumipennis Duda 1925
Karyotype:
1R, 2V
Dobzhansky and Pavan (1943)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: giriensis Prakash and Reddy 1977

Karyotype:
1R, 2V, 1D
Prakash and Reddy (1977)
X is rod-shaped; Y is J-shaped
Listings:
Cytological: Clayton and Guest
Taxonomic: wheeler (1981)

Species: greeni Bock and Wheeler 1972
Karyotype:
1R, 2V, 1D
Lemeunier and Ashburner in Clayton and Guest (1986)
X is rod; Y and dot are short rods
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: guanche Monclus 1976
Karyotype:
3R, 2J
Prevosti (1976)
X is acrocentric; Y is dot
5R
Gonzalez et al. (1983)
Listings:
Cytological: Clayton and Guest
Taxonomic; Wheeler (1981)

Species: helvetica Burla 1948
Karyotype:
1R, 1V, 2J, 1D
Burla (1948)
Miller and Stone (1962)
Like algonquin except Y is rod instead of a J
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: imaii Moriwaki and Okada 1967
Karyotype:
4V, 1D
Moriwaki et al. (1967)
X is V-shaped; Y is a rod
2R, 3V, 1D
Lakovaara and Saura (1982)
X is V-shaped; Y is a rod

Species: insularis Dobzhansky in Dobzhansky, Ehrman and Pavlovsky 1957
Karyotype:
1R, 2V
Dobzhansky et al. (1957)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: jambulina Parshad and Paika 1964
Karyotype:
1R, 2V, 1r
Singh and Gupta (1979b)
X is long rod; Y is J-shaped; dot is short rod with knob
1R, 2V, 1J
Lakhotia and Mishra (1980)
X is a rod; Y is a small J
1J, 2V, 1D
Baimai (1980)
Medium V-shaped Y; X is J-shaped
Listings:
Cytological: Clayton and Wheeler; Clayton and Guest
Taxonomic: Wheeler (1981)

Species: kanapiae Bock and Wheeler 1972
Karyotype:
1R, 2V
Bock and Wheeler (1972)
X is rod; Y is small, densely heterochromatic
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: khaoyana Bock and Wheeler 1972
Karyotype:
1R, 2V
Bock and Wheeler (1972)
X is rod; Y is short rod
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: kikkawai Burla 1954
Karyotype:
2R, 2V
Ward (1949)
X is rod; Y is rod or small v
(Reported as montium)
2V, 1J, 1v
Baimai (1969)
(Reported as montium)
Extensive variation in metaphases.
Baimai and Chumchong (1980)
Large V-shaped Y; J-shaped
X; seven types of chromosome 4 or Y
Listings:
Cytological: Clayton and Wheeler; Clayton and Guest
Taxonomic: Wheeler (1981)

Species: lacteicornis Okada 1965
Karyotype:
2V, lJ, 1D
Baimai (1980)
Rod-shaped Y; X is J-shaped

Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: lamottei Tsacas 1980
Karyotype:
1R, 2V
Tsacas (1980)
X and Y are equal-sized rods
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: leontia Tsacas and David 1977
Karyotype:
lR, 2V, 1D
David et al. (1978)
X is rod-shaped;Y is V-shaped
1R, 2V, lJ
Baimai (1980)
X and Y are J-shaped
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: lini Bock and Wheeler 1972
Karyotype:
1R, 2V, 2r
Bock and Wheeler (1972)
X is rod: Y is short
1R, 2V, 1J
Baimai (1980)
X and Y are J-shaped
Listings:
Cytological: Clayton and Wheeler; Clayton and Guest
Taxonomic: Wheeler (1981)

Species: lowei Heed, Crumpacker and Ehrman 1968
Karyotype:
2R, 2V, 1D
Heed et al. (1969)
X is large V; Y is J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: lucipennis Lin in Bock and Wheeler 1972
Karyotype:
1R, 2V
Bock and Wheeler (1972)
X is rod; Y is small rod
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: lusaltans Magalhaes 1962
Karyotype:
1R, 2V
Magalhaes (1962)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

(lutea = lutescens)

Species: lutescens Okada 1975
Karyotype:
1R, 2V, 1D
Fukatami (1975)
X is acrocentric; (reported as lutea)
Kikkawa and Peng (1938)
Y is a short rod (as lutea)
Listings:
Cytological: Clayton and Wheeler; Clayton and Guest
Taxonomic: Wheeler (1981)
syn. lutea Kikkawa and Peng (1938)

Species: malerkotliana Parshad and Paika 1964
Karyotype:
4V
Kaneshiro and Wheeler (1970)
X and Y are V-shaped; reported as species l0 and ll
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
ssp. pallens Bock and Wheeler 1972
Karyotype:
4V
Kaneshiro and Wheeler (1970)
Y is J-shaped; reported as species l0 and ll
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: mangabeirai Malogolowkin 1951
Karyotype:
1R, 2V
Carson et al. (1957)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: mauritiana Tsacas and David 1974
Karyotype:
1R, 2V, 1D
Tsacas and David (1974)
Lemeumier et al. (1978)
X is acrocentric; Y is sub-acrocentric
Listings:
Cytological: Clayton and Guest

Taxonomic: Wheeler (1981)

Species: mayri Mather and Dobzhansky 1962
Karyotype:
1R, 2V, 1D
Mather and Dobzhansky (1962)
2V, 1J, 1D
Baimai (1980)
X and Y are J-shaped
Listings:
Cytological: Clayton and Wheeler; Clayton and Guest
Taxonomic: Wheeler (1981)

(Species: mcclintockae to subgenus Drosophila)

Species: melanogaster Meigen 1830
Karyotype:
1R, 2V,1D
Metz (1914)
Y is J-shaped
Stevens (1912)
(Reported as ampelophila)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
syn. ampelophila Loew 1862

Species: mimetica Bock and Wheeler 1972
Karyotype:
1R, 2V, 1D
Bock and Wheeler (1972)
X is a rod; Y is small rod
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: miranda Dobzhansky 1935
Karyotype:
3R, 1V, 1D
Dobzhansky (1935)
In males, only nine chromosomes; Y is J-shaped; Xl is V and unpaired; X2 is a rod
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: montium Meijere 1916
Karyotype:
2R. 2V
Kikkawa (1936)
Y is V-shaped; X is rod
(Species identity uncertain)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: nagaholensis Prakash and Reddy 1980
Karyotype:
1R, 2V, 1D
Prakash and Reddy (1980)
X is rod-shaped; Y is short rod
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: narragansett Sturtevant and Dobzhansky 1936
Karyotype:
1R, 1V, 2J, 1D
Sturtevant (1940)
Miller and Stone (1962)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: nebulosa Sturtevant 1916
Karyotype:
1R, 2V, 1D
Metz (1916a); Wharton (1943)
1R, 2V
Pavan (1946); Ward (1949);
Clayton and Ward (1954)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
syn. limbata Williston 1896

Species: neocordata Magalhaes 1956
Karyotype:
1R, 2V
Magalhaes (1956)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: neoelliptica Pavan and Magalhaes in Pavan 1950
Karyotype:
1R, 2V
Pavan (1950)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: neosaltans Pavan and Magalhaes in Pavan 1950
Karyotype:
1R, 2V
Pavan (1950)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: nepalensis Okada 1955
Karyotype:
1R, 2V, 1D
Parshad and Gandhi (1971)
X is rod; Y is J-shaped
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: nesoetes Bock and Wheeler 1972
Karyotype:
1R, 3V
Kaneshiro and Wheeler (1970)
X is V-shaped; Y is J-shaped
(Reported as species 3)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: nigrosaltans Magalhaes 1962
Karyotype:
1R, 2V
Magalhaes (1962)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: nikananu Burla 1954
Karyotype:
1R, 2V, 1D
Lemuenier and Ashburner in Clayton and Guest 1986
2V, 1J, 1D
Baimai (1980)
X is J-shaped; Y is V-shaped
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: obscura Fallen 1823
Karyotype:
2V, 2J, 1D
Frolova and Astaurov (1929)
X is V; Y is rod
2V, 1V, 1J, 1D
Buzzati-Traverso (1941)
2R, 3V, 1D
Ashburner in Clayton and Guest (1986)
Listings:
Cytological: Clayton and Wheeler; Clayton and Guest
Taxonomic: Wheeler (1981)

Species: orena Tsacas and David 1978
Karyotype:
1r, 3V
Lemeunier et al. (1978)

X and Y are metacentric
3V, 2D
Tsacas and David (1978)
X is V-shaped chromosome
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: orosa Bock and Wheeler 1972
Karyotype:
1R, 2V
Bock and Wheeler (1972)
X is a rod; Y is short
Listings:
Cytological: Clayton and Wheeler;
Clayton and Guest
Taxonomic: Wheeler (1981)

Species: pallidosa Bock and Wheeler 1972
Karyotype:
3V, 1v
Futch (1966)
(Reported as light ananassae)
Y is J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: parabipectinata Bock 1971
Karyotype:
3V, 1v
Kaneshiro and Wheeler (1970)
(Reported as species 7)
Listings:
Cytological: Clayton and Wheeler; Clayton and Guest
Taxonomic: Wheeler (1981)

Species: parabocainensis Carson 1954
Karyotype:
1R, 2V
Carson (1954)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: paralutea Bock and Wheeler 1972
Karyotype:
1R, 2V
Bock and Wheeler (1972)
X is rod; Y is short
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: parasaltans Magalhaes 1956

Karyotype:
1R, 2V
Magalhaes (1956)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: parvula Bock and Wheeler 1972
Karyotype:
1R, 2V
Bock and Wheeler (1972)
X is rod; Y is small, densely heterochromatic
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: paulistorum Dobzhansky and Pavan in Burla et al. 1949
Karyotype:
1R, 2V
Dobzhansky and Pavan (1943)
Listings:
Cytological: Clayton and Wheeler
Taxonomic; Wheeler (1981)

Species: pavlovskiana Kastritsis and Dobzhansky 1967
Karyotype:
1R, 2V
Kastritsis and Dobzhansky (1967)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: pennae Bock and Wheeler 1972
Karyotype:
1R, 2V
Bock and Wheeler (1972)
X is long rod; Y is short
Listings:
Cytological: Clayton and Wheeler; Clayton and Guest
Taxonomic: Wheeler (1981)

Species: persimilis Dobzhansky and Epling 1944
Karyotype:
3R, 1V, 1D
Dobzhansky (1935)
X is V-shaped; Y is variable
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: phaeopleura Bock and Wheeler 1972
Karyotype:
4V
Kaneshiro and Wheeler (1970)
(Reported as species 5)

Y is J-shaped

Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: prolongata Singh and Gupta 1977
Karyotype:
1R, 2V, 1D
Singh and Gupta (1979b)
X and Y are rods
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: prosaltans Duda 1927
Karyotype:
1R, 2V
Wharton (1943)
X and Y are J-shaped
Dobzhansky and Pavan (1943)
Rod X and rod Y; X and Y are on one arm of V in some
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (198l)

Species: prostipennis Lin in Bock and Wheeler 1972
Karyotype:
1R, 2V
Bock and Wheeler (1972)
X is rod; Y is short, heterochromatic
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: pseudoananassae Bock 197l
Karyotype:
3V, 2v
Tonomura and Tobari (1978)
Y is rod-shaped
3V, 1v
Tonomura and Tobari (1978)
One autosomal V is absent
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
ssp. nigrens Bock and Wheeler 1972
Karyotype:
5V
Kaneshiro and Wheeler (1970)
X is V-shaped; Y is a rod
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: pseudomayri Baimai 1970

Karyotype:
2V, 1J, 1D
Baimai (1980)
X and Y are J-shaped
Listings:
Cytological: Clayton and Wheeler; Clayton and Guest
Taxonomic: Wheeler (1981)

Species: pseudoobscura Frolova in Frolova and Astaurov 1929
Karyotype:
3R, 1V, 1D
Metz (1916a, 1916b)
(Reported as obscura)
X is V; Y is rod
Dobzhansky (1935)
X is V; Y is variable
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
ssp. bogotana Ayala and Dobzhansky 1974
Karyotype:
3R, 1V, 1D
Ayala and Dobzhansky (1974)
Y is J-shaped
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: pseudosaltans Magalhaes 1956
Karyotype:
1R, 2V
Magalhaes (1956)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: pseudotakahashii Mather 1957
Karyotype:
1R, 2V, 1D
Mather (1956)
(Reported as takahashii)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: pulchrella Tan, Hsu and Sheng 1949
Karyotype:
1R, 2V, 1D
Tan et al. (1949)
Y is J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: punjabiensis Parshad and Paika 1964

Karyotype:
2V, 1J, 1D
Baimai (1980)
X and Y are J-shaped
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species quadraria Bock and Wheeler 1972
Karyotype:
1R, 2V
Bock and Wheeler (1972)
X is a rod; Y is short
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: rectangularis Sturtevant 1942
Karyotype:
1R, 2V
Sturtevant (1942)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: rhopaloa Bock and Wheeler 1972
Karyotype:
1R, 2V, 1D
Bock and Wheeler (1972)
X is rod; Y is short
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: rufa Kikkawa and Peng 1938
Karyotype:
1R, 2V, 1D
Kikkawa and Peng (1938)
1J, 2V, 1D
Baimai (1980)
X and Y are J-shaped
Listings:
Cytological: Clayton and Wheeler; Clayton and Guest
Taxonomic: Wheeler (1981)

Species: saltans Sturtevant 1916
Karyotype:
1R, 2V, 1D
Metz (1916b)
1R, 2V
Sturtevant (1942) (as sellata)
Wharton (1943)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

syn. sellata Sturtevant 1916

Species: seguyi Smart 1945
Karyotype:
2V, 1J, 1D
Baimai (1980)
X and Y are J-shaped
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: septentriosaltans Magalhaes and Bock in Magalhaes 1962
Karyotype:
1R, 2V
Magalhaes (1962)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: serrata Malloch 1927
Karyotype:
1R, 2V, 1D
Mather (1956)
1J, 2V, 1D
Baimai (1980)
X is J-shaped; Y is small v; dots are large
Listings:
Cytological: Clayton and Wheeler;
Clayton and Guest
Taxonomic: Wheeler (1981)

Species: simulans Sturtevant 1919
Karyotype:
1R, 2V, 1D
Patau (1935)
Kikkawa and Peng (1938)
X is a rod; Y is small rod or J
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: sturtevanti Duda 1927
Karyotype:
1R, 2V
Sturtevant (1942)
Dobzhansky and Pavan (1943)
X is V-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
syn. biopaca Sturtevant 1942
syn. earlei Sturtevant 1916

Species: subobscura Collin in Gordon 1936
Karyotype:

5R, 1D
Emmens (1937)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: subsaltans Magalhaes 1956
Karyotype:
1R, 2V
Magalhaes (1956)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: subsilvestris Hardy and Kaneshiro 1968
Karyotype:
2R, 3V, 1D
Knight (1956)
X is V-shaped; Y is J-shaped
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)
syn. silvestris Basden 1954

Species: sucinea Patterson and Mainland 1944
Karyotype:
1R, 2V
Patterson and Mainland (1944)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: suzukii (Matsumura) 1931 (Leucophenga)
Karyotype:
1R, 2V, 1D
Kikkawa and Peng (1938)
X is J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: takahashii Sturtevant 1927
Karyotype:
1R, 2V, 1D
Kikkawa and Peng (1938); Ward (1949)
X is rod; Y is short rod
2V, 1J
Sturtevant (1942)
Dot attached to X; X is J-shaped; Y is a short rod
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: teissieri Tsacas 1971
Karyotype:

1R, 2v, 1D
Lemeunier and Ashburner (1976)
X is a rod; Y is J-shaped
Lemeunier et al. (1978)
Acrocentric X and Y
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: tolteca Patterson and Mainland 1944
Karyotype:
1R, 2V, 1J, 1D
Ward (1949)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: triauraria Bock and Wheeler 1972
Karyotype:
1R, 2V
Bock and Wheeler (1972)
X is rod; Y is short
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: trilutea Bock and Wheeler 1972
Karyotype:
1R, 2V
Bock and Wheeler (1972)
X is rod; Y is short, heterochromatic
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: tristis Fallen 1823
Karyotype:
3V, 1J, 1D
Buzzati-Traverso (1941)
X is V-shaped; Y is J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: tropicalis Burla and Cunha in Burla et al. 1949
Karyotype:
1R, 2V
Burla et al. (1949)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
ssp. cubana Townsend 1954
Karyotype:
1R, 2V
Townsend (1954)

Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: tsacasi Bock and Wheeler 1972
Karyotype:
1R, 2V, 1D
Burla (1954) (as seguyi)
Lemeunier and Ashburner (in Clayton and Guest 1986)
X is a rod; Y is a J;
one autosomal is submetacentric
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: varians Bock and Wheeler 1972
Karyotype:
4V
Kaneshiro and Wheeler (1970)
Y is J-shaped; (reported as species 4)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: vulcana Graber 1957
Karyotype:
1R, 2V, 1D
Bock and Wheeler (1972)
X is a rod; Y is short, J-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: willistoni Sturtevant 1916
Karyotype:
1R, 2V, 1D
Metz (1916b); Wharton (1943)
1R, 2V
Dobzhansky and Pavan (1943)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)
ssp. quechua Ayala 1973
Karyotype:
1R, 2V
Dobzhansky (1975)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: yakuba Burla 1954
Karyotype:
1R, 2V, 1D
Lemeunier and Ashburner (1976)
X is a rod; Y is J-shaped

Lemeunier et al. (1978)
Acrocentric X; submetacentric Y

Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Genus: Drosophila
Subgenus: Spinodrosophila

Species: nigrosparsa Strobl 1898a
Karyotype:
5R, 1V
Burla (1950b)
X and Y are V-shaped
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Genus: Microdrosophila
Subgenus: Oxystyloptera

Species: congesta (Zetterstedt) 1947 (Drosophila)
Karyotype:
2R, 2V, 1D
Okada and Kurokawa (1957)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Genus: Mycodrosophila

Species: claytonae Wheeler and Takada 1963
Karyotype:
1R, 2V, 1D
Wheeler and Takada (1963)
X and Y are V-shaped
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: dimidiata (Loew) 1862 (Drosophila)
Karyotype:
1R, 2V, 1D
Clayton and Ward (1954)
X and Y are V-shaped
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: nigropteropleura Kang, Lee, and Bahng 1965
Karyotype:
1R, 2V, 1D
Kang et al. (1965)
Listings:
Cytological: Clayton and Guest

Taxonomic: Wheeler (1981)

Species: poecilogastra (Loew) 1874 (Amiota)
Karyotype:
5R, 1D
Kang et al. (1964)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: stalkeri Wheeler and Takada (1963)
Karyotype:
1R, 2V, 1D
Wheeler and Takada (1963)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Genus: Samoaia

Species: attenuata Wheeler and Kambysellis 1966
Karyotype:
1R, 2V, 1D
Ellison (1968)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: leonensis Wheeler and Kambysellis 1966
Karyotype:
3V, 1D
Ellison (1968)
X and Y are large metacentrics
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Genus: Scaptomyza
Subgenus: Bunostoma

Species: anomala Hardy 1965
Karyotype:
3R, 1V, 1D
Clayton (1966)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: hamata Hardy 1965
Karyotype:
1R, 2V, 1D
Clayton (1968)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: palmae Hardy 1965
Karyotype:
3R, 1V, 1D
Clayton (1966)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: varifrons (Grimshaw) 1901 (Drosophila)
Karyotype:
3R, 1V, 1D
Clayton (1966)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: xanthopleura Hardy (1965)
Karyotype:
3R, 1V, 1D
Clayton (1966)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Subgenus: Dentiscaptomyza

Species: denticauda Malloch 1934
Karyotype:
1R, 2V, 1D
Brncic (1958)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Subgenus: Elmomyza

Species: argentifrons Hardy 1965
Karyotype:
3R, 1V, 1D
Clayton (1966)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: melancholica (Duda) 1927 (Drosophila)
Karyotype:
1R, 2V, 1D
Brncic (1958)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: waialeale Hardy 1965
Karyotype:

1R, 2V, 1D
Clayton (1966)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Subgenus: Parascaptomyza

Species: adusta (Loew) 1862 (Drosophila)
Karyotype:
3R, 1V, 1D
Metz (1916a)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Species: pallida (Zetterstedt) 1847 (Drosophila)
Karyotype:
1R, 2V, 1D
Okada and Kurokawa (1957)
(as disticha)
Kang et al. (1964)
3V, 1D
Singh and Gupta (1979b)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Subgenus: Scaptomyza

Species: graminum (Fallen) 1823 (Drosophila)
Karyotype:
1R, 2V, 1D
Stalker (1945)
X and Y are rod-shaped
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)
syn. borealis Wheeler 1952
syn. amoena Meigen 1838

Species: noei Brncic 1955
Karyotype:
3R, 1V, 1D
Brncic (1958)
Listing:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Subgenus: Tantalia

Species: albovittata (Malloch) 1938 (Tantalia)
Karyotype:
3R, 1V, 1D
Clayton (1966)

Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Genus: Scaptomyza (Uncertain Affinity)

Species: parva (Grimshaw) 1901 (Drosophila)
Karyotype:
1R, 2V, 1D
Clayton (1966)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981) (1986)

Genus: Titanochaeta

Species: bryani Wirth 1952
Karyotype:
3R, 1V, 1D
Clayton (1968)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981)

Genus: Zaprionus
Subgenus: Aprionus (Wheeler, 1986)

Species: argentostriatus (Bock) 1966 (Drosophila)
Karyotype:
5R, 1D
Bock (1966) (As Drosophila)
Sciandra et al. (1973)
(as multistriatus)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981) (1986)
syn. multistriatus Sturtevant 1927

Species: obscuricornis (Meijere) 1915 (Stegana)
Karyotype:
3R, 2V, 1D
Singh and Gupta (1979b)
X is rod; Y is dotlike
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981) (1986)

Species: silvistriatus (Bock and Baimai) 1967 (Drosophila)
Karyotype:
1V, 3R, 1D
Bock and Baimai (1967)
X is long rod; Y is short rod
Listings:
Cytological: Clayton and wheeler
Taxonomic: Wheeler (1981) (1986)

Subgenus: Zaprionus

Species: bogoriensis Mainx 1958
Karyotype:
5R, 1D
Mainx (1958)
X and Y are double-length rods
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981) (1986)

Species: ghesquierei Collart 1937
Karyotype:
5R, 1D
Mainx (1958)
1R, 1V, 1J 1D
Olembo and Buruga (1973)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981) (1986)

Species: indianus Gupta 1970
Karyotype:
5R, lD
Singh and Gupta (1979b)
X and Y are rods
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981) (1986)
syn. paravittiger Godbole and Vaidya 1972
Karyotype:
5R, 1D
Singh and Gupta (1979b)
X and Y are rods
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981) (1986)

Species: inermis Collart 1937
Karyotype:
5R, lD
Tsacas et al. (1977)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981) (1986)

Species: ornatus Seguy 1933
Karyotype:
5R, 1D
Tsacas et al. (1977)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981) (1986)

Species: sepsoides Duda 1939
Karyotype:
5R, 1D
Tsacas et al. (1977)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981) (1986)

Species: tuberculatus Malloch 1932
Karyotype:
5R, 1D
Tsacas et al. (1977)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981) (1986)

Species: vittiger Coquillett 1901
Karyotype:
5R, 1D
Mainx (1958)
1R, 2V, 1D
Olembo and Buruga (1973)
Listings:
Cytological: Clayton and Guest
Taxonomic: Wheeler (1981) (1986)

Species of Uncertain Affinity:

Species: alexandrei Cordeiro 1951
Karyotype:
3R, 1V
Cordeiro (1951)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: florae Sturtevant 1916 in Metz 1916b
Karyotype:
1R, 2V, 1D
Metz (1916a)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

Species: pagliolii Cordeiro 1963
Karyotype:
3R, 1V, 1D
Cordeiro (1963)
Listings:
Cytological: Clayton and Wheeler
Taxonomic: Wheeler (1981)

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Guide to Authors

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Waters, R.L., J.T. Smith, and R.R. Brown 1990, J. Genet. 47: 123-134.

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Research Notes

Polak, M.,* and T.A. Markow. Department of Zoology, Arizona State University, Tempe, AZ 85287-1501. * Present address: Department of Biology, Lyman Hall, Syracuse University, Syracuse, NY 13244-1270.

A note on the feeding ecology of adult *Drosophila nigrospiracula*, a Sonoran Desert-endemic fruit fly.

The general picture of the breeding ecology of the four species of Sonoran Desert -endemic *Drosophila* is that they all possess a high degree of host plant specificity, controlled in part by host plant toxicity and competitive exclusion processes (Fellows and Heed, 1972; Heed and Mangan, 1986). One of these species, *Drosophila nigrospiracula* Patterson and Wheeler, which belongs to the *repleta* species group, occurs in southern Arizona, northwestern Mexico, and Baja California. It mainly breeds in necroses of saguaro (*Carnegiea gigantea*) and cardón (*Pachycereus pringlei*), but occasionally also in those of hecho (*Pachycereus pecten-aboriginum*) and barrel (*Ferocactus wislizenii*) cacti (Heed, 1978). The diet of both larval and adult *D. nigrospiracula* consists of components of the necrotic cactus tissue and microorganisms, such as a variety of yeast species, that colonize these substrates (Heed *et al.*, 1978; Starmer *et al.*, 1982). Adult *D. nigrospiracula* are commonly seen on the exterior surface of necrotic cacti consuming liquid exuding from the decaying tissue. In numerous samples of flies collected at saguaro cacti in the vicinity of Phoenix, Arizona, U.S.A., we have observed *D. nigrospiracula* and *D. mettleri* of both sexes carrying pollinia of a milkweed vine, *Matelea parvifolia* (Torrey) (Asclepiadeacea), attached to their mouthparts and associated structures. Flies therefore visit flowers of this vine probably to feed, implying that the feeding ecology of adult flies is not as restricted to their host plant as commonly believed.

Table 1. Proportion of flies of both sexes carrying pollinia and mean number pollinia per fly across 5 collection dates.

Date	Sex	Proportion flies carrying pollinia (N)	Mean number pollinia/fly ± SE(N)
Dec. 10, 1994	M	0.027 (74)	1.0 ± 0 (2)
	F	0.020 (98)	1.0 ± 0 (3)
Jan. 14, 1995	M	0.33 (135)	1.64 ± 0.16 (44)
	F	0.39 (43)	1.41 ± 0.15 (17)
Jan. 22, 1995	M	0.30 (89)	1.33 ± 0.11 (27)
	F	0.31 (26)	1.75 ± 0.16 (8)
Jan. 31, 1995	M	0.21 (112)	1.41 ± 0.15 (24)
	F	0.41 (29)	1.50 ± 0.34 (12)
Feb. 11, 1995	M	0.17 (76)	1.23 ± 0.12 (13)
	F	0.042 (24)	1.0 ± 0 (1)
Pooled	M	0.226 (486)	1.42 ± 0.071 (110)
	F	0.182 (120)	1.46 ± 0.12 (41)

In our sampling procedure, flies were netted at necrotic saguaro cacti located within 45 miles E. of Phoenix, aspirated into vials containing banana-agar medium, and returned to the laboratory on the same day of collection where flies were sexed, and number of pollinia on each fly counted. Results of a survey of adult *D. nigrospiracula* based on collections made during 1994-1996 across five different dates are presented in Table 1. Pollinia occurred on flies as early as the beginning of December, increased to a maximum frequency by mid - late January, and decreased thereafter. Sexes did not differ in frequency at which they carried pollinia (Table 1, χ^2 on pooled data = 2.26, 1 d.f., $P > 0.1$), nor did they differ in mean number of pollinia per fly (Table 1, t test on pooled data, $t = 0.12$, 149 d.f., $P = 0.90$). Among flies that carried pollinia, number of individual pollinia per fly ranged from 1 to 5, and mean number ranged from 1.0 to 1.75. On individual flies harboring multiple pollinia, pollinia either had one or both translator arms missing, whereas other pollinia on the same fly appeared fresher (less desiccated) and had both translator arms attached to the corpusculum. This variation in pollinia structure and age suggests that flies make multiple visits to feed from vines over their lifetimes. For comparison with our samples of *Drosophila*, males of two tachinid species, *Chaetonodexodes vanderwulpi* (N = 58) and *Opsoneigenia nana* (N = 19), were collected in Jan 1995 from a hilltop within 200 m of a saguaro cactus at which we took samples of adult *Drosophila*. Individual tachinid flies were pinned on the same day of collection and scored for the presence of *M. parvifolia* pollinia. Neither species was found to carry pollinia.

Our findings suggest that *D. nigrospiracula* feeds on the nectar of asclepiad flowers, perhaps to acquire free sugars which may not occur at sufficient concentrations at cactus necroses. An experimental study of *D. mojavensis* , which breeds primarily in the necroses of *Stenocereus gummosus* (agria) and *Stenocereus thurberi* (organpipe), showed that free sugars are important for adult survival, and that adult flies cannot obtain sufficient amounts of sugars from these cacti (Brazner *et al.*, 1984). Another possible source of free sugars is the ripe, open fruits of *Opuntia* and other cacti. For example, we have often observed the crops of *D. nigrospiracula* during the fruiting period of *Opuntia* to be filled with the wine-colored juice characteristic of its fruit. Extrafloral nectaries (*e.g.*, Blom and Clark, 1980), which exist on columnar and *Opuntia* cacti, might represent another source of sugars utilized by *D. nigrospiracula.*

Acknowledgments: We thank E. Sundell (University of Arkansas-Monticello) for identifying plant material and D. M. Wood (Biosystematics Research Institute, Canada) for identifying tachinid specimens.

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Zak, N.B. Experimental Medicine and Cancer Research, Hebrew University-Hadassah Medical School, Jerusalem, Israel. A note on *tailup*.

Tailup (*tup*) is one of six loci whose zygotic expression is required for germband retraction of the *Drosophila melanogaster* embryo. The *tup* locus has not been cloned. Two ethyl methanesulfonate-induced *tup* alleles have been isolated and the locus was determined to be in map position 54.0. It was cytologically placed between 37A1-B1 and 37B2-8 because it is removed by *Df(2L)137 = Df(2L)36C2-4;37B9-C1* but not by *Df(2L)H68 = Df(2L)36B-C1;37A1-B1* or *Df(2L)TW158 = Df(2L)37B2-8;37E2-F4* (Nüsslein-Volhard *et al.*, 1984). Not surprisingly, we have observed that *tup* is removed by *Df(2L)TW3 = Df(2L)36F7-37A1;37B2-8*. We have tested three lethal loci, each representing one lethal complementation group that is uncovered by this deficiency, for allelism to *tup*. One of them, the ethyl methanesulfonate-induced mutation *l(2)37Aa*, is an additional *tup* allele. *l(2)37Aa* is also known as *l(2)E41*, which was placed in the genetic location 53.1-53.9 (Wright *et al.*, 1976). *l(2)02660r*, a P element insertion allele generated by Paul Lasko at McGill University, falls within the *TW3* interval but is not allelic to *tup*. *l(2)02660r* could serve as a good starting point for "local hopping" into the *tup* locus.

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Hodge, Simon[1,3] **and Paul Mitchell**[2] 1. Dept. of Entomology and Animal Ecology, PO Box 84, Lincoln University, Canterbury, New Zealand; 2. Biology Division, Staffordshire University, College Road, Stoke-on-Trent, ST4 2DE, UK; 3. Author to whom correspondence should be addressed. The concentration of urea in the larval resource and its effect on larval performance.

Introduction: The excretion of metabolic wastes and secretion of enzymes for external digestion by dipteran larvae can alter the biochemical nature of their environment. This habitat modification can have both negative and positive effects on the success of other larvae which simultaneously or subsequently use the resource (Weisbrot, 1966; Dawood and Strickberger, 1969; Budnik and Brncic, 1975; Mitchell, 1988).

Urea has been identified as an excretory product of *Drosophila*, and at high concentrations has been shown to slow down the developmental rate of *Drosophila melanogaster* and reduce larval survival (Botella *et al.*, 1985).

This paper describes the amounts of urea produced by *Drosophila* larvae and re-examines the effects of urea on larval performance.

Methods: All experiments used wild-type stocks of *Drosophila*: 'Kaduna' for *D. melanogaster* and stocks reared from British flies for *D. hydei*. A temperature of 25°C, relative humidity of approximately 45% and a 16:8 hours light:dark regime was used in all cases. The experiments were carried out using standard glass vials (75mm x 25mm diameter) stoppered with foam bungs.

Vials of resource medium were prepared by hydrating 1.0g of ground Instant *Drosophila* Medium (IDM; Blades Biological Ltd., UK) with 4.0ml of distilled water. The vials of IDM were then seeded with three densities of first instar larvae: 0, 25 and 50. At least six replicates of each density were initially set up for both *D. melanogaster* and *D. hydei* (actual replicate numbers for each treatment for each particular assay are given in the Results section). The vials were left until the majority of the larvae had pupated and no larvae were visible in the resource; more specifically 8 days for *D. melanogaster* and 12 days for *D. hydei*. The remaining medium was then freeze-dried and stored at 4°C.

The above procedure was also carried out using 5.0g of mashed banana instead of IDM to examine urea concentrations produced when larvae were reared on a natural resource. The development of the larvae was slightly

elongated in this resource which meant that vials were left for a longer period: 9 days for *D. melanogaster* and 14 days for *D. hydei*.

Urea concentrations were determined using an assay formulated by Newell *et al.* (1967). This assay is extremely sensitive and can detect urea concentrations in the order of 20 μg Nl^{-1}. Samples of culture media to be used in the assay were reconstituted by combining one part freeze-dried resource and four parts distilled water (by mass).

Experiments were also carried out to assess the effect of urea on the performance of *Drosophila* larvae, and a series of urea solutions (0, 250, 500, 1000, 2000μM) were prepared (this range encompasses the urea concentrations found in conditioned medium). 4.0ml of these solutions were used to hydrate 0.8g of IDM, and six replicates of each urea concentration were set up for each *Drosophila* species. Twenty five first instar larvae of either *D. melanogaster* or *D. hydei* were placed onto the surface of the medium after it had been hydrated. To examine whether urea in *Drosophila* medium could initiate a response at high concentrations, a 0.1M treatment was set up for *D. melanogaster* larvae only. Emerged adults were removed from vials every 24 hours and stored in 70% alcohol. The body size of the flies was estimated by a measure of female wing length, using the distance from the anterior cross vein to the wing tip along vein 3, with 10 specimens taken from each vial if available. The mean development time was calculated using all the emergent adults from each vial.

Results: The urea concentrations found varied extensively, and this is reflected by some large standard errors (Table 1). The data were analysed using the Kruskal-Wallis test. IDM and banana with *Drosophila* larvae present tended to have higher urea concentrations than the no-larvae controls. However, the only significant increase occurred in IDM conditioned by *D. melanogaster* larvae (Table 1).

Some 'urea' was found in the control media, where no larvae had been present. This may have been caused by interference with light absorption in the spectrophotometer due to pigments in the media or possibly some urea was produced by microorganisms in the media.

When examining larval performance, survival of *D. melanogaster* remained high for all treatments (Table 2) and was not significantly affected by the urea concentration of the medium. No difference in size of the female flies was found between urea treatments.

The mean development time of *D. melanogaster* larvae showed a significant response to urea concentration of the medium (Table 2); an elongation of the development time occurring when an excessively high urea concentration of 0.1M was used. This treatment was separated from the other urea concentrations using a Tukey test ($P < 0.05$).

No effect of urea concentration was found on *D. hydei* larval survivorship (ranging from 55-75%), or size of the emergent flies (Table 3). Mean development time of *D. hydei* showed a significant response to concentration of urea in

Table 1. Urea concentration (μM) found in medium (mean±SE(N)).

		Number of larvae				
		0	25	50	H	P
D. hydei	IDM	104.0 ± 21.8 (6)	150.2 ± 46.9 (6)	150.2 ± 26.1 (6)	1.45	> 0.45
	Banana	74.0 ± 36.1 (6)	125.8 ± 26.6 (6)	123.8 ± 19.6 (6)	3.32	> 0.15
D. melanogaster	IDM	55.0 ± 11.8 (6)	352.2 ± 71.1 (6)	230.7 ± 26.0 (6)	13.5	< 0.001
	Banana	43.9 ± 04.0 (6)	35.2 ± 02.2 (4)	99.7 ± 33.3 (3)	3.25	> 0.15

Table 2. Effect of urea concentration on *D. melanogaster* and larvae (mean ± SE).

Urea Conc.	0	250 μM	500 μM	1000 μM	2000 μM	0.1M	F	P
Survival	95.2 ± 3.0	98.8 ± 1.3	96.0 ± 2.1	89.2 ± 6.0	96.0 ± 3.3	90.0 ± 4.1	0.9	> 0.45
Wing length	78.8 ± 0.5	79.0 ± 0.6	78.1 ± 0.4	78.2 ± 0.7	79.2 ± 0.5	77.6 ± 0.4	1.1	> 0.35
MDT	10.8 ± 0.2	10.7 ± 0.2	11.0 ± 0.2	10.5 ± 0.1	10.7 ± 0.1	11.3 ± 0.1	3.0	< 0.05

N = 6 (Survival - % larvae to adulthood; MDT, mean development time- days; wing length – graticule units 55 ≡ 1 mm)

Table 3. Effect of urea concentration of *D. hydei* larvae (mean ± SE).

Urea Conc.	0	250 μM	500 μM	1000 μM	2000 μM	F	P
Survival	72.0 ± 3.6	56.8 ± 7.1	69.2 ± 9.4	75.3 ± 5.6	68.8 ± 3.6	1.1	> 0.35
Wing length	96.2 ± 0.3	97.2 ± 0.3	96.3 ± 0.5	96.1 ± 0.4	96.5 ± 0.2	1.4	> 0.25
MDT	14.4 ± 0.2	13.8 ± 0.3	14.2 ± 0.2	14.7 ± 0.1	14.3 ± 0.1	3.5	< 0.05

N = 6 (Survival - % larvae to adulthood; MDT, mean development time- days; wing length – graticule units 55 ≡1 mm)

the medium (Table 3). This difference appears to arise from a probably-spurious reduction in development time when 250μM urea solution was used to hydrate the medium, although a Tukey test failed to isolate this development time from that found for other treatments.

Discussion: Urea has been previously identified as being produced by *Drosophila* larvae (Botella *et al.,* 1985) but the highest concentrations found in those experiments were higher, by about a factor of ten, than the concentrations of urea found in the current experiments. These higher concentrations of urea were probably caused by the higher densities of larvae used in those experiments (*c.f.,* 140 larvae/ml of medium with a maximum of 5/ml in this experiment) and that the experimenters utilised a method of 'larval stop', retaining larvae in the medium for an extended period. However, given these differences, the highest urea concentrations found in this study were still comparable with the lower values obtained by Botella *et al.* (1985).

Botella *et al.* (1985) suggested that urea could have a negative effect on the performance of *D. melanogaster.* However, the concentrations used to produce these responses seemed unrealistically high (0.03M to 0.2M) compared to the concentrations found in conditioned medium by those authors and in experiments carried out here. With the exception of the 0.1M treatment used for *D. melanogaster,* all the concentrations used in our experiment are below those of Botella *et al.* The negative response which *D. melanogaster* displayed to urea at the concentrations they used was linear for development time, a response occurring even at their lowest urea concentration (Botella *et al.,* 1985). The extension of the development time at 0.1M in this study is in accordance with those findings. Apart from the differences in urea concentrations used, there is another problem comparing the current work with that of Botella *et al.* caused by their method of 'larval stop' (Mensua and Moya, 1983). This produced larvae-to-adult development times of 23-35 days at 18°C (*c.f.,* 15 to 17 days for larval and pupal period of *D. melanogaster* at 18°C given in Ashburner and Thompson, 1978). It is possible that the effects of urea identified by Botella *et al.* (1985) are to be found only in the very specific conditions of their study.

It appears that urea can potentially have a deleterious effect on the performance of *Drosophila* larvae. However, these effects only occur at concentrations higher than those found even in contrived high density situations. It seems likely, therefore, that this substance would not occur at sufficiently high concentrations to affect *Drosophila* populations in nature.

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Norry, Fabian M.,* **and Juan C. Vilardi**. Laboratorio de Genética de Poblaciones, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires 1428 Buenos Aires, Argentina. Wing asymmetry and chromosome inversions in *Drosophila buzzatii.*

Abstract: The possible relationship between developmental stability and inversion karyotypes of the second chromosome was examined in the cactophilic fly *Drosophila buzzatii.* The results indicate that developmental stability, as indexed in terms of fluctuating asymmetry of wing length, does not differ among karyotypes in wild-reared flies. Thus, developmental stability is apparently independent of both possible factors: (i) heterozygosity at the karyotypic level of variation, and (ii) any possible genetic coadaptation attributable to these chromosome inversions.

Introduction: Developmental homeostasis is the overall ability of individuals to cope with genetic and environmental stress (Lerner, 1954; Palmer and Strobeck, 1986; Parsons, 1990). In bilaterally symmetrical organisms, this ability may be indexed in terms of fluctuating asymmetry (FA) - side-wise random deviations from perfect bilateral symmetry (Van Valen, 1962). Inbreeding depression has often been thought to be causally associated with low levels of developmental stability (Lerner, 1954; Waddington, 1960, 1966). However, Fowler and Whitlock (1994) demonstrated that FA of sternopleural bristles is not a reliable measure of the degree of inbreeding in experimental populations of *Drosophila melanogaster.* Thus, although the FA level may be a reliable index of developmental stability, the genetic basis of FA remains unclear.

Two well-known hypotheses about the cause of genetic variation in developmental stability are heterozygosity and genetic coadaptation. While the isozyme heterozygosity has been examined with respect to FA in a wide variety of

species (see Palmer and Strobeck, 1986, for a review), chromosome inversions in *Drosophila* were largely unexplored in relation to FA. Here, we compare the level of FA (in wing length) among inversion karyotypes (genotypes) in wild-reared flies of the cactophilic species *Drosophila buzzatii*.

Table 1. Asymmetry of the wing length is given for karyotypes of the second chromosome in wild-reared *D. buzzatii* flies. Values (in mm x 10^3) are given for data pooled across sexes. Statistics are also shown for data pooled within homokaryotypes (Homo-k) as well as heterokaryotypes (Hetero-k). N is the sample size.

	Karyotypes						
Statistics	j / st	j / j	jz3 / st	jz3 / j	jz3 / jz3	Homo-k	Hetero-k
N	27	105	17	104	20	125	148
Mean	4.02	3.84	5.47	5.66	4.65	3.97	5.34
SD	6.92	7.06	7.63	8.38	8.85	7.34	8.03

Material and Methods: A population breeding on *Opuntia vulgaris* at Arroyo Escobar (34°4' S; 58°7' W), Buenos Aires (Argentina), was sampled for this study. This population is polymorphic for inversions on the second chromosome, namely *standard* (st), j, jz^3 and jq^7 (Hasson *et al.*, 1991). During April 1 to 15, 1991, wild-reared flies were collected from rotting cladodes of *Opuntia vulgaris*, as described in Norry *et al.* (1995a). These flies were immediately sexed, placed in vials with culture medium and individually crossed with flies of a homokaryotypic stock as described in Norry *et al.* (1995a). The cytological analysis of eight larvae of the progeny from each cross allowed us to infer the karyotype of the wild parent. Only karyotypic classes with sample sizes larger than 17 individuals were analyzed.

Wing length was scored as the distance from anterior crossvein to distal tip of vein III (see Norry *et al.*, 1995b). Both wings were measured on a microscope slide at 100× magnification, using a Wild M-20 compound microscope. Asymmetry scores were obtained by subtracting the measurement of the left side from that of the right side.

Results and Conclusions: No sexual dimorphism in FA of wing length was detected by the Mann-Whitney test (MEAN $RANK_{MALES}$ = 137; MEAN $RANK_{FEMALES}$ = 135; P = 0.87). The results are therefore reported for data pooled across sexes. Summary statistics for wing asymmetry in wild flies are given for each examined karyotype in Table 1. Among karyotypes, no significant variation in FA was detected by the non-parametric Kruskal-Wallis test (H = 3.14; P = 0.54). Nor was there evidence of karyotypic variation in FA when data were pooled within homo- and hetero-karyotypic classes (both karyotypic classes were compared using the Mann-Whitney test: MEAN $RANK_{homo\text{-}k}$ = 142; MEAN $RANK_{hetero\text{-}k}$ = 131; P = 0.23).

These results suggest that the inversion polymorphism is adaptively independent of developmental stability, as no significant variation in FA was detected among karyotypes. We conclude that developmental stability (as indexed by wing asymmetry) is independent of: (i) heterozygosity at the karyotypic level of chromosomal variation, and (ii) any possible genetic coadaptation attributable to these chromosome inversions.

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Hodge, Simon [1], **and Paul Mitchell**[2]. [1]Department of Entomology and Animal Ecology, P.O. Box 84, Lincoln University, Canterbury, New Zealand; [2]Division of Biology, Staffordshire University, College Road, Stoke-on-Trent, ST4 2DE, UK. The effect of resource quantity and water content, and atmospheric humidity, on the interaction between *Drosophila hydei* and *D. melanogaster*.

Introduction: It has long been known that the form taken by the interaction between two species can be influenced by the abiotic environment (*e.g.*, Park, 1954). Many environmental variables have been found to affect interactions between drosophilids, including; temperature (*e.g.*, Moore, 1952; Ayala 1966), age of resource (Merrel, 1951; Miller, 1954; Mitchell and Arthur, 1990), light intensity (Moth and Barker, 1976; but see Arthur, 1986), ethanol concentration (Arthur, 1980) and amount of resource (Arthur, 1986).

This study investigated how the amount of resource presented to the larvae, the resource water content and the atmospheric humidity affected the interaction between *D. melanogaster* and *D. hydei*. All these factors are associated with resource desiccation, which is known to affect the performance and behaviour of these two species (Arthur, 1996;

Table 1. Interactions between *D. melanogaster* and *D. hydei* based on pair-wise comparisons in mixed and monocultures of (a) larval survival, (b) wing length, and (c) mean development time. (symbol on left represents effect of *D. hydei* upon *D. melanogaster*)

(a)

Humidity	Liquidity	Mass of IDM 0.5g	Mass of IDM 0.8g
Low	Low	0,-	0,0
	High	0,0	0,0
High	Low	0,0	0,0
	High	0,0	0,0

(b)

Humidity	Liquidity	Mass of IDM 0.5g	Mass of IDM 0.8g
Low	Low	0,-	0,0
	High	0,-	-,-
High	Low	0,-	0,-
	High	0,0	0,0

(c)

Humidity	Liquidity	Mass of IDM 0.5g	Mass of IDM 0.8g
Low	Low	0,-	0,-
	High	+,0	+,0
High	Low	0,-	+,0
	High	0,0	0,0

Arthur and Cassey, 1992; Hodge and Wilson, 1997). In addition to examining the interaction under different environmental conditions, different performance measures of the populations have been used to examine how this led to variation in how the interactions were perceived.

Methods: The experiment used standard glass vials (75mm × 25mm diameter) stoppered with foam bungs to house the drosophilids. Two masses (0.5g and 0.8g) of ground Instant *Drosophila* Medium (IDM; Blades Biological Ltd., UK) were used as a resource. The IDM was hydrated with distilled water, using either a 4:1 or 6:1 by mass water:IDM ratio. The relative humidity was either 'high' (RH 45-50%), or 'low' (RH 30-35%), the high value being the ambient humidity in the incubator and the low humidity being maintained using trays of silica gel which were replaced every 24 hours. By combining these parameters factorially, eight 'environments' were created. A temperature of 25^{o}C and a light:dark regime of 16:8 hours light:dark were used in all cases.

The experiment used wild-type stocks of both species: 'Kaduna' from Nigeria for *D. melanogaster* and a stock reared from British flies for *D. hydei*. For each environment, monocultures were set up using 40 first instar larvae. Mixed cultures were set up using 40 first instar larvae of each species, *i.e.*, 80 larvae in total. Between 8 and 11 replicates of each treatment were used. Emerged adults were removed from vials every 24 hours and stored in 70% alcohol. The body size of the flies was estimated by a measure of female wing length, using the distance from the anterior cross vein to the wing tip along vein 3, with 10 specimens taken from each vial if available. The mean development time was calculated using all the emergent adults from each vial.

Results: The effects of the various environmental factors on performance have been analyzed factorially and discussed elsewhere (Hodge, 1995). This paper concerns itself solely with how the populations performed in mixed cultures compared to mono-cultures within each environment. This has been achieved simplistically, using a series of pairwise ANOVAs, differences being declared significant at $P < 0.05$. Data for survival were arcsine transformed before analysis.

Survival of larvae was robust, being unaffected by the presence of the other species in the large majority of cases (Table 1a). Therefore, the interaction between *D. hydei* and *D. melanogaster* based on larval survival tended to be 'neutral', with one amensal interaction occurring when conditions were severe for *D. hydei* (dry atmosphere, 0.5g IDM).

Wing length was a more sensitive measure (Table 1b). Although 'non-effects' still dominated, there appeared four amensal, one competitive and three neutral interactions. *D. melanogaster* only responded to the presence of *D. hydei* on one occasion, whereas *D. hydei* was negatively affected by *D. melanogaster* in over half of the environmental conditions used.

When considering development time, *D. hydei* was found to facilitate *D. melanogaster* on three occasions, *i.e.* development time of *D. melanogaster* was shortened (Table 1c). *D. hydei* on the other hand was, if anything, negatively affected by *D. melanogaster*, the development time being extended under some environmental conditions. This extension of *D. hydei* development time appeared more likely when the resource had a low water content.

Discussion: Inferences made about the interaction between these two species of *Drosophila* were dependent upon the larval environment and which performance measure was used. Generally, *D. hydei* seemed unsuited to dry conditions (see also Arthur, 1986; Hodge, 1995; Hodge and Wilson, 1997) and in the environments which were prone to drying (small amounts of resource, low water content, low humidity), *D. hydei* tended to be inhibited by *D. melanogaster*. The frequency of this inhibition was related to the sensitivity of the parameter used; for example, using survival, inhibition became manifest only in the driest environment, but when using wing size the inhibition of *D. hydei* became a more general phenomenon. In the 'wettest' environments this inhibition did not appear for any of the parameters used.

In some instances the different population measures were contradictory. For example, the effect of *D. hydei* on *D. melanogaster* in the 0.8g, low humidity, high water environment could be neutral, inhibitive or facilitative, depending on what measure was used. In this case, it is possible that development time was shortened as an 'escape response' from

an unfavourable environment, producing smaller adults because the feeding time of the larvae was curtailed. In these situations, conclusions about the interaction must be subjective. A reduction in body size may lower the reproductive output of the female but, when considering animals whose natural habitat is ephemeral and unpredictable, a reduction in development time may represent an important facilitative effect.

The interaction which occurs between two species can be very specific to a given set of conditions (Thompson, 1988; Dunson and Travis, 1991), and it appears that describing the interaction between these two *Drosophila* species in a single manner is almost meaningless (see Arthur, 1986; Hodge, 1995). Compared to variability in nature, only a narrow band of different environments have been used here and these produced four of the six theoretical outcomes between a pair of interacting species. Experiments such as this one produce useful information on the possible range of interactions that can occur between two species and may aid in clarifying the mechanisms via which the interspecific effects are produced (see Tilman, 1987). It is then desirable to put the results into a more realistic context and determine which scenarios are most likely to occur under natural conditions.

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Gandarela, Manuel R., and Emilio Valadé. Dpto. de Bioloxía Fundamental, Facultad de Bioloxía, Universidade de Santiago de Compostela, Spain. Estimation of duplication time between genes *scute* and *asense*.

The *achaete-scute* gene complex (AS-C) is involved in the development of sensory organs and the central nervous system of *Drosophila*. The AS-C is a gene family containing four genes with neurogenic functions: *achaete(ac)*, *scute* (*sc*), *lethal of scute* (*l'sc*) and *asense* (*ase*). AS-C genes encode related proteins containing the basic-helix-loop-helix (bHLH) domain characteristic of a family of transcriptional regulators. Their products confer on cells the capacity to become neural precursors. Besides its neurogenic function, *sc* is also involved in the establishment of the X:A ratio.

It is possible to estimate the date of the duplication event which gave rise to these two members of the gene family. Li and Graur (1991) describe a method to estimate the duplication time of two paralogous genes from the sequences of these two genes from two species when the divergence time between these species is known. In this work we give an estimation of the duplication time between *sc* and *ase*.

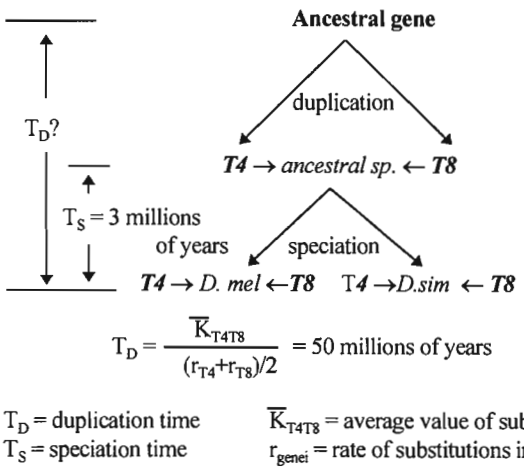

Figure 1. Model for estimating the time of the gene duplication event (Li and Graur, 1991). The matrix of Kimura's two-parameters distances (Table 1) were used to estimate T_D. We consider 3 MYA as the time of speciation between *D. melanogaster* and *D. simulans* (Cariou, 1987).

We amplified by PCR and sequenced a conserved region of *sc* gene from one strain of *D. melanogaster* (Toonda, Australia) and another one of *D. simulans* (Leticia, Colombia). To estimate the duplication time we included two sequences of *ase* obtained from literature: *D. melanogaster* Canton S (Villares and Cabrera, 1988) and *D. simulans* CA-1 (Hilton *et al.*, 1994). We used 3 million years ago (MYA) as the time of divergence between *D. melanogaster* and *D. simulans*. This value is an average of several estimates based on paleo-biogeographic, allozymic, inmunological and nucleotide data (Cariou, 1987).

Table 1. Number of substitutions per site according to the Kimura's two-parameters model.

		T4		T8	
		D. mel Toonda	*D. sim Leticia*	*D. mel Canton S*	*D. sim CA-1*
T8	*D. mel Toonda*	0.0000	0.0408	0.6163	0.6566
	D. sim Leticia		0.0000	0.6064	0.6363
T4	*D. mel Canton S*			0.0000	0.0312
	D. sim CA-1				0.0000

The estimate of the duplication time using Li and Graur's method was 50 MYA (Figure 1). Since most of the estimates consider that *Drosophila* subgenus diverged from the *Sophophora* subgenus 40 MYA, the duplication of the ancestral gene happened likely before the splitting.

To carry out studies about the role of these genes in other species of *Drosophila*, this result should be taken into account.

References: Cariou, M.L., 1987, Genet. Res. 50: 181-185; Hilton, H., R.M. Kliman and J. Hey 1994, Evolution 48: 1900-1913; Li, W-H., and D. Graur 1991, *Fundamentals of Molecular Evolution*, Sinauer Associates, Inc.; Villares, R., and C.V. Cabrera 1987, Cell 50: 415-424.

Noor, Mohamed A. F. Section of Genetics and Development, Cornell University, Ithaca, NY 14853. Courtship songs: a noninvasive method of identifying North American *obscura*-subgroup *Drosophila* males in field collections.

After collecting *obscura*-subgroup *Drosophila* flies along the west coast of North America, one is faced with a challenge when identifying the species. Three of the native North American species are morphologically identical: *Drosophila pseudoobscura*, *D. persimilis*, and *D. miranda*. Generally, females are reared in the laboratory, and their offspring are identified using chromosome squashes or allozymes (Anderson *et al.*, 1977). However, wild-caught males are often not identified and are listed as "*pseudoobscura/ persimilis/ miranda*" in most publications. I report here that male courtship songs can be used to unambiguously identify the species of captured males. Previous studies have noted differences between *D. pseudoobscura* and *D. persimilis* in courtship song elements (Waldron, 1964; Ewing, 1969; Noor and Aquadro, in press), and here I show both that the song of *D. miranda* differs from that of the other two species and that songs can be used to reliably determine the species of wild-caught *obscura*-subgroup *Drosophila* males.

I reared laboratory lines of *D. pseudoobscura* (Flagstaff) and *D. persimilis* (Mount St. Helena) on cornmeal/yeast/agar medium at 21°C. These stocks have been used extensively in laboratory behavioral investigations (e.g., Noor, 1996). Two isofemale lines of *D. miranda* (Mather and Mount St. Helena) were also cultured, both only 2 generations removed from the wild. Individual males from these lines were then paired with conspecific females and recorded in an Insectavox (Gorczyca and Hall, 1987). Courtship songs were analyzed using CANARY (Cornell University Laboratory of Ornithology) software. Interpulse interval (IPI) was defined as the length of time from the beginning of one sound pulse to the beginning of the next in milliseconds. Intrapulse frequency is the frequency of sound within each song pulse in cycles per second. Table 1 presents the results with their standard errors. *D. miranda* strains have a lower intrapulse frequency and a longer interpulse interval than either *D. pseudoobscura* or *D. persimilis* (see Figure 1). The IPI and frequency observed in the *D. pseudoobscura* and *D. persimilis* song are perfectly consistent with those observed in previous studies (Waldron, 1964; Ewing, 1969; Noor and Aquadro, in press). These two song characters can be used together to unambiguously assign wild-caught males to species, but it is important to note the strong temperature-dependence of these phenotypes (particularly IPI- see Noor and Aquadro, in press). Correspondingly, if one is using an Insectavox, one should switch the inside light off, as this light can heat the box substantially, sometimes causing an overlap in song elements between individuals of different species recorded at different times (slight song overlap observed by Noor and Aquadro (in press) in *D. pseudoobscura* and *D. persimilis* resulted from this heating). An individual of known species should be recorded under the same conditions and at the same time to correct for environmental effects.

I used this technique to identify 19 *obscura-subgroup Drosophila* males captured at Mather, California, in June, 1997. These males were brought to the laboratory, isolated for 1 day

Table 1. Courtship song parameters

Recording temp.	Species	IPI (ms)	Frequency (cy/sec)
14°C	*D. pseudoobscura*	57.9 ± 0.7	191 ± 5
14°C	*D. persimilis*	70.0 ± 0.7	300 ± 9
14°C	*D. miranda* (Mather)	116.9 ± 7.4	136 ± 4
19°C	*D. pseudoobscura*	37.7 ± 0.2	213 ± 2
19°C	*D. persimilis*	57.6 ± 1.2	403 ± 12
19°C	*D. miranda* (MSH)	67.7 ± 2.0	155 ± 2

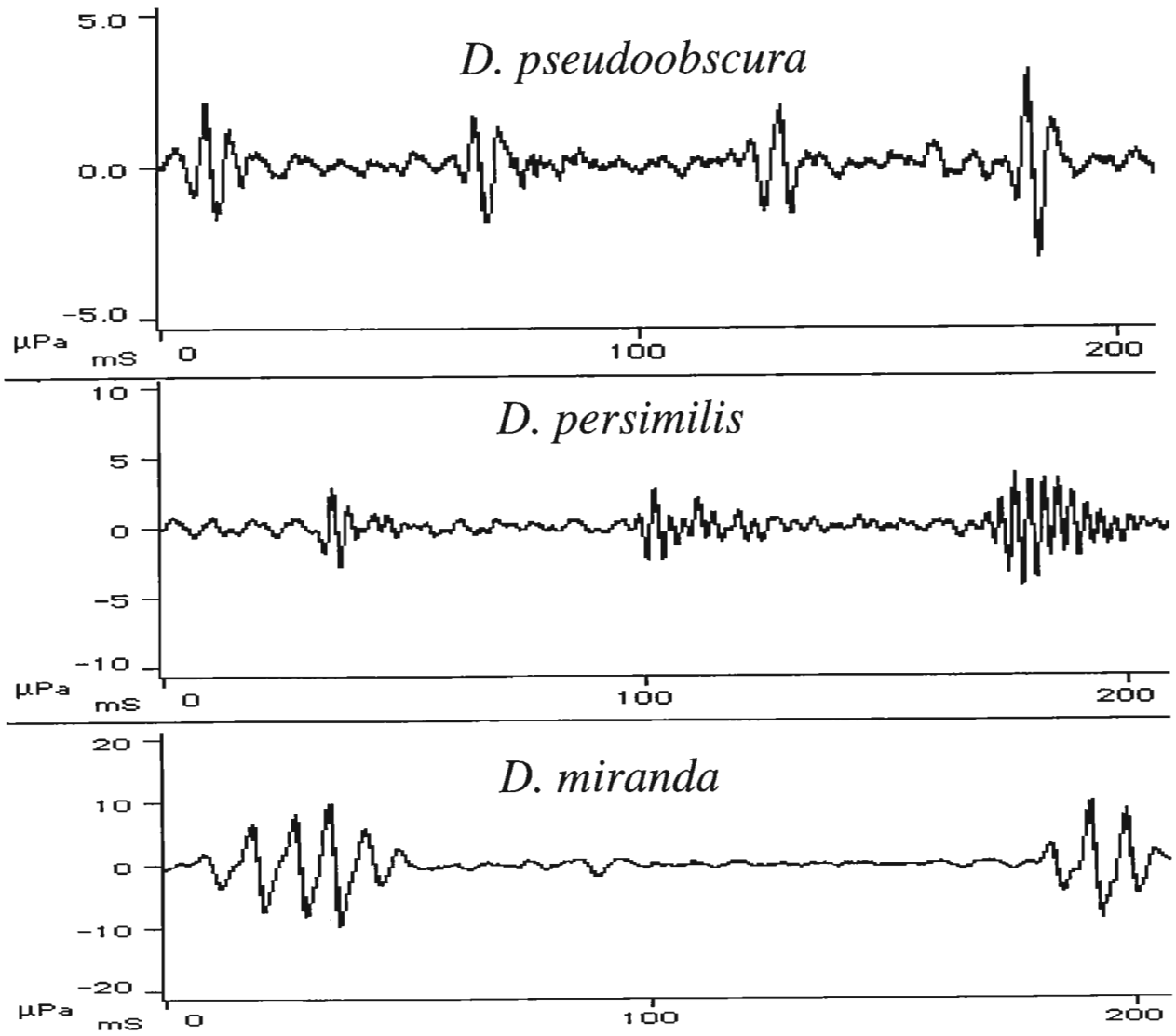

Figure 1.

to prevent crowding-induced courtship inhibition (Noor, 1997), and paired with females in an Insectavox. Figure 2 shows the plot of their mean courtship song IPI and frequency. It is clear that one individual has a lower IPI and frequency than the others. Hence, this individual is likely a *D. pseudoobscura* male. No individuals have very long IPI's but short frequencies, suggesting that there were no *D. miranda* males captured at this time. These data accord with the expected species proportions from previous collections at Mather (Noor, 1995), suggesting the validity of this technique of species identification. I further tested this technique on male offspring from 24 wild-caught females from Mather and Mount St. Helena, California. This technique accurately identified all the males as *D. pseudoobscura* or *D. persimilis*, as shown by subsequent crosses.

Courtship songs can thus be used to determine the identity of wild-caught North American *obscura*-subgroup *Drosophila* males, and may also be used for identifying females if their male offspring are cultured. This method is superior to allozymes and chromosome squashes in that the fly in question does not need to be injured to determine the species identity, and the techniques are both simple and inexpensive once an Insectavox is obtained.

References: Anderson, W.W., F.J. Ayala, and R.E. Michod 1977, J. Hered. 68:71-74; Ewing, A.W., 1969, Anim. Behav. 17:555-560; Gorczyca, M., and J.C. Hall 1987, Dros. Inf. Serv. 66: 157-160; Noor, M.A.F., 1995, Pan-Pacif. Entomol. 71:71-74; Noor, M.A.F., 1996, Anim. Behav. 52:1205-1210; Noor, M.A.F., 1997, J. Insect Behav. 10:305-312; Noor, M.A.F., and C.F. Aquadro, *in press*, Anim. Behav.; Waldron, I., 1964, Science 144:191-193.

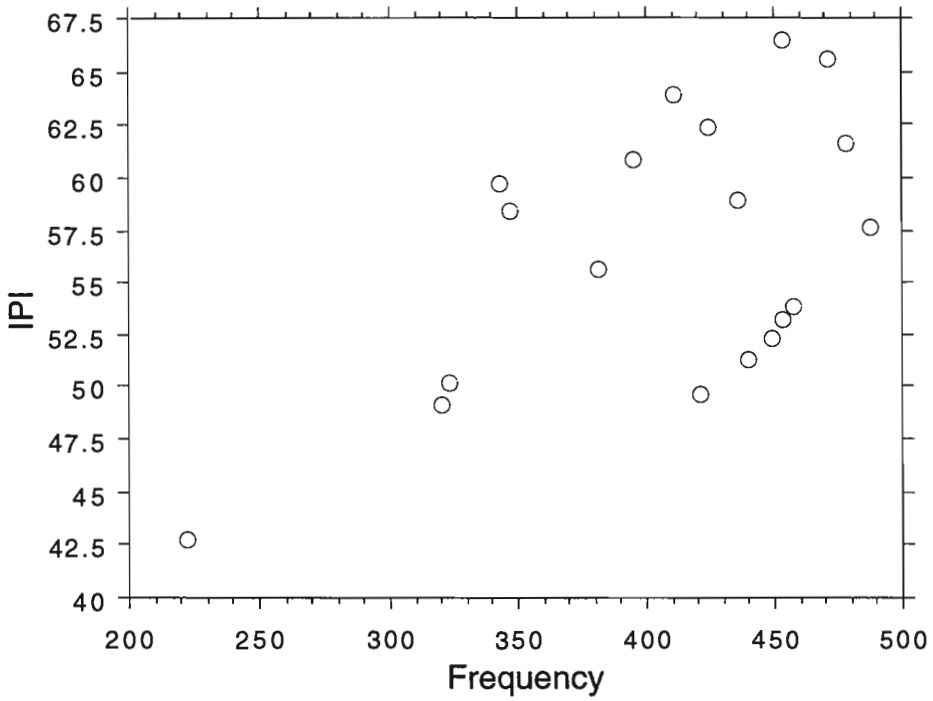

Figure 2. Plot of courtship song elements of wild-caught flies from Mather, California.

Noor, Mohamed A.F.[1], **John R. Wheatley**[2], **Kris A. Wetterstrand**[1], **and Hiroshi Akashi**[3]. [1]Section of Genetics and Development, Cornell University, Ithaca, NY 14853. [2]Department of Psychology, Indiana University, Bloomington, IN 47405. [3]Section of Evolution and Ecology, University of California, Davis, CA 95616. Western North America *obscura*-group *Drosophila* collection data, summer 1997.

We report here our collection data for *obscura*-group *Drosophila* species in Utah, Arizona, and California. Most notable is the rapid introduction and/or rise in frequency of *D. subobscura* in central Utah. Our collection in 1993 at this site in Utah yielded only *D. pseudoobscura*, suggesting this introduction is very recent. Also noteworthy is the drop in the relative abundance of *D. azteca* after a steady increase over several years (Noor, 1995). Finally, four of the ten *D. persimilis* females captured at Mount St. Helena appear to have been inseminated by males possessing the sex-ratio gene arrangement, hence producing all female offspring. In contrast, only 1 of the 30 *D. persimilis* females captured at Mather had been inseminated by an apparently sex-ratio male.

Obscura-group *Drosophila* were collected from five sites in the western United States in June/July, 1997:

American Fork, Utah- 40°26.71'N, 111°42.74'W- July 9-10, flies were collected from the Uinta National Forest in American Fork Canyon. (AFC)

Flagstaff, Arizona- 34°56.58'N, 111°29.53'W- June 20-22, flies were collected from the immediate vicinity of Mormon Lake. (FLAG)

Mather, California- 37°53.12'N, 119°50.78'W- June 26-29, flies were collected immediately outside the cabin maintained by the Carnegie Institute of Washington. (MATHER)

Mount St. Helena, California- 38°39.18'N, 122°35.96'W- July 1-3, flies were collected at Robert Louis Stevenson state park north of Calistoga. (MSH)

Paradise, California- 39°46.20'N, 121°37.58'W- July 6-7, flies were collected from Bille Park. (PARA)

We used buckets of fermenting bananas to attract flies for capture. These buckets were left out of doors overnight, and fresh bananas were added to the fermenting bananas daily. Males were identified to species using morphological criteria (Beckenbach and Prevosti, 1986), and females were identified using chromosome squashes of

offspring (Anderson *et al.*, 1977) and courtship songs of offspring (Noor, 1998). Flies were identified as *D. pseudoobscura* (pseudo), *D. persimilis*, *D. miranda*, *D. subobscura*, and *affinis*-subgroup *Drosophila*.

All *affinis*-subgroup flies captured in California are assumed to be *D. azteca*. Recently, Pascual *et al.* (1997) reported that *D. athabasca* had invaded California. We attempted to use the RAPD markers suggested by Pascual *et al.* (1997) to identify positively the *affinis*-subgroup species that we captured, but several individuals had combinations of bands that were suggested to be unique to each of the two species. Dr. Rhonda Snook positively identified several individuals that we captured as *D. azteca* using a genital comb characteristic.

Table 1. Proportions of *obscura*-group species collected

Site	%pseudo	%persimilis	%miranda	%subobscura	%"azteca"	N*
AFC, UT	24	0	0	75	<1**	252
FLAG, AZ	100	0	0	0	0	94
MATHER, CA	11	69	2	1	17	148/38
MSH, CA	12	8	2	30	47	273/27
PARA, CA	----------26----------			16	58	25***

* The first number is the total number of flies captured, while the second number denotes the number of females used to calculate the relative proportions of *D. pseudoobscura*, *D. persimilis*, and *D. miranda*.
** Probably *D. athabasca*, only 1 male captured.
*** No females that were *D. pseudoobscura*, *D. persimilis*, or *D. miranda* were captured.

The spread of *D. subobscura* into Utah is startling. Genetic studies of the recently established population(s) in Utah, the older populations in the northwest, and the ancestral populations in Europe may yield information on how this species has spread so quickly over such a vast region.

References: Anderson, W.W., F.J. Ayala, and R.E. Michod 1977, J. Hered. 68:71-74; Beckenbach, A.T., and A. Prevosti 1986, Am. Midl. Nat. 115:10-18; Noor, M.A., 1995, Pan-Pacif. Entomol. 71:71-74; Noor, M.A.F., 1998, Dros. Inf. Serv. 81:134-136; Pascual, M., J. Balanya, A. Latorre and L. Serra 1997, Mol. Ecol. 6:293-296.

Jones, C.D., and H.A. Orr. Department of Biology, University of Rochester, Rochester, NY 14620. Test of a *Drosophila simulans* balancer and a remapping of chromosome 3.

Although its genetics does not rival that of *D. melanogaster*, *D. simulans* has an large number of genetic markers, compound chromosomes, and other genetic tools. Most mutations in *D. simulans* are alleles of mutations in *D. melanogaster*. *D. simulans* is also karyotypically quite similar to *D. melanogaster*. However, the two species differ in a large inversion on *3*R. Unfortunately, this inversion difference has caused confusion about the *D. simulans* third chromosome map.

Here, we remap the *D. simulans* third chromosome using a newly created multiply marked stock and test the utility of inversion In(*3*R)*Ubx* (81F1 to 89E) as a balancer for *3*R (Coyne and Sniegowski 1994).

To remap chromosome 3, male *jv st e osp pe* flies were crossed to wildtype females (Solway-Hochman), and F_1 females were then backcrossed to *jv st e osp pe* males. The resulting progeny were genotyped. To test the balancer, male *jv st e osp pe* flies were crossed to female In(*3*R)*Ubx*, *Ubx/Dl*. *Ubx*/+ F_1 females were then backcrossed to *jv st e osp pe* males, and their progeny genotyped. We then compared the recombination distances between markers in these two cross to assess the possible use of In(*3*R)*Ubx* as a balancer.

Remapping of chromosome *3*: Sturtevant showed that *jv*, *st*, and *pe* (an allele of *pink*) are all allelomorphic to *D. melanogaster* mutations (Sturtevant 1929; Sturtevant and Novitski 1941). *e* is also allelic to *ebony* in *D. melanogaster* (J.A. Coyne, pers. comm.). *jv* is the most distal marker shared by both *D. simulans* and *D. melanogaster*. Thus, we anchored our map at *jv* (19.2 cM). The other markers were then positioned according to their recombination distances as determined in the present study (Table 1).

The order of the markers was checked and did not differ

Table 1. Marker map positions (*N* = 1014 flies). Kosambi's formula was used to correct recombination distances ("Corrected map position" column).

Marker	Uncorrected map position	Corrected map position
jv	19.2	19.2
st	46.3	49.5
e	59.4	63.0
osp	68.6	72.3
pe	97.3	104.9

Table 2. Test of In(*3*R)*Ubx* as balancer (*N* = 739 control flies and *N* = 584 experimental flies)

	Percent recombination	
Interval	Control Cross	Experimental Cross
st - e	13.1	12.0
e - osp	9.2	8.6
osp - pe	28.7	21.1

from that of the map reported in Flybase. Moreover, our new map positions roughly agree with the positions of the homologous *D. melanogaster* loci, taking into account the large inversion difference on *3*R.

Test of *3*R balancer: Because it is not linked to *Ubx*, the distal marker *jv* was not scored in this cross. As Table 2 shows, *Ubx* is not a useful balancer. There is no significant suppression of recombination between *st* and *e*, nor between *e* and *osp*. There is weak suppression of recombination between *osp* and *pe* ($\chi 2 = 6.741$, $P = 0.0094$).

References: Coyne, J.A., and P.M. Sniegowski 1994, Dros. Inf. Serv. 75:36; Sturtevant, A.H., 1929, Contributions to the genetics of *Drosophila simulans* and *Drosophila melanogaster*. Carnegie Institute; Sturtevant, A.H., and E. Novitski 1941, Genetics 26:517.

Hegde, S.N., V. Vasudev, V. Shakunthala, and M.S. Krishna. Drosophila Stock Centre, Department of Studies in Zoology, University of Mysore, Manasagangotri, Mysore-570006, India. *Drosophila* Fauna Of Palni Hills: Tamilnadu, India.

Paini hills form the southern part of western ghats and are situated at 10:13° N latitude 77:32°E longitude. The tallest mountain peak has an altitude of 2,333 meters above the sea level, and the foot of the hills has an altitude of just about 300 m. The annual rainfall is about 165 cm and temperature in the area ranges from 8.3 °C to 20 °C. The vegetation consists of shrubby jungles at the foot and, as altitude increases, the forest composition changes to moist deciduous to evergreen type. The moutain peaks have huge eucalyptus trees, *Acanthospermum hispidium*, *Grewia hirsuta, Hibiscus* species, *Euphorbia* species, and so forth.

Table 1. Distribution of different species of *Drosophila* in Palni hills.

Species	Altitude (in meters)											
	350	475	800	950	1050	1150	1450	1650	1750	1800	2300	Total
Subgenus: *Sophophora*												
D. bipectinata	157	24	3	04	9	46	—	—	—	—	—	243
D. malerkotliana	54	150	182	474	461	568	91	—	—	—	—	1980
D. takahashii	—	—	—	—	—	4	4	12	10	—	—	30
D. mysorensis	—	17	4	—	—	16	45	12	12	3	—	109
D. anomelani	—	—	—	—	4	2	—	3	3	—	—	12
D. rajasekari	—	—	12	3	2	2	2	—	—	—	—	21
D. sahyadrii	—	—	—	—	—	—	—	—	2	8	—	10
*D. palniensis**	—	—	—	—	—	—	—	—	—	—	4	04
Subgenus: *Drosophila*												
D.n. nasuta	31	—	19	10	43	35	40	—	—	—	—	178
D.s. neonasuta	14	25	23	06	59	72	57	31	—	—	—	287
D. formosona	—	—	—	—	—	2	34	—	17	8	—	61
D. brindavani	—	1	8	11	9	6	—	—	—	—	—	35
Subgenus: *Scaptodrosophila*												
D. nigra	8	—	—	—	1	—	—	—	—	—	—	09
Genus: *Phorticella*												
Phorticella striata	4	13	20	19	4	4	—	—	—	—	—	64
No. of species	6	6	8	7	9	11	7	4	5	3	1	
Total No. Captured	268	230	271	527	592	757	273	58	44	19	04	3043

* New species

Collections of *Drosophila* were made in hill ranges using net sweeping as well as bottle trapping methods from 11 different altitudes (350, 475, 800, 950, 1050, 1150, 1450, 1650, 1750, 1800 and 2300 m above sea level). These collections yielded a total of 3043 individuals. The catch included twelve species of *Drosophila* and one species of *Phorticella*. Table 1 shows that *D. malerkotliana* with 1980 individuals was the most common and abundant species (65.14%), next was *D. s. neonasuta* with 287 individuals, while *D. bipectinata* was the third largest with 243 individuals (7.89%). The remaining 529 (14.43%) individuals were shared by other species.

Number of flies obtained at 350 m altitude was higher than at 475 m. Then the number of flies increased with increasing altitude up to 1150 m and again declined gradually. From Table 1, it is also clear that some species, namely *D. bipectinata, D. malerkotliana, D. n. nasuta, D. s. neonasuta, D. brindavani* and *Phorticella striata,* were seen only up to 1450 m. *D. takahashii, D. anomelani, D. sahyadrii* were not seen in lower altitude but they were seen sparsely in high

altitude. At the highest altitude of 2300 m a new species was collected. This species belongs to *montium* subgroup of the *melanogaster* species group. The new species has been given the name *D. palniensis* and will be described elsewhere.

Acknowledgments: The authors are grateful to Prof. H.A. Ranganath, Chairman, Department of Studies in Zoology, University of Mysore, for providing facilities and constant encouragement to carry out the work. We also thank Mr. K. Raviram for his kind help during the collection trip.

Marinković, D., V. Stojiljković and S. Stanić.
Faculty of Biology, Belgrade, Yugoslavia. The changes in fitness components in groups of *D. melanogaster* with different rates of embryonic development.

A long-run analysis of the dynamics of preadult and embryonic development, and its correlation with other fitness, physiological, and biochemical characteristics of observed individuals (N > 2000), has resulted in an understanding of complex relationships, based on developmental homeostasis of this species.

Individuals in which development starts with an extremely fast embryogenesis (*i.e.*, less than 15 hours at 22 °C, on the surface of a yeast medium), have, on an average, also faster larval and pupal development, greater wing length, total life-span, and male fertility (Figure 1). On the contrary, the females from that group have somewhat smaller egg-laying capacity, and both larvae and adults have lesser mobility. It comes out that larvae which hatch after more than 30 hours of embryonic development are remarkably more mobile (Jovanovska, 1990) and may search for a new niche, since the surface could be already occupied by larvae having the faster embryogenesis.

As for physiological traits, activity of a majority of studied enzymes is greater in individuals with the shortest preadult development (Marinković *et al.*, 1986; Milošević and Marinković, 1989). This has been correlated with the rDNA spacer lengths (Cluster *et al.*, 1987), *i.e.*, with an eventually greater capacity for production of ribosomal RNA and specific proteins.

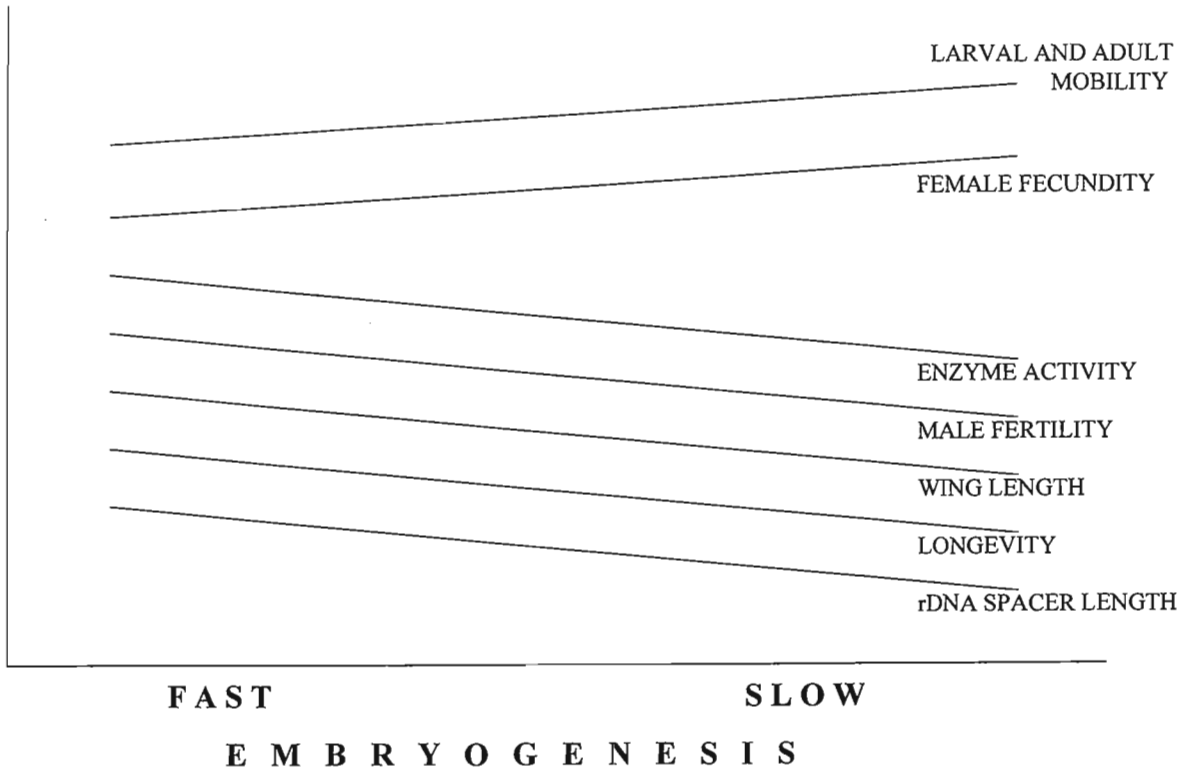

Figure 1. Changes in fitness components in groups of *Drosophila melanogaster* individuals with different rates of embryonic development.

Complex relationships between studied traits are telling us how much could be restricted adaptive biological variation, and which are developmental strategies to reduce enormous potentials of such a variation to efficient ontogenetic programs realized in eclosed adult individuals.

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Koryakov, Dmitry E.[1], Elena S. Belyaeva[2], and Igor F. Zhimulev[1,2]. [1]Department of Cytology and Genetics, Novosibirsk State University, Novosibirsk 630090, Russia [2]Institute of Cytology and Genetics, Novosibirsk 630090, Russia. The new *Drosophila melanogaster* nonlethal inversion, arisen from the *In(2R)bw^{VDe2}*.

In the stock *In(2R)bw^{VDe2}/CyO*, received from the Umea *Drosophila* stock center, part of the flies spontaneously lost lethality in homozygous condition. The new stock, called *In(2R)bw^{VK} - brown Variegated of Koryakov*, was constructed. Cytological analysis has shown that the new stock has the same inversion - 41A-B - 59D6-E1, as the *bw^{VDe2}*.

The viability was studied of heterozygotes of *bw^{VDe2}* and *bw^{VK}* with other rearrangements, displaying *bw-Variegation* phenotype (*In(2R)bw^{VDe1}*, *In(2R)bw^{V1}*, *T(2,3)bw^{VDe4}*, and *T(2,3)bw^{V4}*), and with two lethals *uex^{4}* and *rl^{9}*, located according to Dimitri (1991) in heterochromatin distally and proximally to the break point of the inversion *bw^{VDe2}*, respectively (Table 1). In the case of *bw^{VK}* the expected ratio of the *Cy/Cy^{+}* offspring is 1:1, in the case of *bw^{VDe2}* the ratio is 2:1, and the percentage of surviving flies was calculated from these ratios. It should be noted that the surviving flies *bw^{VK}/bw^{VDe2}* slightly differ in reciprocal crosses. In comparison with *bw^{VDe2}*, viability of heterozygotes of *bw^{VK}* with *bw^{VDe1}*, *bw^{V1}*, and *bw^{VDe4}* is noticeable higher, but with *bw^{V4}* it is lower. Viability of *bw^{VK}/uex^{4}* flies is greatly higher than expected.

Table 1. The viability of heterozygotes of *bw^{VDe2}* and *bw^{VK}* with other rearrangements, displaying *bw-Variegation* phenotype and with two lethals (at 25°C).

		bw^{VK}		*bw^{VDe2}*	
		number of flies	Survived (in %%)	number of flies	Survived (in %%)
bw^{VK}	Cy			124	
	Cy^{+}			116	96.7
bw^{VDe2}	Cy	133			
	Cy^{+}	107	89.2		
bw^{V1}	Cy	121		160	
	Cy^{+}	94	87.4	38	57.6
bw^{VDe1}	Cy	135		164	
	Cy^{+}	19	24.7	0	0
bw^{V4}	Cy	102		110	
	Cy^{+}	41	57.3	33	68.8
bw^{VDe4}	Cy	82		134	
	Cy^{+}	22	42.3	8	17.0
uex^{4}	Cy	82		157	
	Cy^{+}	168	134.4	63	86.3
rl^{9}	Cy	122		153	
	Cy^{+}	126	101.6	80	102.6

In some crosses the flies with wing defects were found in small amounts (Table 2), which is supposedly due to position effect variegation of the *uex* locus, because a very similar phenotype was described for the *uex* homozygotes (Maeda, 1984).

So, losing the lethality for *bw^{VK}* is accompanied by increasing viability with other *bw-Variegated* rearrangements.

Comparison of genetic inactivation as a result of position effect variegation of the *bw*-locus was performed with the inversions *bw^{VDe1}*, *bw^{VDe2}*, and *bw^{VK}*.

Cis-effect of *bw^{+}*-variegation in rearranged chromosomes can be observed in *R(bw^{+})/R^{+}(bw)* heterozygotes where *R* is eu-heterochromatin rearrangement. Precise calculation of pigmented facets is possible if their number is not higher that 30 per eye. Therefore, we established five classes of variegation: 0, 1-10, 11-20, 21-30 and more than 30 pigmented facets per an eye. As a rule, both eyes of the fly

Table 2. A number of flies with wing defects from some crosses.

	number of flies	
	completely unexpanded wings	turbid, crumpled wings
bw^{VK}/ bw^{V4}	7*	23**
*bw^{VK}/ bw^{VDe4}***	4*	5
bw^{VDe2}/ bw^{V1}	0	3
bw^{VDe2}/ bw^{V4}	14	18
bw^{VDe2}/ bw^{VDe4}	3	2

* - Black mouthparts. **- One wing may be smaller than another one, sometimes with bubbles. ***- In this cross a fly with notched wings planted apart (looking like Notch and Dichaete) was found

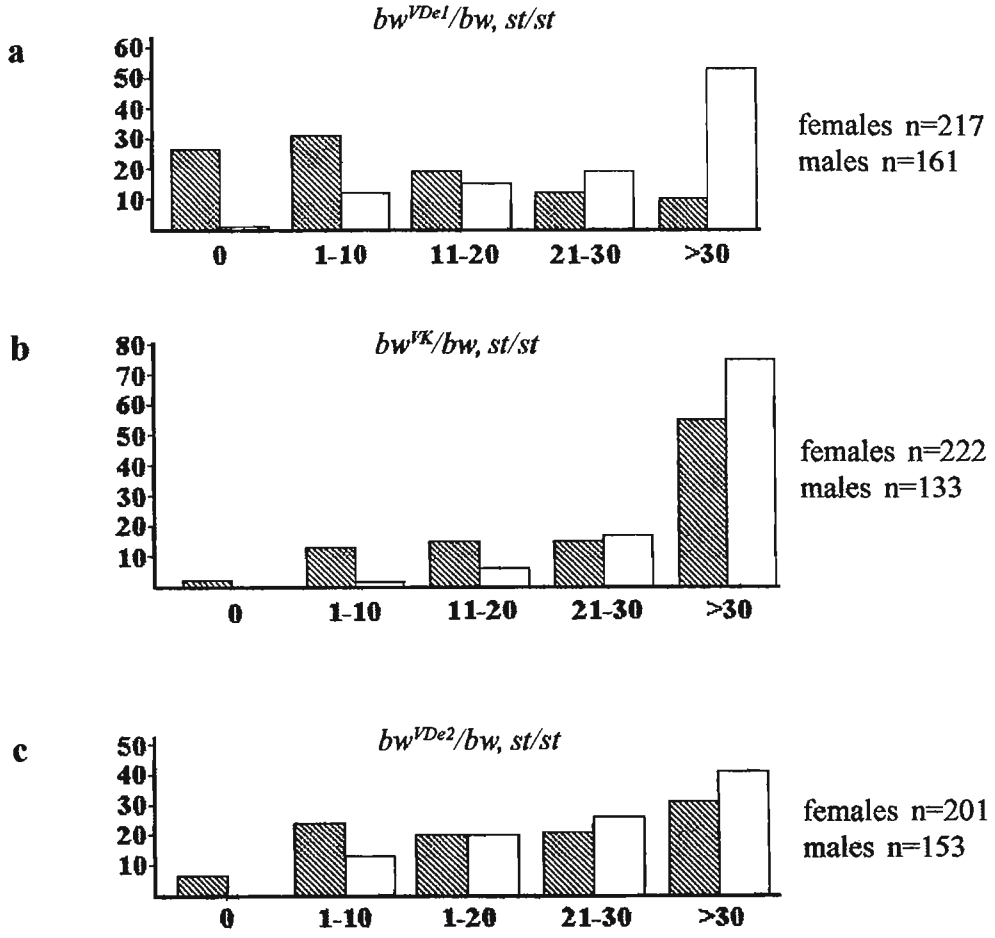

Figure 1. Comparison of the cis-inactivation of bw^+ in different strains at temperature 18°C. Abscissa, classes with different number of pigmented facets; ordinate, number of eyes of every class (in%%). Open columns are males, dashed are females.

belong to the same or to two neighboring classes.

Comparison of bw^+ cis-effect in chromosomes with different inversions gives the following row: $bw^{VDe1}>bw^{VDe2}>bw^{VK}$. Cis-inactivation is stronger in females than in males in all these cases (Figure 1).

Cytological analysis shows that in $X0;bw^{VK}/+$ males at 16°C (maximum enhancement of position effect variegation) only 11% of nuclei shows heterochromatization of the 59E region in rearranged homologue. In these cases the light chromosome region near 59D1-4 disappears and the bands 59D1-4 join to the chromocenter; they have diffuse morphology or are not seen at all. At the same conditions in bw^{VDe2} heterochromatization was found in 66% of nuclei (Belyaeva *et al.*, 1997).

So, the data show that bw^{VK} has noticeably decreased heterochromatization and frequent inactivation of the bw^+ gene in comparison with bw^{VDe2}.

Acknowledgments: The work was supported by grants from "Frontier genetic program of Russian Federation" and RFBR (96-04-50142, 96-15-97749).

References: Belyaeva, E.S., *et al.*, 1997, Chromosoma 106:124-132; Dimitri, P., 1991, Genetics 127: 553-564; Maeda, Y., 1984, Jpn. J. of Genetics 59: 249-257.

Etges, William J. Department of Biological Sciences, University of Arkansas, Fayetteville, AR, 72701. A new pericentric inversion in a natural population of *Drosophila robusta*.

Pericentric inversions should not persist in natural populations because of their predicted effects on gamete production in heterokaryotypes. Should recombination occur within the span of a pericentric inversion, heterozygotes should be semi-sterile because half of the gametes produced will contain duplications and deficiencies. Though pericentrics are much less frequently observed in natural populations than paracentrics, particularly in *Drosophila* species, their predicted depression of fitness in heterozygote females has not been confirmed (Coyne *et al.,* 1993). Furthermore, pericentric inversions have been implicated in chromosomal evolution (Patterson and Stone, 1952), but they remain less studied than paracentric inversions (Powell, 1997). In fact, northern populations of *Drosophila robusta* harbor considerable polymorphism for a pericentric inversion of the third chromosome, 3L-R, reaching frequencies of up to 60% in some populations. Two other pericentrics observed in natural populations involving the second chromosome have been recovered in single individuals only (Carson, 1958; Levitan, 1992).

Figure 1.

A previously undescribed pericentric inversion was observed in the karyotype of a wild-caught female *D. robusta* and her offspring from a population sampled near Mill Creek, Scott County, Arkansas (Etges, 1996). This new gene arrangement is labeled 2LR-3, and includes about 25% of the proximal section of the left arm and almost the entire right arm of chromosome two (Figure 1). This photograph shows the karyotype 2L/2LR-3 from the testcross progeny of the

wild female. The indicated breakpoints of 2LR-3 (①) occur at the approximate borders of regions E and F on the left arm and in region S of the right arm (Levitan, 1992). The chromocenter (②) in this preparation is evident with the second chromosome arms to the right, the third chromosome at the top left (with the small, fourth, dot chromosome next to its centromere), and the X chromosome at the left-bottom in this photomicrograph. This gene arrangement persisted in lab culture for some time, but an effort to recover it from nature again has not yet been made. It would appear that pericentric inversions occur at low frequency in *D. robusta* populations, yet just one has risen to high frequencies in nature. Further study of the fertility effects of 3L-R will hopefully shed light on the fitness consequences of this widespread pericentric inversion.

References: Carson, H.L., 1958, Adv. Genet. 9: 1-40; Coyne, J.A., W. Myers, A.P. Crittenden, and P. Sniegowski 1993, Genetics 134: 487-496; Etges, W.J., 1996, Evolution 50: 2095-2100; Levitan, M., 1992, In: C.B. Krimbas and J.R. Powell (eds.). *Drosophila Inversion Polymorphism*. CRC Press, Inc., Boca Raton. pp. 221-338; Patterson, J.T., and W.S. Stone 1952, *Evolution in the Genus* Drosophila. New York, MacMillan Co.; Powell, J.R., 1997, *Progress and Prospects in Evolutionary Biology: the* Drosophila *Model*. Oxford Univ. Press, New York.

Llopart, Anna, and Montserrat Aguadé. Departament de Genètica, Facultat de Biologia, Universitat de Barcelona. Spain. A new naturally occurring inversion in *Drosophila subobscura*

The species *Drosophila subobscura* is characterized by a very rich inversion polymorphism affecting its five acrocentric chromosomes (A, J, U, E and O). In this species, like in many others, there is some clustering of inversion breakpoints, for example, in bands 64B and 64C for inversions E_2, E_3, E_4, E_5, E_9 and E_{17} of the E chromosome. Also, differences have been observed in the frequencies of short, medium and long-sized inversions in natural populations. In particular, short inversions tend to be rare and endemic, generally known from only one population sample and present at very low frequency. Natural selection has been proposed to explain the observed patterns. In this sense, Krimbas and Powell (1992) concluded in their review of *Drosophila* inversion polymorphism that moderately sized inversions were favored as a result of a trade-off between positive and negative effects of the length of inversions. Long inversions could more easily capture advantageous haplotype combinations but they could also lose them more easily due to their higher rate of double crossovers. On the other hand, short inversions, in addition to possible physical problems in their origin, would have a lower probability of capturing favorable sets of alleles but, if that were the case, they could maintain them more efficiently.

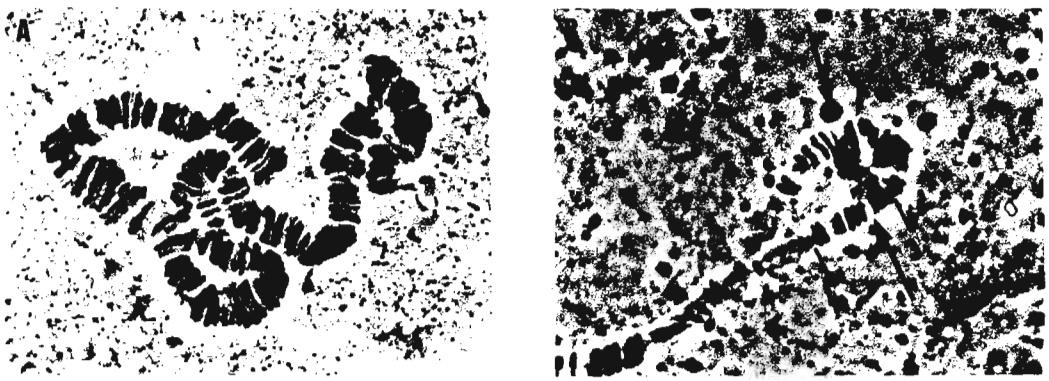

Figure 1. (A) Polytene A chromosomes from a heterokaryotypic (A_{st}/A_{9+2}) female larva. The most centromeric part of the two homologous chromosomes is unpaired; (B) Detail of the inversion loop in a heterokaryotypic female larva. Inversion breakpoints are indicated by an arrow and lines show limits between chromosome sections.

Selective explanations have also been proposed to explain the strong linkage disequilibrium observed between certain non overlapping inversions. Sperlich and Feuerbach-Mravlag (1974), for example, pointed out that the absence of the A_{1+2} arrangement in natural populations of *D. subobscura* was not caused by the inhibition of recombination in doubly heterozygous females (A_1/A_2) due to mechanical reasons but by epistatic interactions.

Until 1993, a total of 67 inversions had been reported in *D. subobscura* (Krimbas, 1993), with eight of these inversions located in the sexual (=A) chromosome. These eight inversions, however, produce only 11 different gene

arrangements of natural origin and occurrence: A_{st}, A_1, A_2, A_{2+3}, A_{2+4}, A_{2+6}, A_{2+3+6}, A_{2+3+5}, $A_{2+3+5+7}$ (SR), A_{2+5+7}, A_{2+8}. The new inversion, A_9, was detected in the progeny of a male that had been crossed to females from a laboratory strain that carried the A_{st} chromosomal arrangement (Figure 1A and 1B). This male, which also carried the A_2 inversion, belonged to an isofemale line recently established from a natural population captured in Bizerte (Tunisia). Inversion A_9 was only present in this male from a total of forty-nine lines studied. Most probably A_9 is a recently arisen inversion as it was not detected by Jungen (1968) in his extensive survey of chromosomal inversion polymorphism (more than 550 sexual chromosome analyzed) in Tunisian populations of *D. subobscura*.

The breakpoints of the new inversion are located in sections 1B and 3B on the Kunze-Mühl and Müller map (1958), respectively. The 1B breakpoint of A_9 is very close, if not coincident, to the proximal breakpoints of inversions A_5 and A_7 which would support the nonrandom distribution of inversions along chromosomes. A_9 is the shortest inversion described in the A chromosome. Also, when we compare A_9 to the autosomal inversions of *D. subobscura*, only J_5, E_{14}, E_{20} and O_{24} are shorter.

In the process of obtaining a homokaryotypic line for A_9 from the progeny of the initial male (A_{st}/A_{9+2} females and A_{st}/Y males), both recombinant classes (A_2 and A_9) were found. This result indicates that, at least under laboratory conditions, crossover between the A_9 and A_2 inversions is not physically inhibited. As expected, the homokaryotypic females for the new inversion (A_9/A_9), as well as the hemizygote males, did not show any viability problems and grew satisfactorily at 18° and 13°C.

Acknowledgments: We thank C. Segarra, M. Papaceit and D. Orengo for technical advice, J. Rozas for sharing flies and J. M. Comeron for useful comments on the manuscript. This work was supported by a predoctoral fellowship from Ministerio de Educación y Ciencia, Spain, to A. LL and by grants PB94-0923 from Ministerio de Educación y Ciencia and 1995 SGR-577 from Comisió Interdepartamental de Recerca i Innovació Tecnològica, Generalitat de Catalunya, to M. A.

References: Jungen, H., 1968, Archiv Julius Klaus-Stiftung Vererbungsforschung Socialanthropologie Rassenhygiene 43: 3-55; Krimbas, C.B., 1993, Drosophila subobscura. *Biology, Genetics and Inversion Polymorphism* pp. 107-139, edited by C.B. Krimbas. Verlag Dr Kovac, Hamburg; Krimbas, C.B., and J.R. Powell 1992, *Drosophila Inversion Polymorphism* pp.1- 20, edited by C.B. Krimbas and J.R. Powell. CRC Press, Boca Raton, Fl; Kunze-Mühl, E., and E. Müller 1958, Chromosoma 9: 559-570; Sperlich, D., and H. Feuerbach-Mravlag 1974, Evolution 28: 67-75.

Cerda, Hugo[1], and Antonio Fontdevila[2]. [1] Simón Rodriguez University Pest Control Laboratory, Apartado Postal (P.O. Box) 47.925, Caracas 1041-A, Venezuela; [2] Universitat Autonoma de Barcelona, Department de Genètica i de Microbiologia, Bellaterra, Spain. Evolutionary Divergence of *Drosophila venezolana* (*martensis* Cluster, *buzzatii* Complex) on Gran Roque Island, Venezuela.

Introduction

The *martensis* cluster (*buzzatii* complex, *repleta* group) comprise a group of four species which emerge from cacti endemic to northern South America (Ruiz and Wasserman, 1992). The species are *D. martensis* (Wasserman and Wilson, 1957), *D. starmeri* (Wassermann, Koepfer and Ward, 1973), *D. uniseta* (Wassermann, Koepfer and Ward, 1973), and *D. venezolana* (Wasserman, Fontdevila and Ruiz, 1983).

D. venezolana was the last species described. Throughout its distribution it has a 2 e^7 chromosomal pattern. This species is symmorphic, and phylogenetically closely related to *D. starmeri* (Cerda and Benado, 1986; Fontdevila and Ruiz, 1983; Ruiz and Fontdevila, 1981). This species has been reported in arid zones of Venezuela, mainly in Falcon State and some small arid coastal zones of Central and Eastern Venezuela, where it feeds on cacti of the *Opuntia* genus (Cerda, *et al.*, 1996; Benado, 1989). On Gran Roque island, the largest island of the Los Roques archipelago, 120 km north of the coast of Venezuela's Federal District, *D. venezolana* is the only species of the *martensis* cluster and breeds and feeds in all the cacti on the island, including *Stenocereus griseus*, *Opuntia wentiana*, and *Melocactus amoenus* (Benado, 1989).

This paper reports the results of a preliminary analysis of chromosomal polymorphism, abdominal band pattern, eye color, and reproductive isolation of *D. venezolana* of Gran Roque, in comparison with the continental populations of *D. venezolana*.

Material And Methods

Location of the Study: *Drosophila* individuals were collected in February 1984 using fermented banana and beer as bait, on Gran Roque island (11° 58' 33" N, 66° 40' 37" W).

Chromosomal Polymorphism and Reproductive Isolation: Polytene chromosomal polymorphism of 22 larvae reared in David medium (1959) was analyzed using acetic orceine staining.

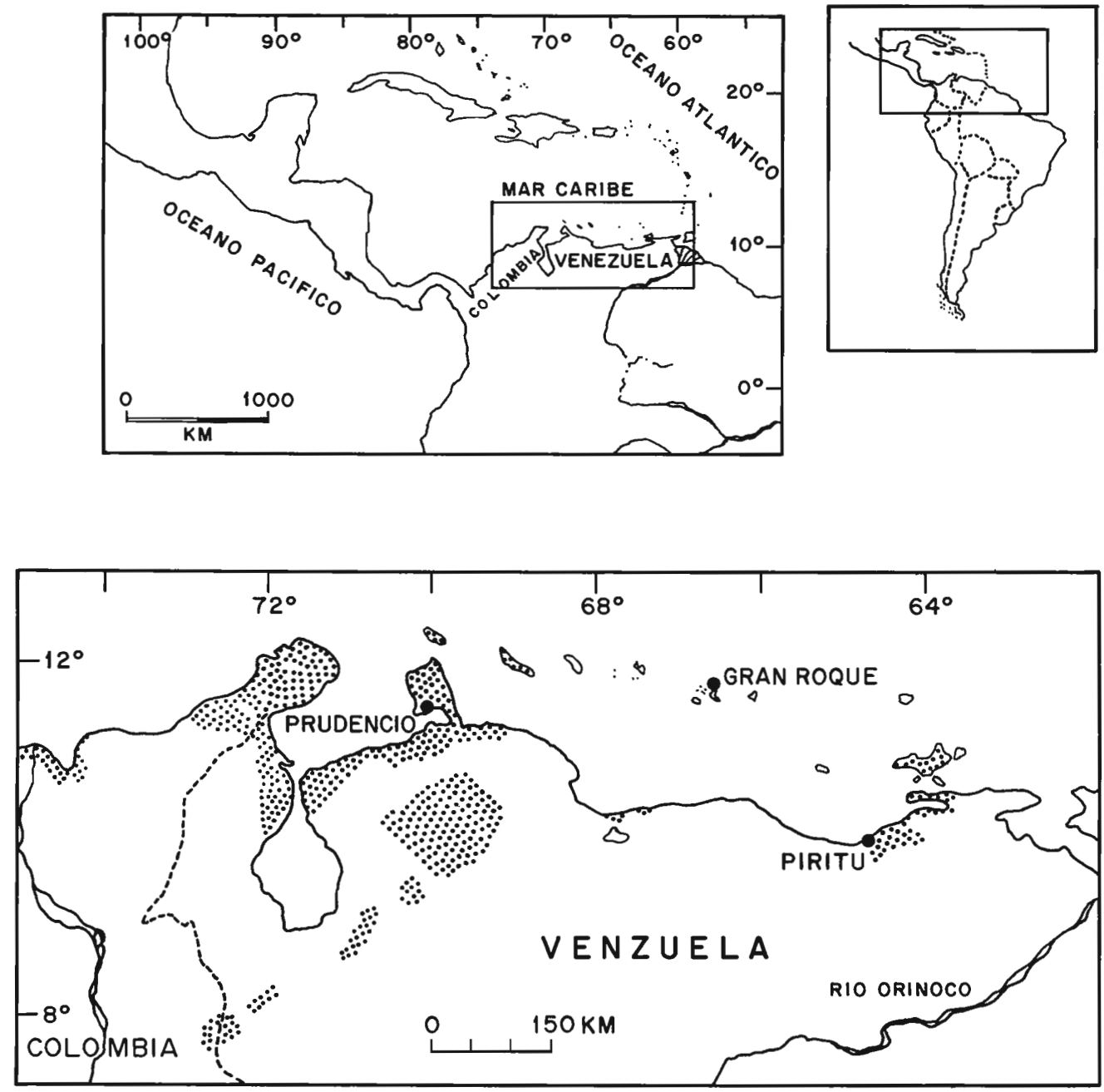

Figure 1. Geographic distribution of *Drosophila venezolana* in the Gran Roque Island, the west continental localities of Prudencio and the East Continental Locality of Píritu.

The study of reproductive isolation was performed through interpopulational crosses between the populations of Gran Roque, Prudencio (11° 39' N, 70° W, Falcón State in western Venezuela), and Píritu (10° 03' N, 65° 03' W, Anzoátegui State in eastern Venezuela) (Figure 1). Five replications were made for each cross of five mating pairs. The adults began to emerge after 15-20 days. Offspring number was counted and the emerged F1 individuals were kept in vials for subsequent crosses to give rise to the F2 generation. Intrapopulational crosses were used for control. The number of descendent was compared with intrapopulation control using Wilcoxon's non-parametric test (Sokal and Rohlf, 1981).

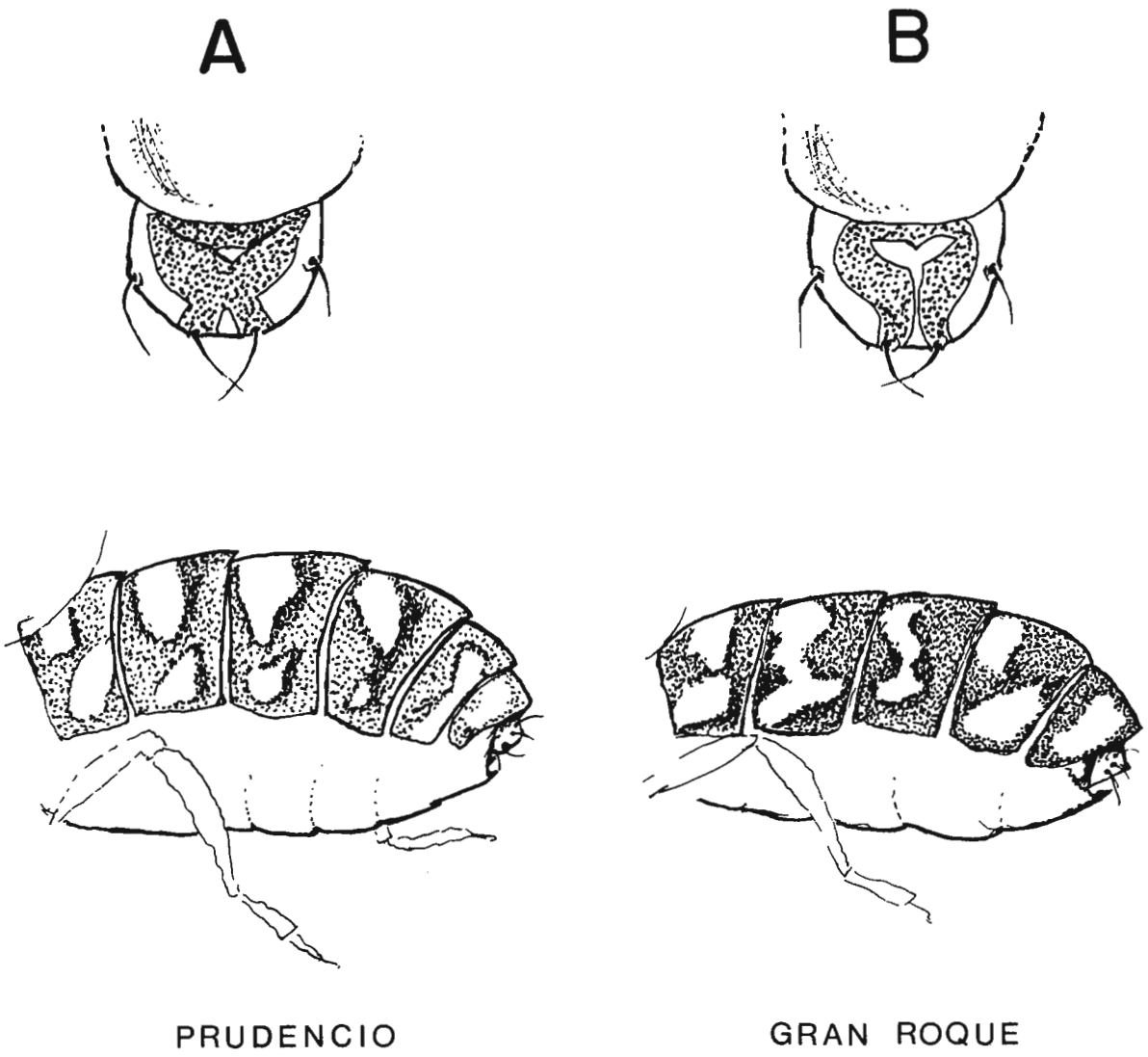

Figure 2. A. The morphological characters, the abdominal band pattern (bottom) and the escutelum (upper), from the locality of Prudencio and B the morphological characters, of the abdominal band pattern (bottom) of the escutelum (upper), from the locality of *D. venezolana* of Gran Roque.

Results And Discussion

The karyotype of the 44 genomes analysed showed the $2e^7$ rearrangement. Morphological characters, namely the abdominal and scutelum band patterns, differ from those of the continental populations (Figure 2). Eye color was opaque red in all the individuals studied, a color never observed before in continental populations.

D. venezolana from Gran Roque revealed reproductive isolation for F1 and F2 crosses with continental populations; the highest values were found for the F2 offspring from crosses with Prudencio (Table 1).

Gran Roque island occupies a small area of 1.7 km^2 and *D. venezolana* is the only cactophilic *Drosophila* species found there; it breeds in all the cacti present on the island. In this paper we give reasons to think that the Gran Roque population has undergone a genetic divergence process, as it is shown by its incipient reproductive isolation and by its morphological divergence. This may be explained by the island's geographic isolation or by a founder effect.

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Table 1. Offspring numbers of crosses (average of five replications ± standard deviation) for the first generation F_1 and the second generation F_2 among populations of the *D. venezolana* species from Piritu (Eastern Venezuela), Prudencio (Western Venezuela), and Gran Roque Island. M indicates males and F indicates females. Asterisk* indicates that the number of control descendents is statistically different from both together of the intrapopulational control group for a Wilcoxon nonparametric test at $p < 0.05$.

A) First generation of F_1 crosses among *D. venezolana* populations from Gran Roque island, Prudencio, and Píritu.		
Breedings	M x F	F x M
Interpopulational crosses:		
Píritu x Gran Roque	219.0 ± 80.5	162.0 ± 86.7*
Prudencio x Gran Roque	162.6 ± 76.7*	138.6 ± 59.0*
Intrapopulational crosses:		
Grand Roque x Gran Roque	322.4 ± 50.8	
Prudencio x Prudencio	338.6 ± 59.0	
Píritu x Píritu	359.0 ± 23.8	
B) Second generation of F2 crosses among *D. venezolana* populations from Gran Roque island, Prudencio, and Píritu.		
Breedings	M x F	F x M
Interpopulational crosses:		
Píritu x Gran Roque	82.2 ± 31.6*	85.2 ± 10.9*
Prudencio x Gran Roque	36.4 ± 16.6*	70.4 ± 32.6*
Intrapopulational crosses:		
Grand Roque x Gran Roque	301.8 ± 32.1	
Prudencio x Prudencio	320.0 ± 50.8	
Píritu x Píritu	342.0 ± 37.0	

and F.D. Wilson 1957, Texas Univ. Pub. 5721: 132-156; Wasserman, M.; H.R. Koepfer and B.L. Ward 1973, Annals of the Entomological Society of America 66: 1239-1242; Wasserman, M., A. Fontdevila and A. Ruiz 1983, Annals of the Entomological Society of America 76: 675-677.

Singh, B.N.,[1] and S. Lata[2]. [1]Department of Zoology, Banaras Hindu University, Varanasi 221 005, India. [2]Zoology Section, Mahila Maha Vidyalaya, Banaras Hindu University, Varanasi 221 005, India. Variation in the number of males and females without apical black patch on their wings in laboratory strains of *Drosophila biarmipes*.

Drosophila biarmipes males possess a dark apical black patch on their wings. There is variation in the male apical black wing patch and the males without the patch are also found (Singh and Chatterjee, 1987). Singh *et al.* (1995) reported for the first time the presence of apical black patch on the wings of females in two laboratory stocks of *D. biarmipes*. However, in females, the patch is lighter and does not touch the margin of wings. Behavioral studies have been carried out in *D. biarmipes* by Singh and his coworkers (Singh and Chatterjee, 1987; Singh and Pandey, 1993, 1994; Srivastava and Singh, 1996, 1997). Males with wing patch have greater mating success than those without wing patch which provides evidence for the role of visual stimuli in mating behavior of *D. biarmipes* (Singh and Chatterjee, 1987). Males without a patch lack the visual element of courtship display and they have to court the females for a longer time to stimulate the females beyond the acceptance threshold and achieve copulation (Chatterjee and Singh, 1991).

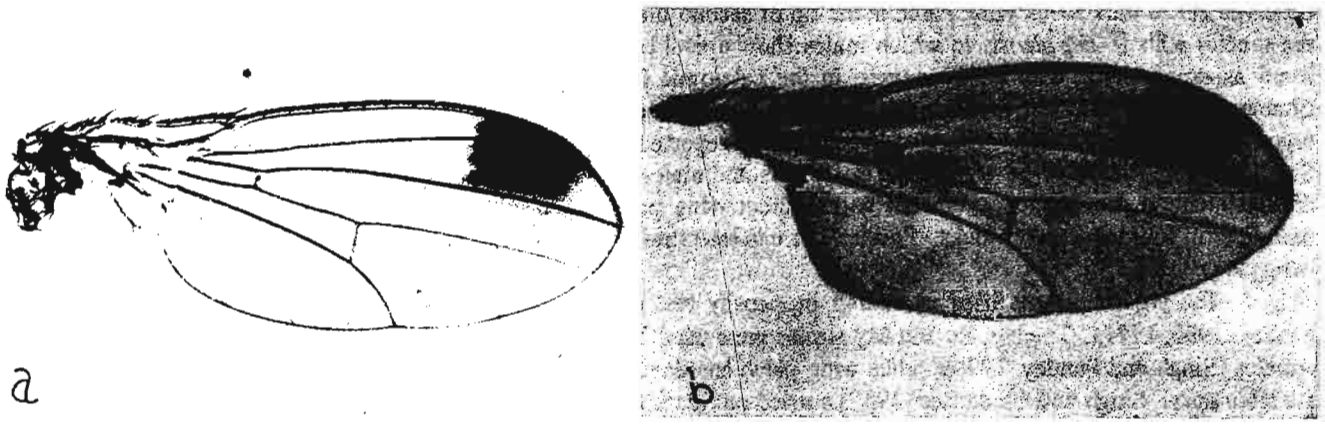

Figure 1. Wings of *D. biarmipes* showing apical black patch: a - male, b - female

We scored the number of females and males with/without wing patch in four laboratory strains of *D. biarmipes*. The strains used are:

1. My - obtained from Mysore Stock Centre in 1993 (origin-Mysore, Karnataka).
2. BR Hills - obtained from Mysore Stock Centre in 1993 (origin - B.R. Hills, Karnataka).
3. Ng - established from a female collected from Nagpur, Maharashtra in 1990.
4. Rc - raised from a female collected from Nagpur, Maharashtra in 1990.

In each of the four strains, females and males were taken randomly from culture bottles for observation of black patch on their wings. In females, the wing patch is small, lighter and does not touch the margin of wing in all the four strains analyzed. We observed variation in male apical black wing patch in all the four stocks. Males were classified into four groups: dark patch, dark patch not touching third vein, faint patch and without patch. Wings of a male and a female showing the apical black patch are depicted in Figure 1. Table 1 shows the number of males and females with wing patch and without wing patch. Frequency of males without wing patch varies from 0.74 percent (BR Hills) to 4.32 percent (Mysore). Thus the males without wing patch occur in low frequency in all the four strains. Frequency of females without wing patch ranges from 42.94 percent (Mysore) to 89.92 percent (Ng). As compared to males, the frequency of females without wing patch is higher in all the four stocks. Interstrain variation in the number of males and females with/without wing patch has been tested by calculating 2x4 chi-square values. Interstrain differences are significant for males ($\chi^2 = 9.89$; $p < 0.05$) as well as for females ($\chi^2 = 140.82$; $p < 0.001$). Thus there is interstrain variation with respect to apical black wing patch in both sexes of *D. biarmipes*.

Table 1. Number of males and females with wing patch and without wing patch in different wild laboratory strains of *Drosophila biarmipes*.

Strain	Males						Females		
	With dark patch	With dark patch not touching III vein	With faint patch	Total number with patch	Total number without patch	Total number of males scored	With patch	Without patch	Total number of females scored
My	120	7	6	133	6 (4.32)	139	97	73 (42.94)	170
BR Hills	226	25	16	267	2 (0.74)	269	54	147 (73.13)	201
Ng	314	49	13	376	3 (0.79)	379	40	357 (89.92)	397
Rc	297	24	26	347	8 (2.25)	355	57	168 (74.67)	225
	$\chi^2 = 9.89$, df = 3, $p < 0.05$						$\chi^2 = 140.82$, df = 3, $p < 0.001$		

Values given in the parentheses are percentages.

D. biarmipes was described by Malloch (1924) from Coimbatore, India. Malloch mentioned no black wing patch in males, although he observed a slightly darker tinge at the tip of the second vein. Reddy and Krishnamurthy (1968) described a new species *D. rajasekari* from Mysore, India, in which males have an apical black patch on the wings not touching the third vein. However, certain glaring similarities between *D. rajasekari* and *D. biarmipes* were noted by these authors. A new species, *D. raychaudhurii* was described by Gupta (1969) from Varanasi, India, who observed apical dark black wing patch at the tip of second the vein extending to the third vein. Gupta (1969) compared the species with *D. nepalensis* in which males show apical black wing patch. *D. rajasekari* and *D. raychaudhurii* are listed as synonymous with *D. biarmipes*, in the catalogue of world fauna prepared by Wheeler (1981). Singh and Chatterjee (1987) observed variation in the wing patch in males of *D. biarmipes* and males without wing patch were also found in a laboratory stock. None of these authors reported the presence of wing patch in females of *D. biarmipes*. Singh *et al.* (1995) observed wing pitch in females and males in two laboratory stocks of *D. biarmipes*. However, they did not mention the frequency of flies with/without wing patch. We have observed wing patch in both sexes of *D. biarmipes* in four laboratory stocks. Further, there is interstrain variation with respect to the number of flies with/without wing patch.

The Ng strain was used in mating propensity test but wing patch was not observed in females (Singh and Pandey, 1993, 1994). The Ng, Rc and My strains were used in sexual isolation tests but wing patch was not observed in females (Singh and Pandey, 1994). Thus wing patch has developed in females in the stocks during their maintenance in the laboratory. Singh and Chatterjee (1987) carried out mating propensity tests with two types of males of *D. biarmipes*, with patch and without patch using a laboratory stock established from a female collected from Bhagalpur, Bihar.

Several males without wing patch were regularly found in that stock. However, the number of males without patch is very low in all the stocks used during the present study. Thus the number of males without patch is gradually decreasing in the stocks during their maintenance in the laboratory. We have initiated selection experiments to study the genetic basis of wing patch in *D. biarmipes*.

References: Chatterjee, S., and B.N. Singh 1991, Dros. Inf. Serv. 70:47-48; Gupta, J.P., 1969, Proc. Zool. Soc, (Calcutta) 22:53-61; Malloch, J.R., 1924, Memb. Dep. Agr. Ind. 8:63-65; Reddy, G.S., and N.B. Krishnamurth 1968, Proc. Ind. Acad. Sci. 68:202-205; Singh, B.N., and M.B. Pandey 1993, Indian J. Exp. Biol. 31:932-933; Singh, B.N., and M.B. Pandey 1994, Indian J. Exp. Biol. 32:482-485; Singh, B.N., and M.B. Pandey 1994, Dros. Inf. Serv. 75:58-59; Singh, N.N., T. Srivastava, and A.K. Sundaran 1995, Dros. Inf. Serv. 76:129-130; Srivastava, T., and B.N. Singh 1996, Biol. Res. 29:355-360; Srivastava, T., and B.N. Singh 1997, Rev. Bras. Biol. 57:571-577; Wheeler, M.R. 1981, In: *The Genetics and Biology of* Drosophila (eds. Ashburner, M., H.L. Carson, and J.N. Thompson, jr.), Academic Press, New York, 3a:1-97.

Pavković Lučić, S., and V. Kekić. Institute of Zoology, Faculty of Biology, University of Belgrade, Studentski trg 16, 11000 Belgrade, Yugoslavia. *Drosophila* (*Lordiphosa*) *miki* Duda, first record for Yugoslavia.

In our investigations of *Drosophila* fauna in habitats on the Yugoslav coasts of the river Danube, we have found 26 species (Kekić, 1997). Continuing these investigations, in June 1996, we have collected flies on the locality of the Kamarište (about 1360 km far from the mouth of the Danube to the Black Sea). Following the methodology applied in previous researches, flies were collected by sweeping net over fruit-fermenting baits (banana, apple, small amount of seasonal fruit with baker's yeast and sugar) distributed over the studied habitat - in a quite dense green belt following the river, where the trees of old willows and poplars dominate. The habitat of Kamarište, compared to the other investigated habitats along the Danube, is most wild. For illustration, while collecting flies we have seen wild pigs and deer. So far we have analyzed only a part of a rather rich Drosophilidae collection, and among them we have identified a male of *Drosophila* (*Lordiphosa*) *miki* Duda, 1924 (see also Laštovka and Máca, 1978). *D.* (*L.*) *miki* is a very rare European species whose taxonomic status is not yet defined (Gimaldi, 1990; Máca, 1991); up to now it was only recorded in Austria (Duda, 1924), Hungary (Papp and Pescenye, 1988), Czechoslovakia (Máca, 1991), Switzerland (Bächli, 1996) and now in Yugoslavia.

Acknowledgments: We are grateful to Dr. G. Bächli for the help in determination of the examined Drosophilidae species.

References: Bächli, G., 1996, Mitt. Ent. Ges. Basel 46(4): 135-138; Duda, O., 1924, Ent. Medd. 14: 246-313; Grimaldi, D., 1990, Bull. Amer. Mus. Nat. Hist. 197: 1-139; Kekić, V., 1997, Dros. Inf. Serv. 80: 11-13; Laštovka, P., and J. Máca 1978, Acta Entomol. Bohemoslov. 75: 404-420; Máca, J., 1991, Acta Entomol. Bohemoslov. 88: 415-423; Papp, L., and K. Pescenye 1988, Acta Biol. Debrecina 19(1986-87): 55-90.

Sultana, F. Institute of Low Temperature Science, Hokkaido University, Sapporo, Japan. Drosophilidae from Bangladesh.

The family Drosophilidae is a large family of muscomorphan Diptera, containing very nearly 3,500 species around the world (Wheeler, 1986; Toda, pers. comm.). A considerable number of species have been recorded from the Indian subcontinent and its surrounding areas: 262 spp. from India (Gupta, 1993; Singh and Fartyal, 1997), 71 spp. from Nepal (Okada, 1966), 148 spp. from Sri Lanka (Okada, 1988; Toda, pers. comm.), 127 spp. from Myanmar (Toda, pers. comm.) and 20 spp. from Bhutan (Gupta and Abhijit De, 1996).

Our knowledge about drosophilid flies of Bangladesh is still very meagre and fragmentary, in comparison with other countries of the Indian subcontinent. It remains as a virgin field to be explored. Only eight species of Drosophilidae have been recorded from Bangladesh: *Drosophila* (*Sophophora*) *kikkawai, D.* (*So.*) *ananassae, D.* (*So.*) *bipectinata, D.* (*So.*) *melanogaster, D.* (*Dorsilopha*) *busckii, D.* (*Drosophila*) *repleta, D.* (*D.*) *latifshahi,* and *Scaptodrosophila meijerei* (Anwara Begum *et al.*, 1977).

The present report deals with the result of a preliminary survey carried out at two localities, Dhaka and Rajshahi (Table 1). Flies were collected near human habitations by traps baited with various kinds of fruits in 1997. The collected species were mostly domestic ones. In total, 750 flies were caught, belonging to seven species and three subgenera of the genus *Drosophila.* Two species, *D.* (*So.*) *takahashii* and *D.* (*D.*) *sulfurigaster albostrigata,* were recorded for the first

Table 1. Numbers of drosophilid flies collected from Bangladesh

Genus	Subgenus	Species	Dhaka Total (M / F)	Rajshahi Total (M / F)
Drosophila	*Sophophora*	*kikkawai*	172(105 / 67)	180 (113 / 67)
		ananassae	166 (67 / 99)	158 (79 / 79)
		melanogaster	17 (8 / 9)	23 (10 / 13)
		takahashii	8 (2 / 6)	13 (5 / 8)
		bipectinata	- - -	3 (1 / 2)
	Dorsilopha	*busckii*	5 (2 / 3)	4 (2 / 2)
	Drosophila	*sulfurigaster albostrigata*	- - -	1 (1 / 0)
Total			368 (184 / 184)	382 (211 / 171)

time from Bangladesh. The present data in no way provide a complete picture about the drosophilid fauna of Bangladesh since many areas of this country still remain unexplored.

Acknowledgments: My hearty thanks are due to Prof. M.J. Toda (Institute of Low Temperature Science, Hokkaido University, Japan) and Dr. V.S. Sidorenko (Institute of Biology and Pedology, Russia) for their help in identification of the species and their valuable advice. Special thanks are also due to Prof. Sohorab Ali (Zoology Department, Rajshahi University, Bangladesh) for providing necessary facilities and constant encouragement to carry out the work.

References: Ammen, M., and M.F. Huq 1973, J. Nat. Hist. 7: 633-652; Anwara Begum, N. Quraishi, G. Zannat, S.K. Shaha, and S.M.H. Kabir 1977, J. Asiatic Soc. Bangladesh (Sc.), 2: 1-6; Gupta, J.P., 1993, Dros. Inf. Serv. 72: 87; Gupta, J.P. and Abhijit De 1996, Entomon 21: 177-186; Okada, T., 1966, Bull. British Mus. (Nat. Hist.) Suppl. 6: 1-129; Okada, T., 1988, Ent. Scand. Suppl. 30: 111-151; Singh, B.K., and R.S. Fartyal 1997, Dros. Inf. Serv. 80: 43-45; Wheeler, M.R., 1986, Additions to the catalog of the world's Drosophilidae. In: *The Genetics and Biology of Drosophila 3a* (eds. Ashburner, M., H.L. Carson, and J.N. Thompson, jr.) 395-409, Academic Press, London.

Acharyya, M., and R.N. Chatterjee. Department of Zoology, University of Calcutta, 35 Ballygunge Circular Road, Calcutta, India. Differentiation of the male specific internal reproductive organs of *Drosophila melanogaster* does not require the sex determining gene *transformer* (*tra*).

In the adults of *D. melanogaster*, the last abdominal segments, the anal plates, and the genitalia show a strong sexual dimorphism. Clearly, all the structures derived from the genital disc in the female are different from those in the male. Various lines of evidence indicate that the sexual dimorphism which appears at the end of the second instar larva gives rise to different structures according to the positions of the cells within each disc (Nothiger *et al.*, 1977; Lauge, 1980, 1982). Littlefield and Bryant (1979) noted that male and female genital discs begin development with initially identical arrays of positional values *i.e.*, they represent a single field. Later, the anal plates develop in response to the same positional values in both sexes whereas the genitalia would develop in response to different subsets of positional values according to the sexes. Thus, the entire adult terminalia (*i.e.*, the analia and the whole genital apparatus without gonads) are produced by the genital disc.

Several lines of evidence indicate that the recessive mutation *tra* (*transformer*) alleles have no effects on males but transform females into sterile pseudomales that are identical to males in every respect except for their non-functional gonads and female size (Sturtevant, 1945; Baker and Ridge, 1980; Baker and Belote, 1983). In order to know how sexual morphology of XX, *tra/tra* flies is specifically sculpted the internal organization of terminal abdominal segments, we have examined the histological structures of the terminal segment of the body of XX, *tra/tra* flies of *D. melanogaster*.

For the investigations, the histological preparations of terminal abdominal segments of adult flies were made as described by Miller (1950). The tissues were stained in eosin hematoxylin as described by Chayen *et al.* (1973).

Figure 1a-d shows the histological differences including the skeletal musculature pattern of wild type male and female. As noted earlier (Miller, 1950), our data also reveal that there is strong sexual dimorphism in the internal organization and histological structures of the adult flies. Segment specific muscle patterns are also apparent in the histological sections of the adult flies of the two sexes (Figure 1a,c), although the muscles of the genital organs are comparatively few and are concerned with the morphological interpretation of some of the skeletal frame work of the body to such an extent that they should be considered. Most of the muscles appear to be special adaptations to the functions of the fly genitalia. In males, two pairs of movable structures are associated with the genitalia. These are the

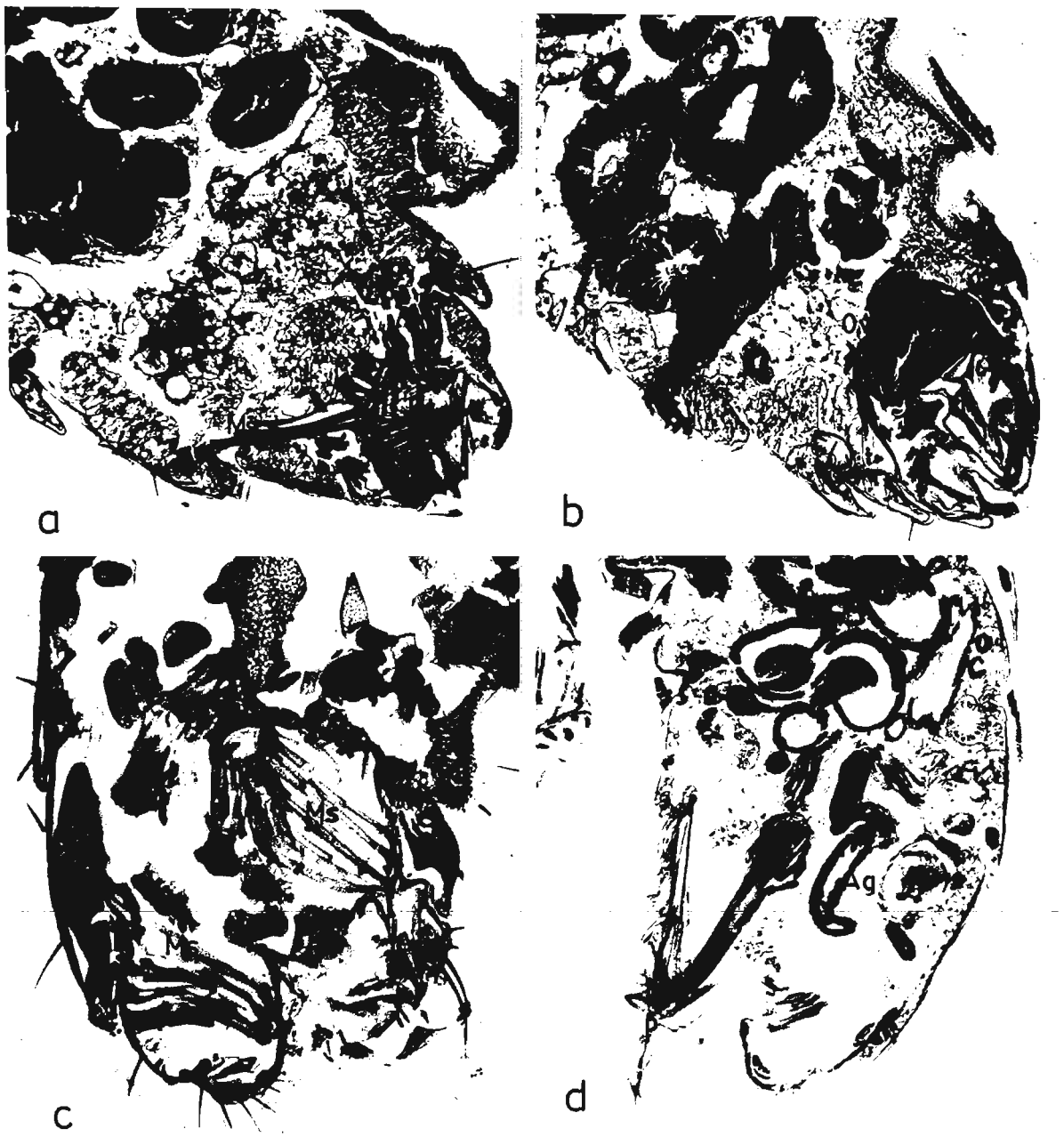

Figure 1a-d: Longitudinal sections through the terminal abdominal segments of adult male and female. (a) Longitudinal section showing some female specific musculature pattern of terminal abdominal segment of a female, (b) Longitudinal section showing female reproductive system, (c) Longitudinal section showing male specific musculature pattern of the terminal segment of a male and (d) Longitudinal section showing terminalia and genitalia of the male. Note the penis structure of the male in Figure (d). Ms-muscles; Ut-uterus; Ov-ovary; p-penis; Ag-accessory gland.

styles or claspers of the ninth segment with their coxites and the parameres. The second pair of muscles originates upon the inner face of the basal phargma of the nine sternite and insert upon the base of the penis itself (Figure 1d). These evidently function to retract that structure and they may therefore be designated as the retractor muscles of the penis. Similarly, in females, comparatively few muscles are concerned with the morphological interpretation of some of the skeletal parts of the body (Figure 1a).

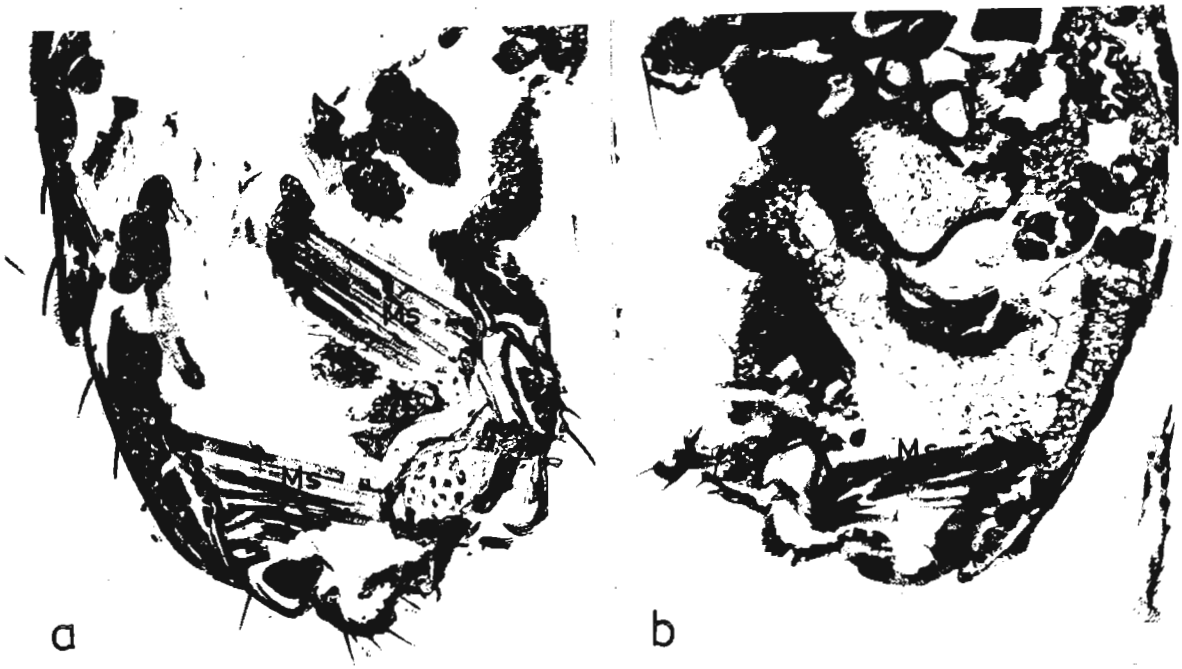

Figure 2a-b: Longitudinal sections through the terminalia of (a) an XY, *tra/tra* male and (b) an XX, *tra/tra* pseudomale. Note that the male specific abdominal muscles (a) were not affected by *tra/tra* mutation. Symbols as in Figure 1.

When the internal organization and histological structures of XY, *tra/tra* males were examined under the microscope, it has been noted that all male limited internal organization and histological structures are apparent in the tissues of the terminal segment of *tra/tra* males (Figure 2a). However, not all male specific muscles were developed in sexually transformed, XX, *tra/tra* females as they regulate the differentiation of sex specific cuticular structures. Some internal organization of the terminal segment of the body and the muscle patterns are male like (Figure 2b). These observations clearly suggest that determination of male specific internal organization of the terminal segment of the body (including muscle patterns) does not require the sex determining gene *tra*.

As the sexual dimorphism which appears at the end of the second instar larva gives rise to different structures according to the positions of the cells within each disc, it is expected that the growth dynamics of primordia of *tra/tra* females is set before second instar larval development. However, as it appears from our data presented in Figure 2a,b, the *tra* gene is not required for the development of internal organization of male flies (as there are no significant changes of internal organization of histological structures of XY, *tra/tra* male). It is, therefore, reasonable to believe that the *tra* gene is not required for the development of the internal organization of the terminal segment of the males including muscle patterns as they regulate the differentiation of sex specific cuticular structures in null mutation of *tra*.

Acknowledgment: This work has been supported by UGC research grant [Grant no. F.3-10/95 (SR-II) dated 23-12-95] to RNC.

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Band, H.T. Dept. of Zoology, Michigan State University, E. Lansing, MI 48824. Changes in mating duration in *Chymomyza amoena* stocks over time.

Band (1995) reported that duration of copulation (DC) in *C. amoena* populations was not species specific. Laboratory populations established from flies bred from substrates collected in Virginia (acorns, apples) and from apples collected in E. Lansing, Michigan, had an average DC of 17 to 20.5 minutes. A laboratory population established from flies bred from apples collected at Iron Mountain, MI, had a significantly shorter DC, 14.7 ± 3.4 minutes. Laboratory populations established from *C. amoena* from the Maggia Valley, Canton Ticino, Switzerland, showed similar heterogeneity in DC. The stock established from flies bred from nuts had a DC of 22.0 ± 7.1 minutes, but the stock established from flies coming to bait at the same site had a significantly shorter DC, 16.0 ± 2.8 minutes. Results, however, paralleled the early reports of Wheeler (1947) and Spieth (1952) on mating duration in *C. amoena* of 14 minutes and 21 minutes, respectively.

The fact that DC was significantly shorter in one population from each of two different countries also suggested that a genetic basis for the polymorphism might exist. Data included in Band (1995) had been completed by May, 1994. It was necessary to determine that DC remained significantly more rapid in one or both stocks. Work was undertaken in October 1995 on the Swiss stock and in November and December 1995 on the Iron Mountain, MI, stock. Single pair matings were used.

Table 1. Duration of copulation (DC) observed in laboratory stocks of *Chymomyza amoena* from Iron Mountain, MI, USA and the Maggia Valley, Switzerland in Oct.-Dec. 1995. Time in minutes. Minimax values also given.

Population	N	Duration of copulation Mean ± SE	Min.	Max.	Pairs not mating
Iron Mountain	8	20.2 ± 1.9	15	32	16
Maggia Valley-B	13	18.1 ± 1.4	11	28	4

As shown in Table 1, the average DC increased in both stocks. Minimum and maximum duration has also increased from previously reported values (Band, 1995; Table 2), although only half as many matings have been scored per stock in the current trials.

There were more nonmating than mating pairs among the Iron Mountain, MI, flies. Individually, five females given new males mated; five females given new males still did not mate. Also, whereas termination of copulation and separation had been abrupt in the early work, in the later experiments individual females showed more evidence of restlessness and attempts to dislodge the male before pairs finally separated.

DC has been argued to be controlled by the male. Difficulty in separation would certainly add to the increased length in observed mating duration. Neither laboratory stock now approaches the lower DC found by Wheeler (1947) for this species, but are at or below the mean DC observed by Spieth (1952) and in other *C. amoena* populations (Band, 1995).

References: Band, H.T., 1995, Mitt. Schweiz ent. Ges. 68: 23-33; Spieth, H.T., 1952, Bull. Am. Mus. Nat. Hist. 99: 395-474; Wheeler, M.R., 1947, Univ. Texas Publ. 4720: 78-115.

Amador, A., and E. Juan. Department de Genètica, Universitat de Barcelona, Diagonal 645, 08071 Barcelona, Spain. Morphology of mouth hooks and anterior spiracles during larval development of *D. funebris*.

The characteristics used to stage larvae in *Drosophila* are the morphology of mouth parts and the presence and appearance of anterior spiracles (Bodenstein, 1950). The interspecific variability in these characteristics makes it necessary to describe them for each single species. Studies of temporal gene regulation in *D. funebris* require the exact staging of larvae along development.

Flies were allowed to lay eggs on ethanol-acetic acid agar-medium (1.4%) seeded with live yeast for 6 hours, and 10 drops of a 10% glucose solution were added daily until first instar larvae appeared. Further larval development on this medium is delayed so larvae older than two days were collected from bottles with cornmeal-sugar agar-medium previously seeded with 100 eggs. The development took place at 23°C.

Larvae hatched at about 18 hours after the eggs were laid. The mandibular hooks of first instar larvae usually had 3 teeth of uniform size (Figure 1A), although approximately 20% of individuals presented 4 teeth. The first moult occurred three days later. At this time anterior spiracles were apparent but had no papillae; hooks had doubled in size and showed 3 teeth. The second tooth was longer and sharper than the other two (Figure 1C). Two days later, the second moult took place, the size of hooks had doubled again and two big sharp teeth were observed (Figure 1D). At this time

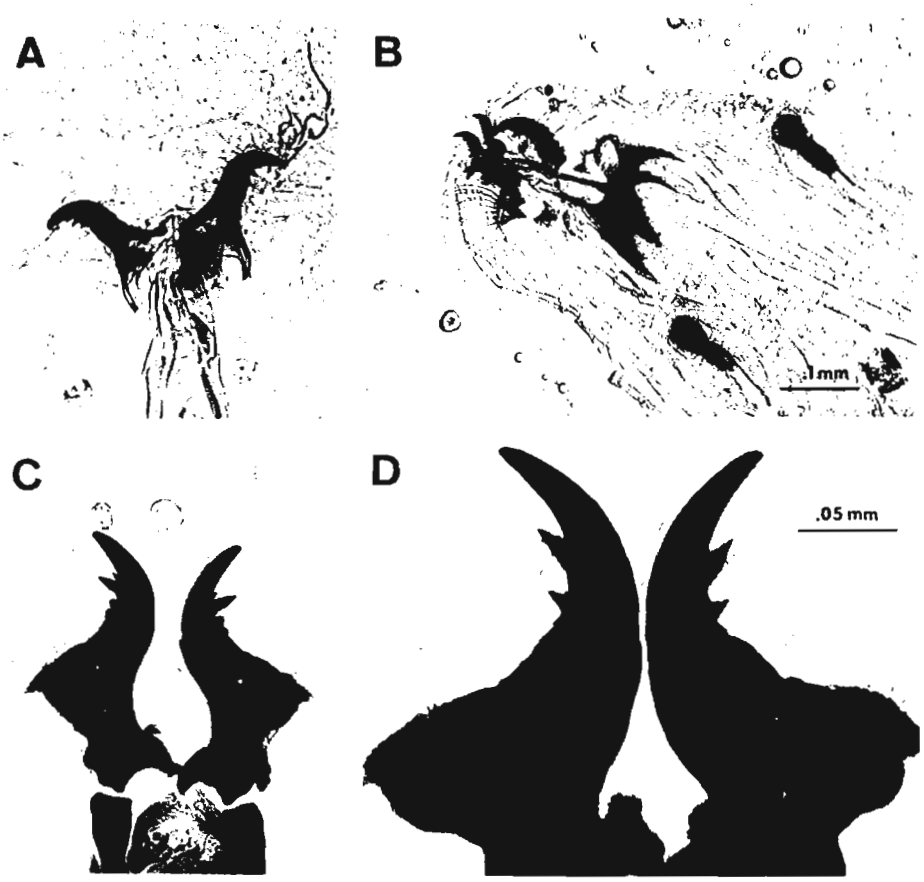


Figure 1. Larvae were squashed between a slide and a coveslip in a drop of water and viewed under a Zeiss microscope. **A,**. First instar larvae. **B,**. Transition from first instar to second instar. **C,**. Second instar larvae. **D**, Third instar larvae. A, C and D at a magnification of 400×, B at 160×.

spiracles showed finger-like papillae. Eight days after hatching, pupariation began, and five days later began eclosion. The shortest life cycle from egg to adult was 14 days at 23°C.

References: Bodenstein, D., 1950, In: *Biology of Drosophila.* Demerec M. (ed.), JohnWiley and Sons, p. 275.

Kosuda, Kazuhiko, and Akira Sekine. Biological Laboratory, Faculty of Science, Josai University, Sakado, Saitama, Japan 350-02. The viability reduction as a correlated response to selection for body weight in *Drosophila melanogaster*.

Artificial selection experiments for light and heavy adult body weight in *Drosophila melanogaster* were carried out for eight generations. The egg to adult viability was also examined as a correlated response to selection for body weight. It was shown that the genetic variations which decrease and increase body weight have deleterious effects on viability.

Flies from a natural population in Katsunuma, Yamanashi, Japan, were used for the present selection experiment. Two replicate selection lines were made in both directions (HA and HB for high lines and LA and LB for low lines). Random samples of 50 virgin female and male flies were taken and maintained in yeast-sugar-molasses medium separately for two days. Then they were weighed at the age of two days old every generation. Five pairs of females and males with the extreme body weight were selected for parents of the next generation in each selection line. These selected flies were transferred to fresh vials with the medium every one or two days in order to avoid a high larval density. These selection procedures were repeated for eight successive generations. The control line was also maintained from five pairs of flies which were randomly taken each generation.

For measuring the egg to adult viability, the following procedure was employed. A glass slide with culture medium on its surface was inserted into a large plastic vial. Female and male flies from each line were put together into the vials and were allowed to lay eggs. After several hours, portions of the medium with 50 eggs were transferred to

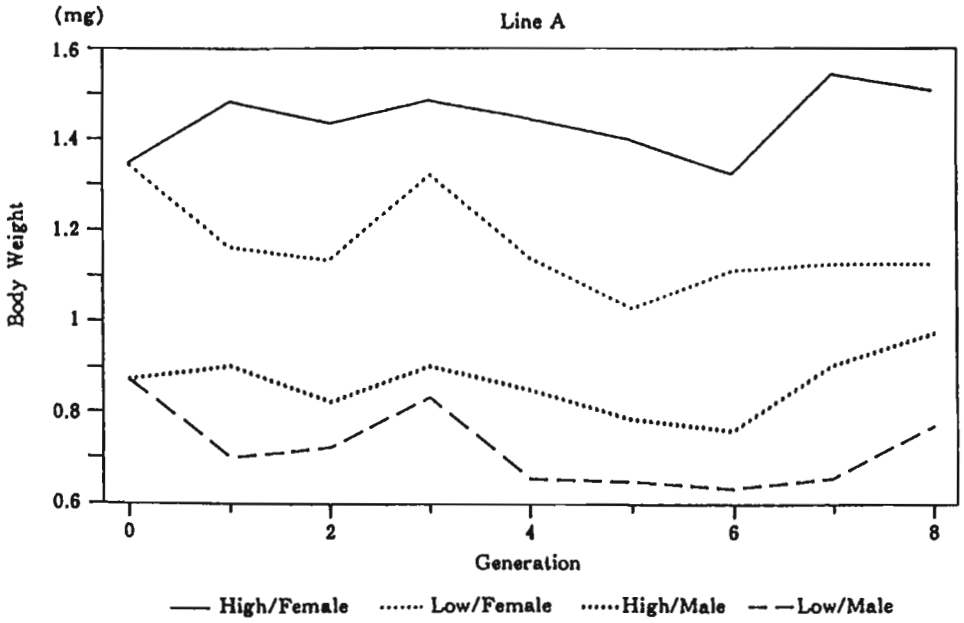


Figure 1. Selection for body weight in the line A.

culture vials. Eclosing flies from these vials were completely counted after 10 to 14 days and the proportion of emerged flies was used as a measure of egg to adult viability. About 20 replicates were made for each measurement.

Experimental results are graphically given in Figures 1 and 2. It should be noted that responses to selection in the line B in both directions was much greater than those in the line A both for female and male, although they are replicate selection lines. The reason for this difference between two replicates is not clear. These figures also show that the selection response in females was remarkably larger that males. The realized heritability was estimated to be 0.17-0.22.

Egg to adult viability was examined as a correlated response to selection for body weight. The viability in the original line was high at 0.899. The linear regression of egg to adult viability on generation did not significantly differ from 0 in the control line ($Y = -0.0063X + 0.914$, $t_3 = 8.63$, $P > 0.05$). On the other hand, it was smaller than 0 in four selection lines without exception. The highly significant regression line of $Y = -0.0346X + 0.920$ was obtained for all selection lines ($t_{26} = 5.37$, $P < 0.001$). Egg to adult viability in the line HB at generation eight was only 0.369. These

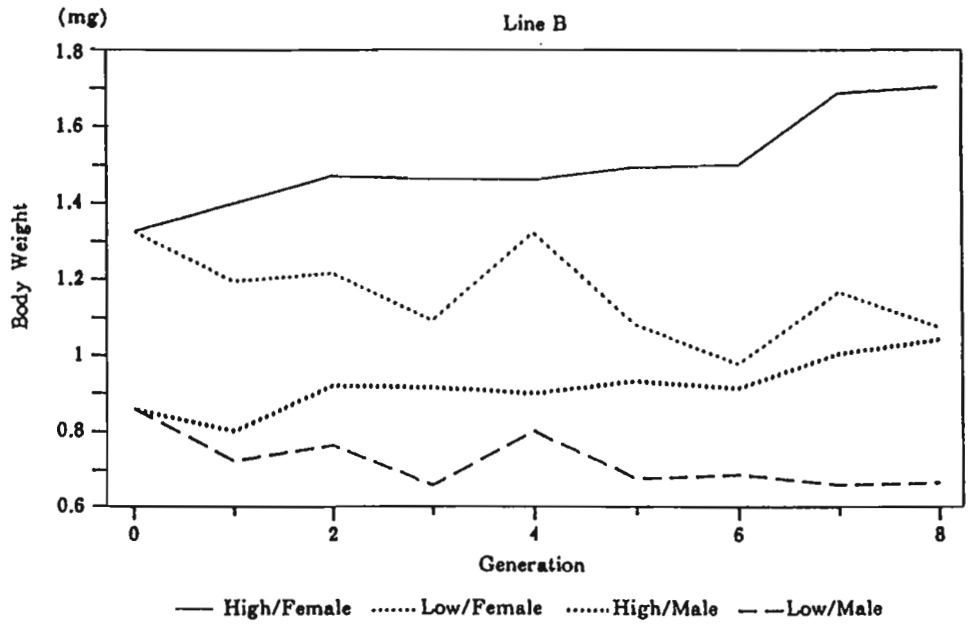


Figure 2. Selection for body weight in the line B.

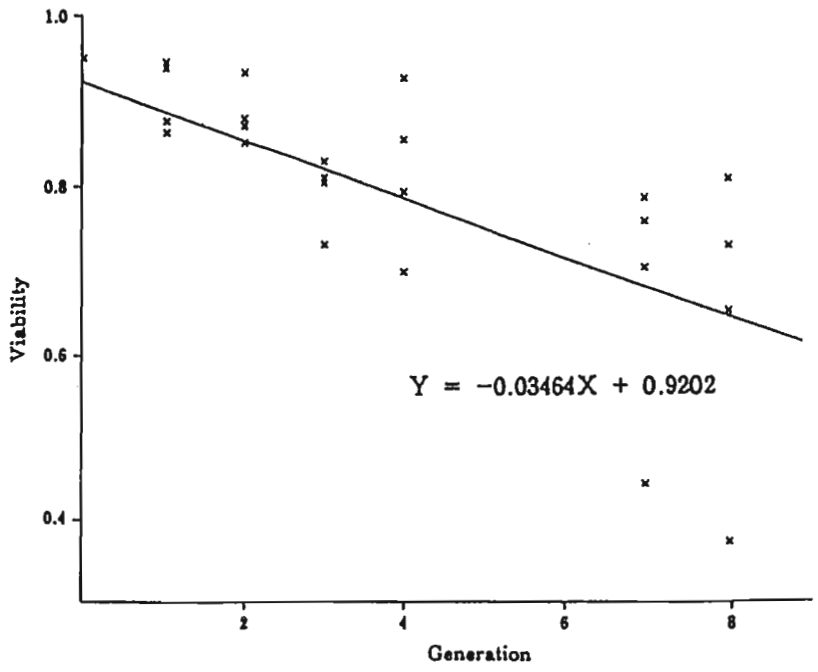


Figure 3. Regression line of viability on generations in selection lines.

facts imply that the artificial selection for light and heavy body weight has deleterious effects on egg to adult viability and suggest the existence of the stabilizing selection for body weight in *D. melanogaster*.

Regner, L.P.,[1] A. Zaha,[2] E. Abdelhay,[3] and V.L.S. Valente[1]. [1]Departamento de Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul (UFRGS). Caixa Postal 15053, CEP 91501 - 970. Porto Alegre, RS, Brazil; [2]Departamento de Biotecnologia, 1. Biociências, UFRGS, CEP 91501 - 900 Porto Alegre, RS, Brazil; [3]Laboratório de Biologia Molecular, Instituto de Biofísica, Universidade Federal do Rio de Janeiro (UFRJ). CEP 21949 - 900. Rio de Janeiro, RJ, Brazil. *P* elements in natural populations of *Drosophila willistoni* from different geographical origins.

The present study involved a screening of several strains of *Drosophila willistoni* from different places of its geographical distribution, analyzed by Southern blot for the presence of *P* elements.

Drosophila willistoni is among the most abundant drosophilid species inhabiting the hot, humid South American forests, with a wide Neotropical distribution extending from Mexico and Florida to North Argentina and from the Atlantic to the Pacific Oceans (Ehrman and Powell, 1982).

P elements have been shown to be widely distributed in this species, as well as in several others of the subgenus *Sophophora* (Lansman *et al.*, 1985; Daniels and Strausbaugh, 1986; Daniels *et al.*, 1990; Kidwell, 1994). It has been noticed that virtually all strains of *D. willistoni* studied show the presence of *P* elements, in contrast to *D. melanogaster*, where strains may (P strains) or may not (M strains) have the complete *P* sequences.

Molecular analysis of *D. melanogaster P* elements has permitted the identification of two structurally distinct types: complete elements and defective ones (O'Hare and Rubin, 1983). The complete *P* elements are 2.9 kb in length and encode two known polypeptides. Depending on the pattern of pre-mRNA splicing, a complete element may produce a transposase or a transposition-repressor protein (for a review, see Rio, 1990). Defective elements are deletion-derivatives of complete ones, and have lost their capability to encode transposase themselves but can be mobilized if a source of transposase is provided to them. It has been suggested that some truncated forms of transposase produced by internally deleted elements can act as negative regulators of transposition in *D. melanogaster* (Black *et al.*, 1987; Robertson and Engels, 1989).

The behavior of *P* elements and their effects on the host provide means by which evolutionary changes may come about, but it is not yet completely known how *P* elements behave in *D. willistoni*. Pursuing this issue we asked how widespread *P* elements are in *D. willistoni* populations and if it is possible to found any *P* free population among samples from different origins than those screened by the former authors.

In an attempt to contribute to the knowledge of the evolutionary history of this mobile element system, we performed a survey of geographically distinct strains available in our laboratory, searching for the presence or the absence of *P* elements in *D. willistoni*.

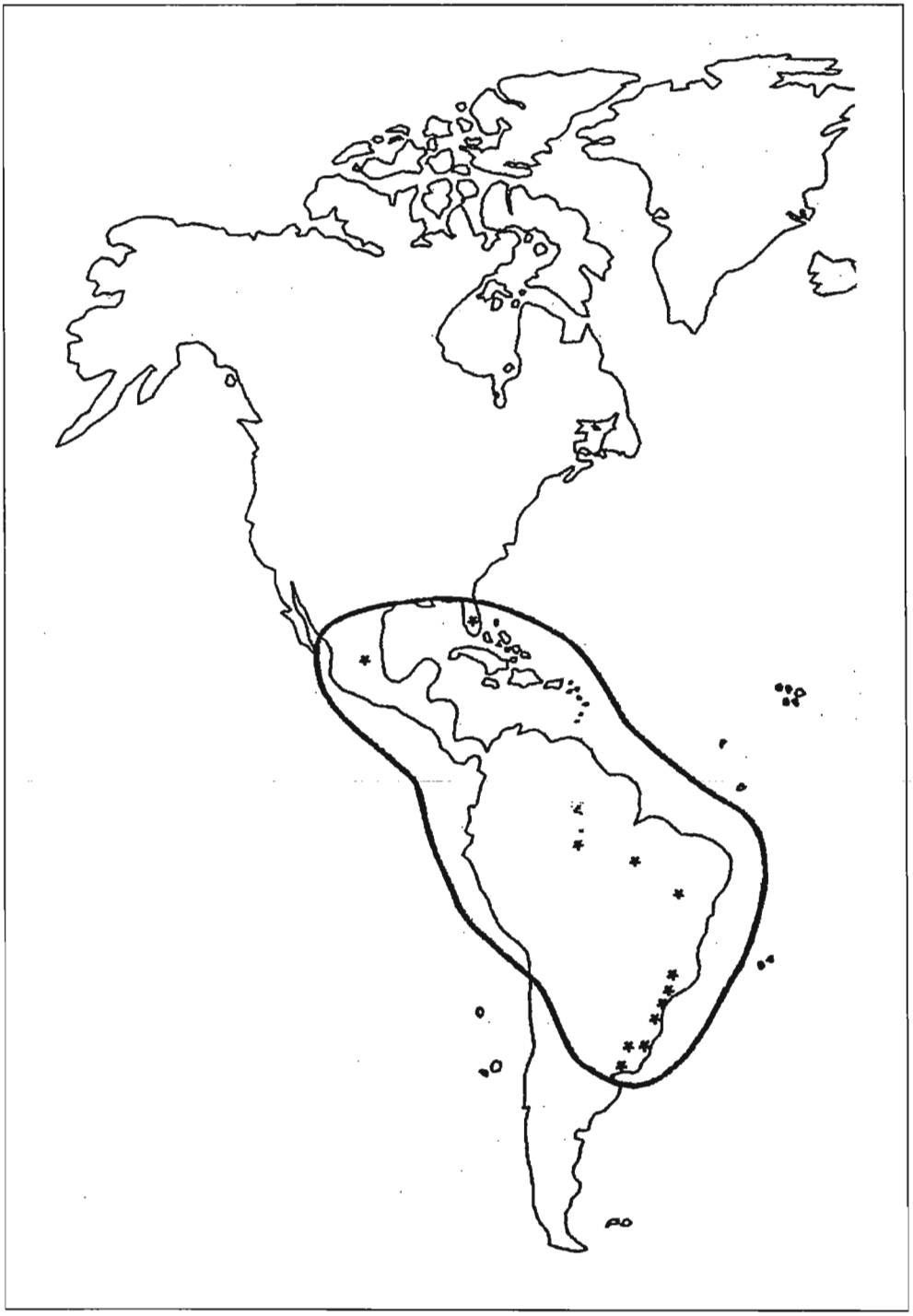


Figure 1. Geographical distribution of the *Drosophila willistoni* populations (Table 1), screened for the presence of *P* sequences. The species distribution range (according to Spassky *et al.*, 1971) is delimited by the line.

The *D. willistoni* stocks employed in this study are listed in Table 1. All of them were screened for the presence of *P* homologous sequences by Southern blotting. The fly stock cultures were maintained by mass matings on standard *Drosophila* culture medium (Marques *et al.*, 1966) at 17 ± 1°C. The Southern blot analysis was performed as follows: to assay for *P*-homologous sequence, *Pst*I, *Pvu*II, *Eco*RI/*Sal*I, or *Ava*II digests of genomic DNA were probed with the 2.4 kb *Acc*I internal fragment derived from the *P* element contained in the plasmid pπ25.1 kindly provided by Dr. Alfred M. Handler (USDA-ARS, Gainesville, USA). Genomic DNA was prepared from approximately 200 adult flies (Jowett, 1986) and digested with appropriate enzymes. The fragments were separated on 0.8% or 0.9% agarose gels, transferred to nylon membranes, and hybridized to the DNA probe labelled with ^{32}P-α-dATP and ^{32}P-α-dCTP by random priming. The specific activity of the probes was about 10^8 cpm/μg DNA. Hybridizations were carried out at 42°C for 24 h in the presence of 50% formamide. Filters were washed with 0.5×SSC and 0.1%

SDS, at 50°C and exposed to X-ray film for 48 h.

Figure 1 shows a map with the geographical distribution of *D. willistoni* according to Spassky *et al.* (1971). The strains analyzed for the occurrence of *P* elements in this study came from representative places of the distribution range of the species and their origins are plotted on the map.

Sequences homologous to *P* elements were found in all stocks examined. Southern blot analysis (Figure 2) showed DNA segments with the expected sizes for the complete canonical *P* element. Also noticeable is the systematic occurrence in almost all *D. willistoni* stocks of smaller DNA fragments besides the one expected for a complete *P* element when DNAs were digested with *Pst*I and *Ava*II. These DNA fragments may reflect the presence of internally deleted elements that might play a role in the genomic positions of *P* elements in *D. willistoni*. There is a great deal of intraspecific variation in the genomic positions of *P* elements in *D. willistoni*. In all cases, the hybridization patterns indicate a reduced number of elements relative to the number in a *D. melanogaster* P strain (compare with the first lane in each autoradiogram).

Table 1. Fly stocks of *Drosophila* employed in the present study.

Stock	Location	Collection Date
(1) *D. melanogaster* Harwich Positive Control		
(2) *D. willistoni* Manaus	Amazonas State, Brazil	1986
(3) *D. willistoni* Marabá	Pará State, Brazil	1984
(4) *D. willistoni* WIP-4	Bahia State, Brazil	1961
(5) *D. willistoni* WITA	Rio de Janeiro State, Brazil	1971
(6) *D. willistoni* WE27	São Paulo State, Brazil	1987
(7) *D. willistoni* WIC	Paraná State, Brazil	1983
(8) *D. willistoni* WUBA	Santa Catarina State, Brazil	1972
(9) *D. willistoni* WSPe	Rio Grande do Sul State, Brazil	1960
(10) *D. willistoni* Florida	Florida, USA, Bowling Center	?
(11) *D. willistoni* Mexico	Mexico, Bowling Center	?
(12) *D. willistoni* Montevideo	Montevideo, Uruguay	1991
D. melanogaster Canton S Negative Control	Bowling Center	

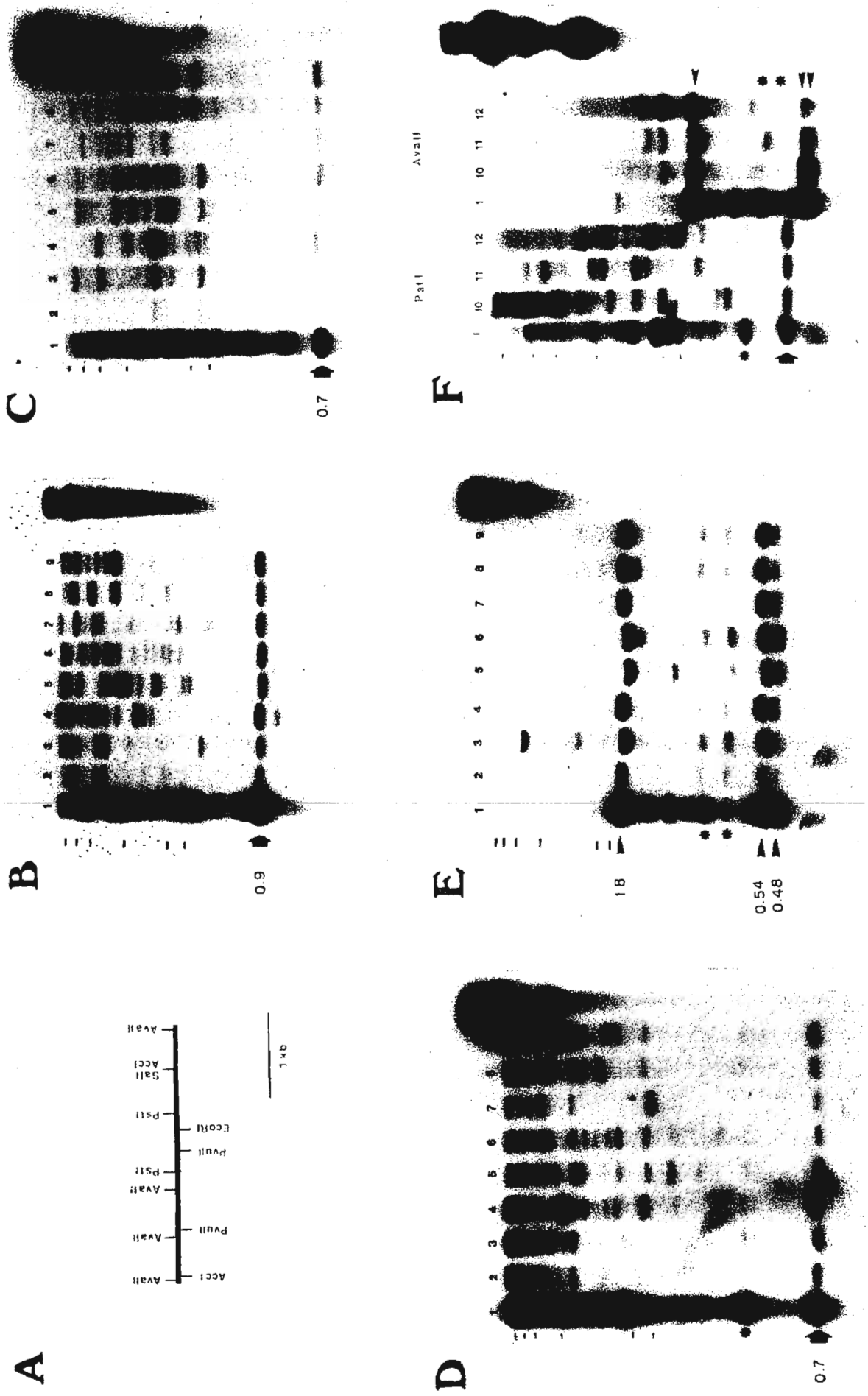
An especially careful analysis of *Ava*II restriction fragments hybridized to the *P* element probe shows that all strains analyzed presented at least one complete element in addition to a probably deleted *P* element (asterisk marked on the figure) and some elements polymorphic for *Ava*II. Particularly interesting is the presence of two fragments (about 6 kb and about 3 kb) in the Marabá strain (lane 3, Figure 2E) that are not present in the other strains. The same can be said for fragments of about 1 kb and 5.9 kb in lane 5 (WIT A); fragment of about 1.7 kb in lanes 6 and 8 (WE27, WV BA); fragment of about 2.3 kb in lanes 10, 11, 12, and corresponding to samples from Florida, Mexico and Uruguay, respectively (Figure 2F); and a fragment of approximately 3 kb in lanes 11 and 12 (samples from Mexico and Uruguay, in opposite borders of the geographical distribution of the species).

By roughly analysing these findings with the geographical origin of the strains, it appears that strains more distant from the putative center of *D. willistoni* distribution (Central Brazil) show a higher degree of polymorphism while strains closer to the origin have large numbers of deleted elements. It is also interesting to note that strain WIC (lane 7) contains only the canonical *P* element. Looking at the results of other restriction hybridizations it is possible to observe that in this strain the element has at least 3 insertion sites in the *D. willistoni* genome (lane 7, Figure 2B, C, D).

Certain other similarities were also observed among strains from geographically closer locations. The samples in lanes 2, 3 and 4 of Figure 2 correspond to strains from the States of Amazonas, Pará and Bahia (North and North East regions of Brazil); the samples in lanes 5 and 6 correspond to strains from the States of Rio de Janeiro and São Paulo (South East region), and the samples of lanes 8 and 9 are of strains from the States of Santa Catarina and Rio Grande do Sul (South region). Strain WIC (lane 7) seems to be more distantly related to the other strains, a fact that may be partially explained by its place of origin (Ilha das Cobras), an Atlantic island.

From all studies performed until now (Daniels and Strausbaugh, 1986; Daniels *et al.*, 1990; Lansman *et al.*, 1985), including our own, it is clear that the number of copies of *P* elements in *D. willistoni* is much lower than in *D. melanogaster*. The presence of complete *P* elements in all studied populations adds support to the idea that in the absence of a homologous M strain, those elements cannot undergo transposition, a fact that ould explain the poor

Figure 2 (see facing page). Southern blots of *Drosophila willistoni* populations. (A) Map of pertinent restriction enzyme sites of 2.9 kb intact *P* element contained in the pπ25.1 plasmid. Genomic DNA samples were digested with *Pvu*II (B), *Eco*RI and *Sal*I (C), *Pst*I (D and F), and *Ava*II (E and F), and probed with the 2.4 kb *Acc*I fragment of the *P* element. Fly stocks are designated by the numbers listed in Table 1. The approximate positions of the 23.1, 9.4, 6.6, 4.4, 2.3, 2.0 kb fragments generated from a *Hind*III digest of lambda DNA are indicated on the left. The last lane in each panel correspond to the pπ25.1 plasmid. The arrows indicate the internally derived fragments that are produced whenever a complete *P* element is digested. The asterisks point to other low molecular weight bands peculiar to most populations.


A
AvaII
AccI
SalI
PstI
EcoRI
PvuII
PstI
AvaII
AvaII
PvuII
AccI
AvaII
1 kb
B
0.9
C
0.7
D
0.7
E
18
0.54
0.48
F
AvaII
PstI

number of insertions in the genome. However, previous results from *in situ* hybridization analysis have shown some differences in the position of *P* elements in several *D. willistoni* subgroup species (Daniels and Strausbaugh, 1986; Lansman *et al.,* 1985). In some cases *P* mapped at the chromocenter while in others at few euchromatic sites.

Recently we have analyzed two other *D. willistoni* strains (17A2 and WIP 11A) by Southern blot and by *in situ* hybridization with *P* elements and we found strong differences between a freshly collected strain (17A2) and an old laboratory stock (WIP 11A) concerning *P* element genomic position (Regner *et al.,* 1996). These differences are reflected by the finding of 24 euchromatic insertion sites in the 17A2 strain and the unique chromocenter mapping of *P* in the old stock WIP 11A. These studies suggested that wild strains are still capable of transposition, while old stocks are not, probably because of the insertion of *P* elements in heterochromatin, as proposed by Stofford (1976), Spradling and Rubin (1983), and Devlin *et al.* (1990).

If transposition is still able to occur in *D. willistoni* under certain special conditions, is it possible that invasion and re-invasion of the populations by *P* elements would result in periods of genomic disturbances proportional to the amount of complete elements, followed by their accumulation in heterochromatic "hot spots"? Responses to such questions probably should be done by studies including a wide spectrum of *D. willistoni* natural populations, and the present is a preliminary attempt to do that. By the other hand, strains coming from places closer to the limits of the *D. willistoni* geographical distribution show higher polymorphism in *P* sequences probably because they are subject to lower selective pressures when in heterochromatin. In contrast, those strains coming from Central and South Brazil show lower polymorphism, but a large quantity of deleted elements. Those deleted *P* elements may be a potential source for transposition induction under appropriate environmental conditions.

Recently, however, Clark *et al.* (1995) found the presence of four major *P* element families in the genome of *D. willistoni*, being possible the coexistence of more than one member of these subfamilies in the same genome. Such findings need to be considered in order to establish evolutionary relationships among species, groups of species, and other upper taxa, as done by Clark and Kidwell (1997). So, a finer characterization of the *P*-homologous sequences present in the natural populations of this species needs to be done before raising hypotheses to explain variability such as that here described.

Acknowledgments: This work was supported by grants and fellowships from the Brazilian agencies FAPERGS, CNPq, FINEP, PROPESQ-UFRGS, and FAPERJ.

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Singh, B.K., and R.S. Fartyal. Cytogenetic Laboratory, Department of Zoology, Kumaun University, Naini Tal, India. Drosophilidae collected from Chaubatiya Garden, Ranikhet, Kumaun, India.

The Drosophilidae is a large family of world wide distribution. In recent years, our studies particularly in Kumaun region, which is located in the north-eastern periphery of the state Uttar Pradesh of the Indian subcontinent, have yielded considerable data on the Indian species (Singh and Bhatt, 1988; Singh and Negi, 1989, 1992; Singh and Dash, 1993). However, the authors believe that these data in no way furnish a complete picture of the Drosophilid species inhabiting this region since a vast area still awaits exploration. This note deals with the Drosophilid survey of Chaubatiya garden from May 1996 to April 1997.

Chaubatiya garden is located in Ranikhet, Almora district of the Kumaun region at an elevation of about 7025 feet from the sea level. It has an area of about 30 acres and is mainly characterized by the presence of *Quercus* sp.,

Table 1. Drosophilidae collected from Chaubatia Garden from May 1996 to April 1997

Drosophilid species	Number of flies collected												Total number of flies		Sex ratio
	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	Male	Female	(approx.)
Genus—*Drosophila*															
Sub-genus—*Drosophila*															
D. immigrans	72	87	118	195	109	95	60	35	15	11	36	64	408	489	4:5
D. lacertosa	26	35	61	79	31	08	—	—	—	—	—	—	99	141	3:4
Sub-genus—*Sophophora*															
D. nepalensis	68	85	101	176	181	105	65	18	—	—	—	28	487	340	5:4
D. bifasciata	21	28	62	74	30	09	—	—	—	—	—	—	114	110	3:3
D. jambulina	28	41	86	41	18	22	—	—	—	—	02	15	136	117	3:3
D. suzukii indicus	15	29	65	48	31	15	10	—	—	—	—	02	129	86	3:2
Sub-genus— *Dorsilopha*															
D. busckii	39	55	61	28	08	—	—	—	—	03	48	85	211	116	5:3
Genus— *Zaprionus*															
Z. indianus	45	53	78	69	25	19	01	—	—	—	—	—	111	179	3:5
Genus — *Leucophenga*															
Leucophenga sp.*	—	—	—	03	02	—	—	—	—	—	—	—	03	02	3:2
												Total	1698	1580 = 3278	

Note: The species marked with (*) is not identified and expected to be a new species.

Rhododendron arboreum, *Pinus roxburghii*, *Cedrus deodara*, *Rubus laciocarpus*, *Myrica esculenta*, *Pyrus communis*, *Pyrus malus*, *Prunus* sp., *Prunus domestica*.

Several traps, usually small tin containers containing fermenting banana, pineapple, or some other local fruits, were placed at different places under cool and shady areas. Flies were obtained by net sweeping over these traps as well as by sweeping over natural habitat. In order to procure the maximum number of flies, collections were made several times during the day. The collected flies were then sorted out, some were transferred to culture vials containing *Drosophila* food medium for raising their progeny, and the rest were preserved in 70% alcohol for further study.

A total of 3278 flies belonging to different genera of the family Drosophilidae were collected since May 1996 to April 1997 (Table 1). Our observation shows that during summer and rainy seasons all the species shown in the table were present but during winter only a few species were present in the collection, *e.g., Drosophila immigrans* and *Drosophila nepalensis*, which shows that these species have marked preference for the colder climate. Besides the known species, one species belonging to the genus *Leucophenga* was not identified and it is expected that it is a new species.

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Additional Information:

Research Staff: 1. Rajendra Singh Fartyal - Senior Research Scholar (U.P., C.S.T.) 2. Ritu Pandey - Junior Research Scholar.

Stock List:

Wild Stocks

1. *Drosophila immigrans*
2. *Drosophila nepalensis*
3. *Drosophila jambulina*
4. *Drosophila melanogaster*
5. *Zaprionus indianus*

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The *mei* mutations intensify rDNA mobility in polytene nuclei of *Drosophila melanogaster*.

In a *Drosophila melanogaster* polytene nucleus, all chromosomes are gathered in a structure called a chromocenter. Two nucleolus organizers (NOs) of the nucleus, located either on the X or on the Y chromosome, are united together and form a single nucleolus. Nucleoli non-associated with the NOs, but connected to various chromosomal sites, have also been observed to occur with definite frequency in the wild type polytene nuclei (Semionov *et al.*, 1978). The ribosomal DNA (rDNA) of such mobile nucleoli contains all types of the ribosomal gene repeats (Semionov and Kirov, 1986) and displays active replication and transcription (Ananiev *et al.*, 1981).

Our previous data (Semionov and Kirov, 1986; Toshev and Semionov, 1987) show that the frequency of formation of mobile nucleoli is substantially increased under conditions of rDNA dose compensation, which provoke intensive recombination in rDNA (Tartof, 1971). The results presented here (Table 1) reveal that conditions of repair deficiency lead to the same effect. The formation of mobile nucleoli is enhanced by genotypes deficient either in the excision repair (alleles *mei-9* and *mei-9^a*) or in the post-replicative repair (alleles *mei-41* and *mei-41^{195}*), as compared to the wild-type (Canton-S males and females). The combinations of the repair deficiency and the rDNA dose compensation condition (genotypes *Xmei-41^{195}/O* and *Xmei-41/Y^{bb-}*) do not show cumulative effect on the feature analysed (Table 1).

Table 1. Frequency of the salivary gland cells with mobile nucleoli in wild-type and in mutant genotypes.

Genetic constitution	Number of nuclei analysed	Frequency of nuclei with mobile nucleoli (% ± 2 SEM)
X/Y	931	40.3 ± 3.2
X/X	608	39.3 ± 4.0
Xmei-9/Y	829	70.8 ± 3.2
Xmei-9^a/Y	768	68.9 ± 3.3
Xmei-41/Y	771	70.7 ± 3.3
Xmei-41/Xmei-41	500	67.8 ± 4.2
Xmei-41^{195}/Y	638	65.8 ± 3.8
Xmei-41^{195}/O	419	67.5 ± 4.6
Xmei-41/Y^{bb}	574	53.8 ± 4.2

The distribution of the rDNA-specific insertion sequence type 1 (see Glover, 1981), revealed by the *in situ* hybridization within polytene nuclei of the mutant genotypes, was very similar to that found earlier in the compensating *Drosophila* (Semionov and Kirov, 1986). In particular, numerous labeled inter- and intrachromosomal ectopic fibers, asterisk-like shaped nucleoli scattered throughout the genome sited, where only a part of the chromosome diameter is labelled, were observed.

Acknowledgments: I thank Dr. K. Tartof for kindly providing me with the *Drosophila* stocks and Dr. D. Glover and Dr. A. Kolchinsky who kindly provided me with the rDNA clones used as hybridization probes.

References: Ananiev, E.V., V.E. Barsky, Y.V. Ilyin, and N.A. Churikov 1981, Chromosoma 81: 619-628; Glover, D.M., 1981, Cell 26: 297-298; Semionov, E.P., and N.K. Kirov 1986, Chromosoma 93: 477-482; Semionov, E.P., A.F. Smirnov, and A.V. Rodionov 1978, Cytologia (Russ.) 20: 411-414; Tartof, K.D., 1971, Science 171: 294-297; Toshev, L.B., and E.P. Semionov 1987, Chromosoma 95: 258-262.

Hartley, Stephen, Roger Butlin, and Bryan Shorrocks. School of Biology, University of Leeds, Leeds, LS2 9JT, UK. E-mail: bgysh@leeds.ac.uk.
Preliminary results from an allozyme survey of *Drosophila phalerata* using cellulose acetate electrophoresis.

This paper reports early results obtained from screening three distinct laboratory stocks of *Drosophila phalerata* for allozymic variation across nineteen enzyme systems. Some preliminary data from F1 rearings of individuals from natural populations are also presented. This is the first allozymic study of this species, and the first reported use of cellulose acetate (CA) electrophoresis with a *quinaria*-group species

Since their initial use in the 1960s (*e.g.*, Lewontin and Hubby, 1966) electrophoretic variation in allozymes has proved to be a valuable tool for studying genetic population structure. Starch gels and polyacrylamide gels have been the most widely used media, although more recently cellulose acetate membranes have been gaining in popularity, due to their easier preparation and reduced run-times (Easteal and Boussy, 1987).

Stocks of *D. phalerata*, of three separate origins, were maintained in the laboratory for at least nine months by continuous culture on cereal based media (Shorrocks, 1972). The first strain originated from wild flies collected in

Table 1. Mobility and staining characteristics of allozymes of *D. phalerata*.

enzyme	buffer[a]	run-time[b] (mins)	stain intensity [c]	resolution of bands	No. of alleles detected
ACP	C	120	3-5	high	2+
AD	TG	25	5	med-low	1
ADH	TG	50	1	med	1
AO	TG	25	5	med-low	1
EST	TG	65	0-5	med	3+
FUM	TG	60	3-4	med-high	2
GLC	TG	?	0	—	0
α-GPDH	TG/C	60	5	high	2
GPI	C/TG	100	5	low	1
HEX	TG/C	?	0	—	0
LAP	C	100	0-3	low	1
LDH	C/TG	30	2-3	med	1
MDH1	C	110	4	high	2
MDH2	C/TG	110	4	high	2
ME	C/TG	110	4	high	2
ODH	TG	40	0-1	med	1
PEP1	TG	50	3-4	med-low	2
PEP2	TG	25	3-4	med-low	2+
PGM	TG	70	3-5	high	5
SOD	TG	?	0-3	low	1+
XDH	TG/C	40	3-5	med-high	1

[a]Buffer systems: TG = Tris glycine, pH 8.5; C = CAAPM, pH 7.0. (see Hebert & Beaton, 1993) Where two buffers give good results, the preferred buffer is given first.
[b]Run times required with the preferred buffer to achieve a migration of 2-4 cm. TG buffer run at 200v, CAAPM at 130v. (All allozymes migrate anodally with the exception of MDH1 with CAAPM buffer.)
[c]Stain intensity: subjective assessment from 5 (high intensity) to 0 (no staining).

suburban gardens near the centre of Leeds, England, National Grid reference SE 27-35-, (=Leeds strain). The second strain originated from larvae reared from mushroom baits placed in Arthington wood, ten kilometres north of Leeds, SE 27-45-, (= Arthington strain); and the final strain was a laboratory stock supplied from Leiden University, Netherlands, originating from locally caught, wild flies (Sevenster, pers. comm.) (= Leiden strain). One would expect each strain to be highly inbred, since laboratory populations are relatively small, but due to their separate origins they would not necessarily be fixed for the same electromorphs (alleles). To investigate levels of polymorphism in natural populations, first generation wild-type flies were obtained by rearing larvae from baits of domestic mushroom, *Agaricus bisporus*. The baits had been exposed for fourteen days in various woodlands within 30km of Leeds, using a method similar to Bingley and Shorrocks (1995).

As a prelude to future genetic population studies, the three laboratory strains were screened for allozymic variation across nineteen enzyme systems (21 putative loci) using cellulose acetate membranes (Helena cat. # 3023 and 3024) and the methods and staining recipes outlined in Hebert and Beaton (1993). Additional recipes were adapted from starch gel electrophoresis (Pasteur 1988) by increasing the concentrations and reducing the volumes of the necessary reagents.

Flies were removed from a -70 °C freezer, defrosted, and had their wings removed, before being ground in 15µl of distilled water. The homogenate was centrifuged at 13000 rpm for 30 sec and then 8µl of the supernatant was pipetted into the sample wells of a Helena sample well plate (Helena cat. # 4085 or 4095). A fixed quantity (approx. 1µl) of supernatant was transferred onto the cellulose acetate membrane using a Z-8 or Z-12 applicator (Helena cat. # 4084 or 4090). Occasionally the volumes or concentrations of some of the reagents were varied to achieve stronger or weaker staining as required. The mobility, variation and general performance of the different enzyme systems and staining recipes are detailed in Table 1.

Migration was slightly slower when run in a cold room at 4 °C rather than at room temperature, but for certain enzymes, such as α-GPDH, this produced neater, more even bands.

Further details for each enzyme system are as follows:

ACP: Up to three distinct bands of activity may appear for inbred individuals, decreasing in intensity from slow to fast. However, usually only the slowest migrating band is visible for females, and the slowest two for males; the fastest band always being very faint, if at all visible. Parkash *et al.* (1992) repeatedly observed 3 bands in inbred laboratory stocks of *D. busckii* from India, which they interpreted as fixed alleles at two separate loci producing compatible subunits for a dimeric enzyme. In one cross between two different laboratory strains of *D. phalerata*, a heterozygote banding pattern (three bands of relative intensity 1:2:1) appeared at the slowest zone, whilst the faster two banding zones were too faint to score. One possible explanation for the patterns currently observed in *D. phalerata* is that there are two independent loci, the faster of which has lost most of its activity, and is only regularly expressed in males. Nonetheless, the faster, "null" allele can still interact with the active slow allele to form a heterodimer of reduced activity.

AD and AO: Produced streaky and indistinct bands that could not be scored

ADH: Compared to most fruit-breeding *Drosophila*, *D. phalerata* has extremely low levels of ADH activity (Jacobs, 1994). Consistent with the findings of Jacobs (1994), staining is slightly stronger using iso-propanol as a substrate rather than ethanol, but still not sufficient for reliable scoring.
EST: Extremely variable in the number and quality of bands that appear. There is certainly some polymorphism at this locus/loci, but interpretation is problematic.
FUM: Is a tetrameric enzyme as revealed by a 5-banded heterozygote. The rare electromorph has a slower relative mobility (approx. 60%).
GLC: As of yet no activity has been detected, but may be worth further experimentation.
α-GPDH: The quality of the bands is susceptible to the temperature at which the plate is run, better results being obtained at lower temperatures. A rare electromorph with approximately 120% relative mobility was detected from natural populations.
GPI: Produces streaky bands with both standard buffers.
HEX: No activity detected, the reasons for this are unclear.
LAP: Weak, indistinct bands, not suitable for analysis.
LDH: Fairly weak bands, but could be useful, no polymorphism detected yet.
MDH: MDH1, the mitochondrial form migrates cathodally in CAAPM buffer. MDH2, the supernatant form, migrates anodally. Alternative (slower) electromorphs have been detected occasionally at both loci, from natural populations.
ME: Can be run in combination with MDH. A rare allele (slower electromorph) was detected from natural populations.
ODH: Stains very weakly, often shows up ADH bands as well.
PEP: Stains more strongly when using *leu-ala* rather than *leu-gly* as the dipeptide substrate. Two independent loci, both of which seem to harbour considerable polymorphism, however, the bands are often wide and of similar mobility and consequently difficult to score reliably.
PGM: Inter-strain crosses confirmed that this is a monomeric enzyme with simple Mendelian inheritance. Five readily distinguishable electromorphs were recovered from F1 rearings, originating at Arthington Wood. The three commonest electromorphs were present in the laboratory strains.
SOD: This enzyme does not stain well on cellulose acetate membranes (see Hebert and Beaton, 1993), and is often very streaky and sporadic.
XDH: Stains reasonably well with TG or CAAPM buffers and may be run in conjunction with MDH and/or LDH. No polymorphism detected in *D. phalerata* to date.

Discussion: The closest phylogenetic relative to *D. phalerata* for which allozyme information exists is *D. falleni* and, with the exception of LDH, all of the above have been found to be polymorphic in *D. falleni* when assayed with starch-gel electrophoresis (Lacy, 1982a,b, 1983; Shoemaker and Jaenike, 1997). Shoemaker and Jaenike (1997) also found EST, LAP, MDH1, MDH2, PEP1 and PGM consistently polymorphic for two other *quinaria*-group species. Offenberger and Klarenberg (1993) found no polymorphism in alpha-amylase, when comparing eight German lines of *D. phalerata*.

The low levels of variation observed in this study relative to Lacy (1982) or Shoemaker and Jaenike (1997) may be due to three main reasons. Firstly, by chance, all three laboratory strains may have been fixed for the same alleles. This is most likely where one allele is much more frequent than the others in natural populations. Nonetheless, one might expect the Dutch stocks to differ more substantially from the two stocks originating near Leeds, England. Secondly, CA may detect less variation than starch gel electrophoresis, a possibility that has been suggested by Jaenike (pers. comm.) although Easteal and Boussy (1987) suggest the opposite, namely, that CA electrophoresis often results in superior separation. Thirdly, it may indeed be the case that, relative to the studies on North American *quinaria*-group species, *D. phalerata* does possess low levels of genetic variation.

Conclusion: Of the enzyme systems tested to date PGM shows by far the greatest amount of allozymic variation using CA electrophoresis, and could profitably be used in studies of genetic population structure of *D. phalerata*.

Acknowledgements: We thank Rosie Sharpe for initial guidance in setting up the cellulose acetate electrophoresis system.

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Oliver, Brian, and Daniel Pauli. Department of Zoology and Animal Biology, University of Geneva, Geneva, Switzerland. Correspondence to: Brian Oliver, LCDB NIDDK NIH, 6 Center Dr. MSC 2715, Building 6 Room B1-13, Bethesda MD 20892. Email: oliver@helix.nih.gov. Duplications of *ovo*$^+$ do not result in XY germline death or sex transformation.

The *ovo* locus encodes C_2H_2 sequence-specific DNA-binding proteins (Mével-Ninio *et al.*, 1991; Garfinkel *et al.*, 1992; Lü *et al.*, 1998) that have been implicated in germline sex determination based on XX-specific germline death and germline sex transformation phenotypes; and on genetic interactions with mutations in *Sxl*, *snf*, *otu* and *stil* (Oliver *et al.*, 1987, 1990; Pauli *et al.*, 1993; Staab and Steinmann-Zwicky, 1996; Penetta and Pauli, 1997). At the molecular level, the *ovo*$^+$ locus is required for female-specific splicing of *Sxl*$^+$ mRNAs in the germline (Oliver *et al.*, 1993) and for high level expression of *otu* and *ovo* reporter genes (Oliver *et al.*, 1994; Mével-Ninio *et al.*, 1995; Lü *et al.*, 1998).

Given that decreased *ovo*$^+$ activity results in XX germline death and the differentiation of some surviving germ cells as spermatocytes (Oliver *et al.*, 1990, 1993), we were interested in determining if, conversely, increased *ovo*$^+$ activity would result in XY germline death and male to female transformation. This is especially pertinent given that *ovo* is an X-linked gene and is, therefore, present in two copies in females, but only one copy in males. The dose of *ovo*$^+$ was elevated using *ovo*$^+$ transgenes that fully rescue the ovo$^-$ germline phenotype in females and result in functional OVO proteins in male germ cells (Andrews *et al.*, 1998; Lü *et al.*, 1998). The most striking phenotype seen in females homozygous for strong loss-of-function *ovo* alleles is the extreme reduction of germ cell number (usually none) in XX flies (Oliver *et al.*, 1987, 1994; Staab and Steinmann-Zwicky, 1996). We did not see a corresponding germ-cell-death phenotype in males bearing three to five copies of *ovo*$^+$. There were rare individuals (less than 1%) with few or no germ cells, but germ cell death was not characteristic. Most testes had wildtype gross morphology, and flies with up to five copies of *ovo*$^+$ were at least semi-fertile. At the cellular level, the morphology of the germ cells appears to be male in hundreds of examined testes.

However, keeping *ovo*$^+$ expression levels low may be important for late steps in spermatogenesis (Figure 1). Nearly all XY male flies with five copies of *ovo*$^+$ showed spermatid cysts with defects and other spermatid cysts that appeared wildtype. The most common defect was cells with a single large mitochondrial derivative surrounded by four spermatid nuclei. This phenotype is similar to that found in a number of male-sterile mutations and is believed to be due to defects in the completion of cytokinesis during meiosis (reviewed by Fuller, 1993). More rarely we saw altered spermatid nuclei sizes. Because nuclear size is dependent on chromosome content, non-uniform nuclei could be due to non-disjunction (Gonzalez *et al.*, 1989). If so, defective sperm were not successfully transmitted, as we found little XY or 2nd chromosome non-disjunction (few *+/+/B^{s}Y, +/0*, or *2^2/0* progeny). Meiosis in *Drosophila* is sexually dimorphic (reviewed by Hawley, 1993) and a female meiosis in a male germ cell might well be expected to result in gross defects in spermatogenesis. Indeed, *ovo* mutations disrupt recombination in female germ cells, which indicates that *ovo* is required for proper sex-specific meiosis (Cook, 1993). However, we do not know if the defects we saw in XY males with increased copies of *ovo*$^+$ were due to reciprocal sex-specific defects in meiosis. The salient point for this work is that high *ovo*$^+$ copy number was not sufficient for overt of XY germ cells. Absence of *ovo*$^+$ in females and extra copies of *ovo*$^+$ in males do not result in reciprocal phenotypes.

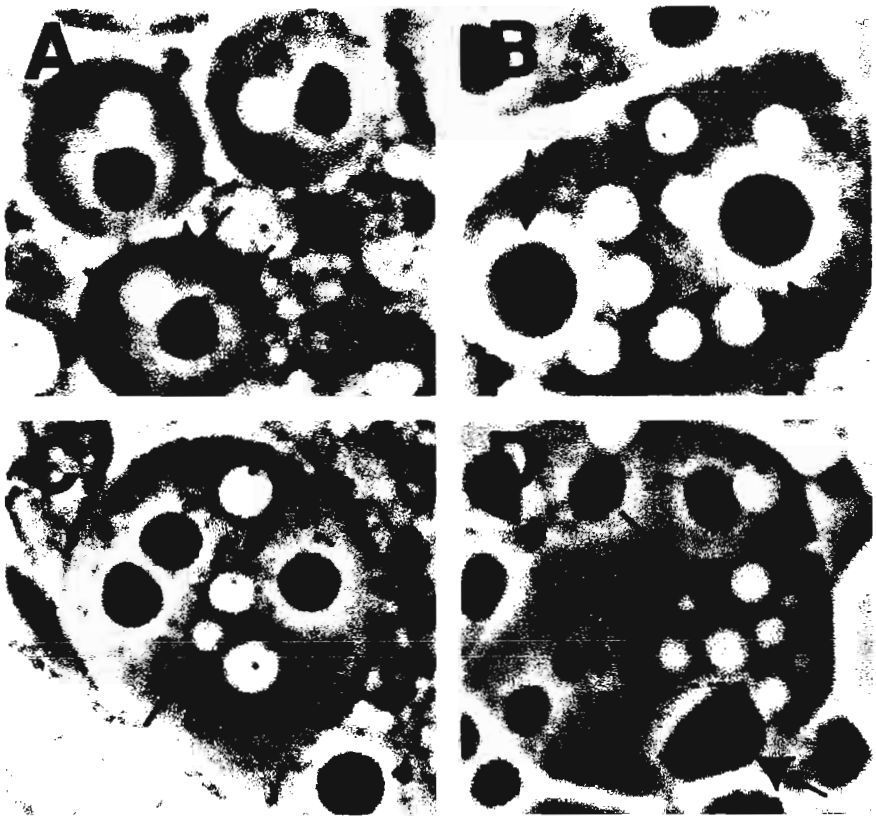

Figure 1. XY males bearing duplications of *ovo*$^{+}$ show defective spermatids. A. Early wildtype spermatids showing phase dark mitochondrial derivatives (filled arrows) and phase clear nuclei (open arrows). B-D. Spermatids from XY males with five copies of *ovo*$^{+}$. B. Examples of four spermatid nuclei associated with a single large mitochondrial derivative (filled arrows). This was the most common phenotype observed. The dark spots in the nuclei are proteoid bodies also found in wildtype spermatid nuclei. C. Less commonly there were defects in spermatid nuclear volume (open arrow). D. Spermatids with altered mitochondrial derivatives (filled arrows) and nuclear volumes (open arrows and outlined). Genotypes: (B) *X/Y; P{w*$^{+}$ *ovo*$^{+10.0}$*}13D P{w*$^{+}$ *ovo*$^{+10.0}$*}E* (C) *X/Y; P{w*$^{+}$ *ovo*$^{+10.0}$*}13D P{w*$^{+}$ *ovo*$^{+10.0}$*}Y/P{w*$^{+}$ *ovo*$^{+10.0}$*}13D P{w*$^{+}$ *ovo*$^{+10.0}$*}E*, (D) *X/Y; P{w*$^{+}$ *ovo*$^{+10.0}$*}13D P{w*$^{+}$ *ovo*$^{+10.0}$*}Y*.

Given that germline sex determination requires both germ-cell-autonomous signals and somatic induction (Schüpbach, 1985; Steinmann-Zwicky *et al.*, 1989), XY males with extra copies of *ovo*$^{+}$ might be competent for female germline development but un-induced. XY flies expressing a *tra* female cDNA driven from an *hsp70* promotor (*tra*$^{hs.PM}$) are transformed into females (McKeown *et al.*, 1988) with germlines that remain male (Steinmann-Zwicky *et al.*, 1989). Strikingly, XY *tra*$^{hs.PM}$ bearing flies are able to support nearly normal development of transplanted XX germ cells into only slightly defective eggs (Steinmann-Zwicky, 1994), indicating that TRA expression in the soma and a XX germline karyotype is sufficient for female germline sex determination and provides nearly all the information that is needed for full female germline differentiation. We tested whether XY germ cells with extra copies of *ovo*$^{+}$ act like XY or XX germ cells when in a female soma. XY somatically female flies with three *ovo*$^{+}$ doses usually showed male germline development indistinguishable from controls bearing a single copy of *ovo*$^{+}$ (Figure 2). Very rare germ cell chambers were found that contain larger nuclei, that did not look like male germ cells. These might be examples of sex transformed germ cells, but because they were degenerating, we were unable to determine their sexual identity unambiguously. Regardless of the nature of these larger nuclei, it is quite clear that duplications of *ovo*$^{+}$ are not equivalent to a XX karyotype. We never saw advanced stages of oogenesis or eggs in *X/Y; tra*$^{hs.PM}$ flies with three copies of *ovo*$^{+}$. Thus, the absence of *ovo*$^{+}$ in XX germ cells results in altered sex determination and cell viability, but

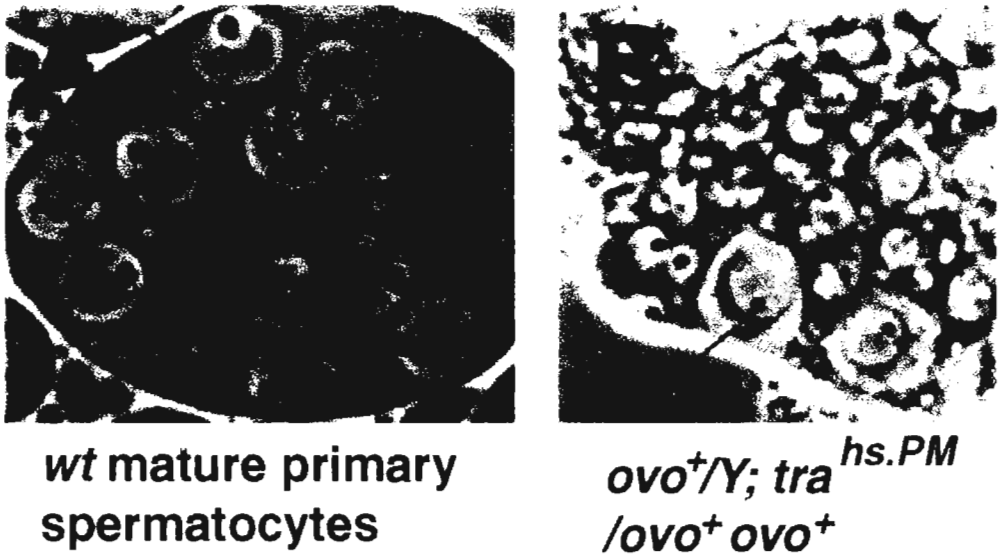

Figure 2. Duplications of *ovo*$^{+}$ do not switch the sexual identity of XY germ cells in a female soma. A. Wildtype spermatocytes towards the end of the growth phase. B. Germ cells showing spermatocyte morphology in a XY fly transformed from male to female somatic identity. Note the mitochondrial condensation near one of the larger nuclei in the polar primary spermatocyte (star). Polar primary spermatocytes are in early growth phase. The majority of the germ cells are earlier growth phase primary spermatocytes or spermatogonia. Genotype: *+/B^{s}Y; P{ry^{+} tra$^{hs.PM}$}/ P{w^{+} ovo$^{+10.0}$}13D P{w^{+} ovo$^{+10.0}$}Y.*

increased *ovo*$^{+}$ activity in XY germ cells is insufficient to impair germline viability (four extra copies), and is probably insufficient to alter sexual identity (two extra copies).

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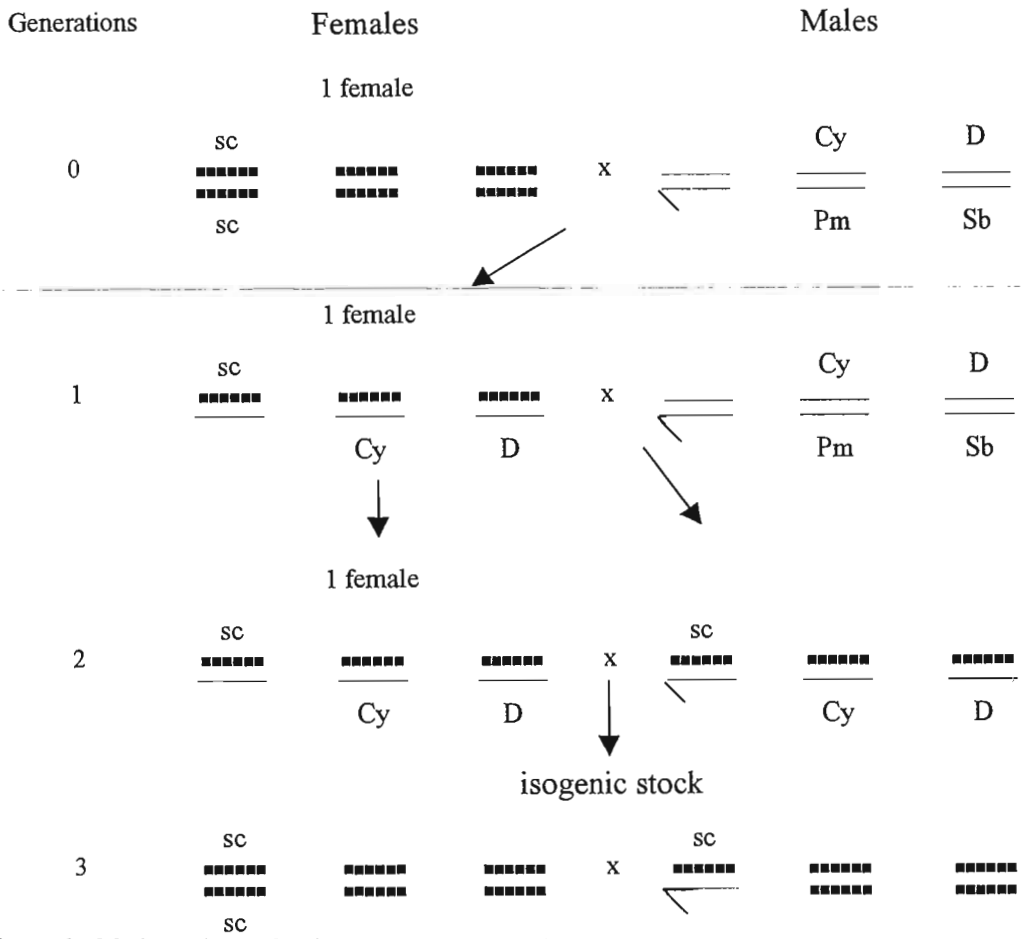
Furman, D.P., and T.A. Bukharina. Institute of Cytology and Genetics, Siberian Department of the Russian Academy of Sciences, Novosibirsk 630090, Russia. Transposable elements reveal crossing-over.

Balancing chromosomes are widely used in the genetic experiments which require preservation of the genetic constitution of homologous chromosomes, in particular, in developing isogenic lines. When coupled with the standard sequence homologues, inverted balancers are believed to suppress recombination effectively; the homologues should therefore remain intact (MacIntyre and Wright, 1966).

Meanwhile, events of exchange in such heterozygotes as these take place and viable crossover gametes occur either by single exchange in inversion-free regions or by double exchange in inverted regions. Visible mutations may not help identify these events, but transposable genetic elements (TEs), which appear to be more sensitive indicators, perhaps can do that. By comparing the location of transposable elements in the balanced, balancing, and derived lines, it is possible to answer the question as to whether there is an exchange between the balancer chromosomes and their normal homologues. The interchanged chromosomal regions will show up clearly: the labeling sites either appear on or disappear from the chromosomes of the derivative, which is free from inverted balancing chromosomes.

Cy/Pm;D/Sb, a balancer line from the collection of the SD RAS Institute of Cytology and Genetics, contains multiple inversions, lethal and visible mutations (Lindsley and Zimm, 1990). Our interest was to see whether recombination is absolutely suppressed in between-line crosses with *Cy/Pm;D/Sb*.

Isogenic lines are perhaps the best we can use in this crossing-over study, because no within-line polymorphism for the location of transposable elements has been observed in them as yet (Furman *et al.*, 1993). *Mdg1*, *Dm412*, *copia*, and *B104* served as markers. Their location was determined by *in situ* hybridization on salivary gland chromosomes; not less than 4-5 preparations with not less than 10 nuclei on each were assayed. The mating scheme was a routine (Scheme 1). Analysis covered the balancing, balanced, and derived isogenic lines.



Scheme 1. Mating scheme for developing the isogenic line. Crosses 1 and 2 with the same female.

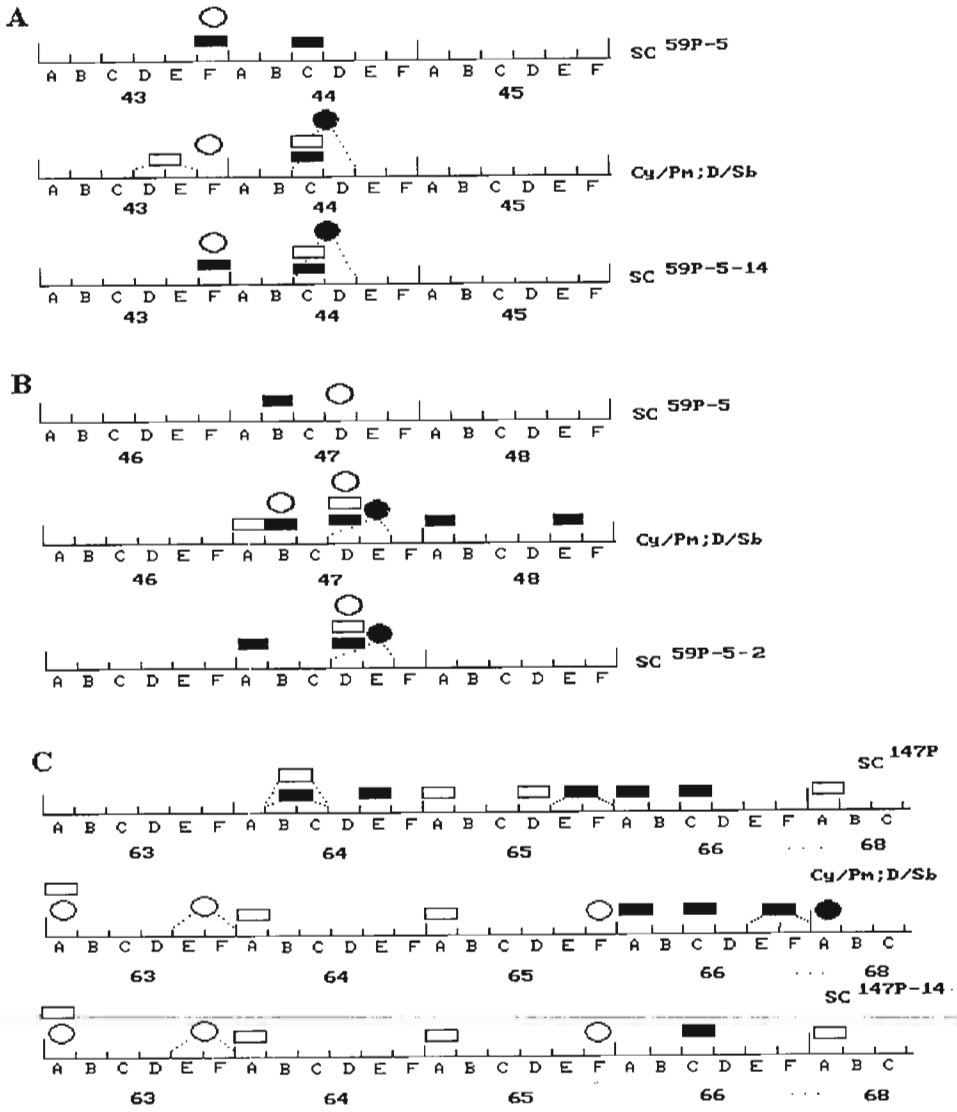


Figure 1 a-c. Examples of chromosomal exchange between *Cy/Pm;D/Sb* (balancing line) and homologues from *sc59-5* and *sc147*: black ovals - *Mdg1*, white ovals - *Dm412*, black rectangles - *copia*, white rectangles - *B104*.

Exchange between the balanced and balancing chromosomes was assumed if a set of transposable elements was found to occupy either the same site in an isogenic derivative and the balancing line or the same sites over a more or less extending region of the respective chromosomes in the isogenic derivative and the balancing line.

The presence/absence of a single transposable element at/from a separate site of the derivative line was not looked at as evidence for exchange. Phenomenologically, either looks like insertion/excision, and therefore may have been preceded by crossing-over, but not necessarily.

The most convincing examples of chromosome exchange are presented in Figure 1a-c. Figure 1a-b depicts fragments of chromosomes from *Cy/Pm;D/Sb*, *sc59-5*, and its two isogenic derivatives, *sc59-5-14* and *sc59-5-2*. Region 44C, labeled by three elements inserted in *sc59-5-14* (Figure 1a), and region 47DE with four inserts in *sc59-5-2* (Figure 1b), are from the balancer. Because no increase in the mortality rate was observed with the *sc59-5* × *Cy/Pm;D/Sb* offspring, double exchange can be assumed within the inverted regions.

The six inserts running in succession on chromosome 3L of *sc147-14* (Figure 1c) might be resulting from crossing-over (site 66A in *Cy/Pm;D/Sb* is polymorphic and hence our explanation is still consistent). The region in question lies off In(3L)69D3-E1;70C13-D1.

Therefore, it would be good to know how the transposable elements are distributed in the balancing line and balanced lines. It would be then easy to foresee crossing-over effects on the polymorphism in derivatives and to estimate correctly the frequencies of TE transpositions in between-line crosses of that sort. This could be of especial importance when TEs alone are markers of genomic regions in experiments of populational genetics, for example, in studying correlations between the pattern of TE distributions and the profile of alteration of polygenic traits.

Our data, however, are insufficient to say by which exact mechanism crossing-over takes place here. For example, recombination frequency may be dependent on whether inserts, especially multiple ones, are present at the same sites of the homologous chromosomes of the lines being crossed. Constructions of that kind either make chromosomes easily breakable and repairable at the homologous regions of TEs or allow exchange at long terminal repeats of TEs (McGinnis and Beckendorf, 1983; Goldberg *et al.*, 1983; Davis *et al.*, 1987; Tsubota *et al.*, 1989).

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Jarman, M.G., and J.I. Collett. School of Biological Sciences, University of Sussex, Brighton, Sussex, BN1 9QG, U.K. Siting the genes of *Dipeptidase-B (Dip-B),* several 'recessive-lethal' complementation groups and the breakpoints of chromosomal deficiencies within the region 87F12- 88B1 of *D. melanogaster.*

This analysis was undertaken to establish the chromosomal site of *Dip-B* and to assess the possibility of association of mutant alleles of the complementation groups of *l(3)88Aa* and *Ac*, isolated by Mortin *et al.* (1992), with the expression of the *Dip-B* gene. At the outset of this analysis the status of the mapping of *Dip-B* was as follows. Electrophoretic analysis of heterozygotes of electrophoretic variants of *Dip-B* in *D. pseudsoobscura* (Collett, unpublished) had indicated a homotetrameric structure and Onishi and Voelker (1981) had shown a map position within the region 87F12-88C1 of Df(3R)red-31. Subsequent aneuploid mapping of about 80% of the genome by Hall (1983) confirmed the position of *Dip-B* at this site and failed to reveal any other affecting *Dip-B* expression. Following the screen for recessive lethal mutations in the region of Df(3R)P52 (88A3-88B3), Mortin *et al.* (1992) assigned mutations to a number of complementation groups throughout the region including those of *l(3)88Aa* and *Ac*. Several mutant alleles of these two genes had shown some sign of affecting Dip-B activity in heterozygotes. Here, following definition of deficiency breakpoints, *Dip-B*, three previously identified genes and at least one new gene are assigned to sites throughout the region, and the identity of one of these genes found by both mutation and transposon insertion is established.

Compiled in Table 1 are the chromosomal deficiencies used here, the methods and sources of identification of their breakpoints and the results of complementation analysis to confirm, to define and to refine the positions of their breakpoints. All deficiencies and mutant alleles were maintained in stocks with either the 'balancer' TM3 or TM6B. Included among these are two, M36 and M49, which were thought to be small deficiencies by Mortin *et al.* (1992) on the basis of the complementation pattern. This expectation was confirmed here, as indicated below, and thus their inclusion in Table 1. The alleles designated M and Z (Figure 1) were isolated by Mortin *et al.* (1992) and supplied by M. Mortin. The allele *k43* of l(3)*88*Ab was supplied by A. Shearn and the transposon induced allele of *l(3)03477, j1D1*, was supplied by the *Drosophila* Genome Center (Baltimore).

Complementation was deemed to have failed when no unmarked flies were found among at least 100 progeny from more than one cross, or, in several cases, when results in smaller progenies were consistent with the progenies of similar crosses of flies with other deficiencies or with other alleles within a complementation group (Jarman, 1997). Among the M and Z mutants, each was complementation-tested in pairwise matings. *k43* was tested with each M allele, and similarly, the allele of *l(3)03477* was tested with both M and Z mutants. These alleles are listed in Figure 1 in complementation groups according to the results of these tests. Those listed are consistent with Mortin *et al.*'s (1992) assessment with the exception of the mutant M30. This was found to be in the region of Df(3R)P1. But, in addition, these analyses revealed allelism between the M mutants of *l(3)88Aa* and the P-transposon induced allele *j1D1*. Thus the

Table 1. Complementation of Deficiencies Demarcating Chromosomal Region 87F12 - 88B1

		Deficiencies, Df(3R):									
Df(3R):	Assignment of Breakpoints	126c	urd	red-31	$293^{\gamma 5}$	$293^{\gamma 7}$	red-P52	M49	M36	su(Hw)[7]	red-P1
126c	87E1-2; 87F11-12(c)[1]	*		+	+	+	+				
urd	87F1; 87F15(dfh)[2]		*	-		+					
red-31	87F12-14(c)[1]; 88C1-3(c)[1]			*	-	-	-				
$293^{\gamma 5}$	87E2; 88A5-6(m)[3,4,5]				*	-	-	-	+	+	+
$293^{\gamma 7}$	88A1(m,g)[4,5]; 88A4-6(m,g)[4,5]					*	-	-	+	+	+
red-P52	88A3-4(g)[3]; 88B3(m)[6]						*	-	-	-	-
M49	88A3-4(ca); 88A6-7(ca)							*	-		+
M36	88A5-6(ca); 88A8-10(ca)								*	+	+
su(Hw)[7]	88A9(c)[7]; 88B3(m)[9]									*	-
red-P1	88B1(dfh,m)[8,9]; 88D3-4(c)[2]										*

c, cytological observation of polytene chromosomes; dfh, complementation analysis of deficiency heterozygotes; g, *in situ* hybridised genic DNA excluded from/included in deficiency region; m, molecularly-defined breakpoint; ca, complementation analysis presented here.
1. W.M.Gelbart cited by Hall and Kankel (1976). 2. Lindsley and Zimm (1992). 3. Dalton *et al.* (1989). 4. R.Kelley, pers. comm.
5. synonym: fs293gamma7. 6. Parkhurst *et al.* (1988). 7. R.Coyne, pers. comm. 8. Hamilton *et al.* (1993). 9. Breen and Harte (1991). The sources of stocks carrying the Deficiencies were as follows: 126c and red-31 , Umea *Drosophila* Center; urd,red-P52, su(Hw)[7], red-P1 Bloomington *Drosophila* Stock Center; $293^{\gamma 5 \text{ and } 7}$, R.Kelley; M49 and M36, M. Mortin. '+' indicates complementation and '-' its failure.

formerly identified gene named *P03477* is one and the same as *l(3)88Aa* and should be named according to its earlier mutational identification.

The positioning of the 'recessive lethals' *88*Aa, *Ab, Ac* and *Ad* follows from the landmark positioning of the *P*-alleles of *l(3)88Aa* by *in situ* hybridisation to88A4-5 (Spradling *et al.,* 1995). The argument runs as follows. Since M36 and *88Ac* mutants complement the 293 Deficiencies, their positions are distal to 88A6. Since M49 fails to complement alleles at the locus 88A4-5 as well as M36, it must be a deficiency. Then, since M36 fails to complement both M49 and the *88Ac* mutants, it too must be a deficiency. Thus the loci of these two complementation groups are restricted to the regions indicated in Figure 1. However, the failure of M36 and M49 to complement also indicates at least one more 'recessive lethal' complementation group in the region of their overlap. Following Mortin *et al.*'s(1992) earlier surmise and naming, *88Ad* should be recognised to exist within the cytological region 88A6-8, and the locus of *88Ac* must be to its right and bounded distally by the chromosomal region of Df(3R)su(Hw)[7]. Finally, the position of the complementation group *l(3)88Ab* is defined by its inclusion in the region of Df(3R)P52 and its exclusion from the region of Df(3R)M49. These relationships are laid out in the map presented in Figure 1.

The assignment of the *Dip-B* gene to a chromosomal site was made by the visualisation of Dip B activity following electrophoretic separation of preparations of flies heterozygous for a null allele of *Dip-B* (in a stock of *raised* flies) and each of the first six deficiencies listed in Table 1. Using the method of separation and identification of Dip B activity (Collett, 1989), null and active alleles were identified in at least 4 samples of each heterozygous genotype consisting of three 4-10 day old adult females, maintained at 18°C. until sampling. The results in every sampling were consistent with the deficiency breakpoints indicated in Figure 1. Thus *Dip-B* is within the region of 87F12-15.

In establishing the demarcation of the chromosomal region 87F12-88B1 (Bridges, 1938) by a set of deficiency breakpoints at intervals throughout the region, the map positions of Dip B and four 'recessive lethal' genes have now been defined within chromosomal segments equivalent to lengths of DNA cloned in P1-plasmids. These deficiencies should continue to be useful in similarly defining the map sites of other genes, identified and not yet identified, throughout the region. All stocks carrying mutant alleles and the deficiencies used here (except Df(3R)M49, now lost) are, or are soon to be, available in a *Drosophila* stock Center.

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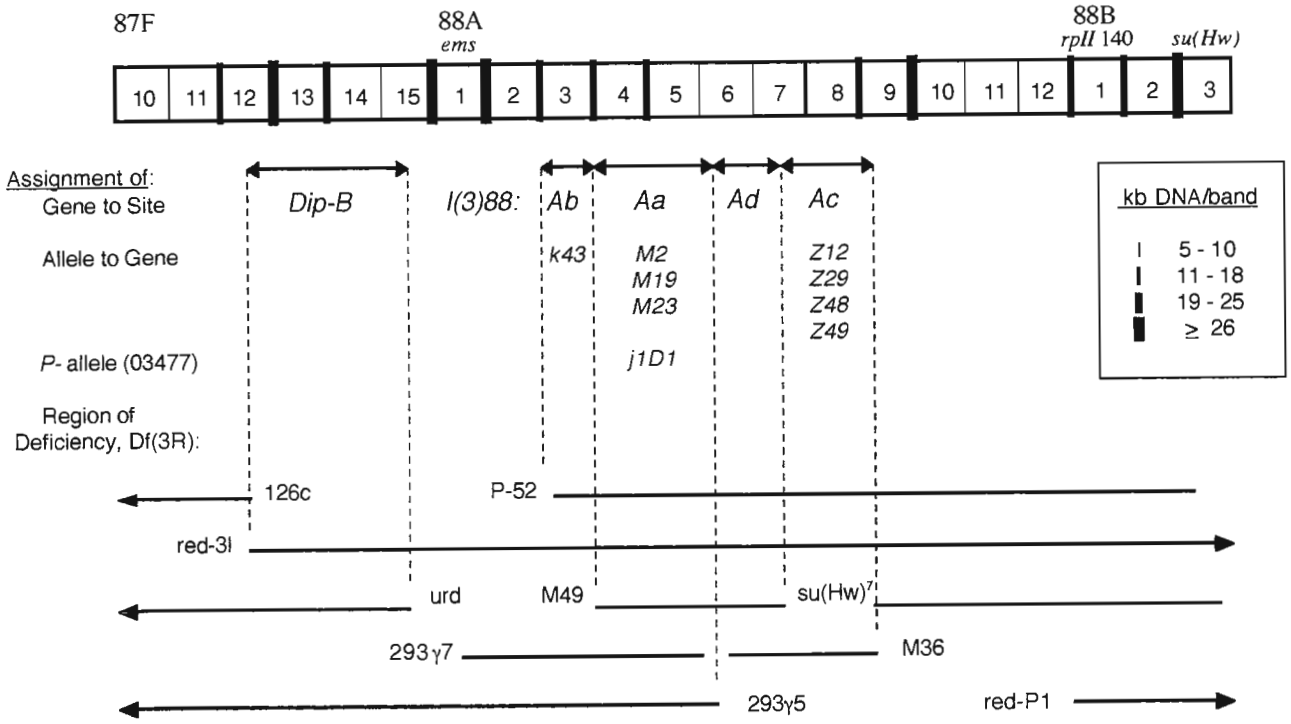

Figure 1. Sites of *Dipeptidase*-B and *l(3)88Aa-Ad* in the region 87F12-88B. The widths of the vertical demarcations of polytene bands represent the constituent amounts of DNA (Heino *et al.*, 1994), as indicated. The genes *ems, rpII140* and *su(Hw)*, assigned to single polytene bands (Flybase, 1997) and used in establishing the positions of deficiency breakpoints are also indicated. Heavy lines indicate the regions of deficiency (see Table 1 and text). Arrows indicate breakpoints in adjoining regions. The M and Z alleles and the *P* - allele *j1D1* of *l(3)03477*, located at 88A4-5 by *in situ* hybridisation (Spradling *et al.*,1995), were assigned by deficiency complementation analysis to the 'recessive lethal' genes *l(3)88Aa-Ac*. In addition, failure of the deficiencies M49 and M36 to complement indicates at least one further 'recessive lethal' gene site, designated l(3)88*Ad*. The vertical lines are guides to the boundaries of the regions of each gene as defined by deficiency breakpoints.

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Crowley, Thomas E. Department of Biological Sciences, Columbia University, code 2407, 1212 Amsterdam Ave., New York, NY. email: tc45@columbia.edu; phone, 212-854-4835; fax, 212-865-8246. Mutations near the *Trf* cluster cause a premeiotic defect in the *Drosophila* male germ line.

Abstract:

In situ hybridization, P transposase-mediated mutagenesis, and stage-specific markers are used to examine the regulation of expression and function of the *Trf* cluster during spermatogenesis in *Drosophila melanogaster*. The temporal regulation of the presence of the three mRNAs during sperm development is determined, and the effect of various mutations at the *Trf* site on the premeiotic stages is described. The molecular nature of each mutation is then established, and possible functions of *Trf* cluster gene products are discussed, in particular a potential role in cell cycle regulation is suggested.

Introduction:

TBP-Related Factor, or TRF, is a sequence-specific DNA-binding protein in *Drosophila* which resembles the TATA box Binding Protein (TBP) in structure and DNA sequence-specificity (Crowley *et al.,* 1993). TBP binds to the promoter of Pol II transcribed genes in eukaryotic cells in one of the first steps of transcription initiation. In addition TBP plays a critical role in Pol I and Pol III transcription making it a universal general transcription factor (Baumann *et al.,* 1995; Nikolov and Burley, 1994; Burley, 1996). The observed similarity in structure and DNA-binding specificity of TRF and TBP suggested that TRF might be a transcription factor, and in fact the recent findings of Hansen and coworkers (1997) reveal that TRF can substitute for TBP to provide basal level Pol II transcription from an adenovirus or *Drosophila Adh* promoter in an *in vitro* assay which includes the other general transcription factors. They also demonstrate that in the *Drosophila* embryo, TRF is associated with several TRF-Associated Factors (nTAFs) which are distinct from the TBP-Associated Factors and the TRF/nTAF complex can also substitute for TBP to provide basal level transcription in an *in vitro* system. In addition to the *in vitro* experiments, Hansen and coworkers expressed TRF in cultured *Drosophila* cells and observed TRF-mediated activation of a reporter gene by the neurogenic transcription factor, NTF-1.

The fly gene coding for this protein, *Trf*, was discovered in an enhancer-trap screen and cloned by plasmid-rescue via the transposon, P-lacW, which had inserted nearby. Screens of embryo and testis cDNA libraries with genomic DNA probes including 5 kb of sequence on either side of the insertion site revealed that *Trf* is flanked on either side by a gene of unrelated sequence. All three genes are on one side of the insertion and there appear to be no transcripts produced in the embryo or testis from within 5 kb on the opposite side (Figure 1). The transcription reporter in the transposon indicates that this genomic site is transcribed in embryonic neuroblasts which give rise to the CNS, and primary spermatocytes in the adult testis. The transposon insertion at *Trf* causes two phenotypes when homozygous: male-sterility due to a lack of motile sperm and leg-shaking in ether-anesthetized adults (Crowley *et al.,* 1993). This allele is designated P[lacW]Trf for convenience since *Trf* is the best characterized of the three genes, although it is not yet known which gene's expression is actually affected by the insertion. The transcription of the *Trf* cluster in spermatocytes and the male-sterile phenotype of P[lacW]Trf suggest that at least one and possibly all three of the proteins coded at this site play a critical role in spermatogenesis.

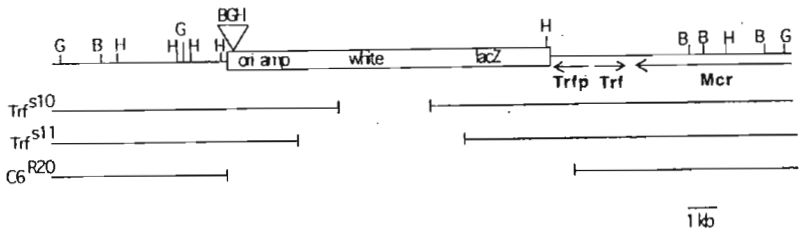

Figure 1. Molecular map of P[lacW]*Trf*. The three transcribed segments are shown and the transposon insertion is depicted as an open rectangle on the left side of the gene cluster. The component genes of P-lacW are indicated within the rectangle. The transcription initiation site for *Mcr* is off the right side of the map, and the direction of transcription for *Trfp* is based on the presence of a 252 codon CDS in a right to left reading of the transcribed segment which is significantly longer than any CDS found in a reading in the opposite direction. The portion of P-lacW removed by Trf^{s10}, Trf^{s11} and $C6^{R20}$ is shown below the map. B = *BamH*I, G = *Bgl*II, H = *Hind*III.

Male germ cell development in *Drosophila* is an excellent system for examining the properties of stem cells, regulation of mitotic proliferation, cell growth, meiosis, alterations in chromatin components, nuclear and cytoplasmic elongation, and the formation of a motile flagellum (Lindsley and Tokuyasu, 1980; Lifschytz, 1987; Fuller, 1993). The study of mutations which cause male-sterility due to inhibition of motile sperm production may shed some light on the mechanisms regulating these processes. To establish the importance of *Trf* cluster expression in spermatogenesis, *in situ* hybridization is used to assay transcription regulation in the male germ cells, P transposase-mediated mutagenesis is used to create new mutations near the *Trf* cluster, and male germ cell-specific markers are used to assay the abundance of the early stages of sperm development in mutant testes. Finally, the molecular nature of each mutation is determined and mechanisms are suggested which may explain how these mutations affect expression of the *Trf* cluster.

Materials and Methods:

Detection of RNA via in situ *hybridization:*

Testes were dissected from *Oregon R* wild type adults, less than 24 h post-eclosion, then fixed in 5% formaldehyde, 0.1 M $NaPO_4$, pH 7.2 for 30 min. Tissue was then washed in 10 mM $NaPO_4$, 140 mM NaCl, pH 7.2 (PBS) four times for 5 min each. The testes were then dehydrated in 1:1 ethanol:PBS for 10 min, followed by four 5 min washes in pure ethanol, and stored at -20°C until hybridization was performed. Proteinase treatment of the tissue, hybridization of the cDNA probe and detection of the signal were carried out as described by Tautz and Pfeifle (1989). The probe was created by labeling a cDNA with digoxigenin-uridine following the supplier's protocol (Boehringer Mannheim Genius Kit).

P element deletion: The source of transposase was the P[ry$^+$ delta 2-3](99B) insertion on the 3rd chromosome described by Robertson *et al.* (1988). This transgene was introduced into the P[lacW]Trf genome by using a *Sp*/CyO; *Sb delta 2-3*/TM6 stock, then removed by standard genetic crosses.

Spermatocyte Labeling with EXU/GFP: An X chromosome carrying a P-element construct which includes the *exu/Gfp* gene fusion was introduced into the *Trf* mutant stock via standard genetic crosses. The expression of the encoded fusion protein, its properties and the method for visualizing it in *Drosophila* oogenesis are described in Wang and Hazelrigg (1994). For images of whole testes, tissue was removed from adults, placed on a slide in a drop of DB, and covered with a coverslip. Images of the fluorescence in the live cells were obtained with a Biorad Confocal Imaging system by performing a z-series, then creating a projection of this series. The z-series consisted of 8 images at 3 μm intervals taken through the 10X objective of the microscope, employing the BHS/fluorescein filter. For individual cysts of primary spermatocytes, the testis epithelium was opened before applying the coverslip in order to allow the premeiotic cysts to flow out. Images were obtained with a conventional fluorescence-detecting microscope using a filter providing 550 nm excitation and 615 nm emission.

Staining for Enhancer-trap Reporter Expression: Testes were dissected from adults in a physiological saline, placed in PBT for 5 min, fixed in 1% glutaraldehyde, 50 mM cacodylate pH 7.3 for 15 min, washed in PBT 3x5 min then stained in X-gal as described in Hiromi *et al.* (1985).

Characterization of Transposase-induced Deletions: Genomic DNA was purified from adult flies, digested with restriction enzymes, and Southern blots performed with standard techniques. The genotypes examined were:
y w; P[lacW]*Trf*
y w; *Trf*$^{\delta 10}$
y w; *Trf*$^{\delta 11}$
and probes consisted of genomic subclones from the *Trf* region or P-lacW plus the *Trf* region which includes the three transcribed segments.

Results and Discussion:

Detailed cytological description of *Drosophila* spermatogenesis can be found in Lindsley and Tokuyasu (1980), Lifschytz (1987), and Fuller (1993), so only a brief summary will be given here. Each testis is a long narrow tube, closed at the end where the germ line stem cells are found (apical), and open at the basal end where mature elongated spermatids pass into the seminal vesicle. As their name indicates, the stem cells divide asymmetrically, regenerating themselves and producing a spermatogonium which then undergoes 4 rounds of mitosis, resulting in a cyst of 16 primary spermatocytes which then enter a 90 h growth period in the absence of cell division. The enlarged primary spermatocytes, each 25 μm in diameter, are now prepared to enter meiosis, which will give rise to clusters of 64 haploid spermatids. The early round spermatids now begin the process of elongation, by which the streamlined mature sperm will be formed, with each batch of 64 sister spermatids held together in a bundle by the two somatic cyst cells which have enclosed the developing cluster of germ cells since just after stem cell division. As development of the sperm proceeds, the cells move through the testis tube from apical to basal end, so that the mitotic spermatogonia are clustered near the apical end while the enlarged primary spermatocytes fill the region 2-27% of the testis length measured from the apical end, and the elongated spermatid bundles fill the remaining volume down to the basal end.

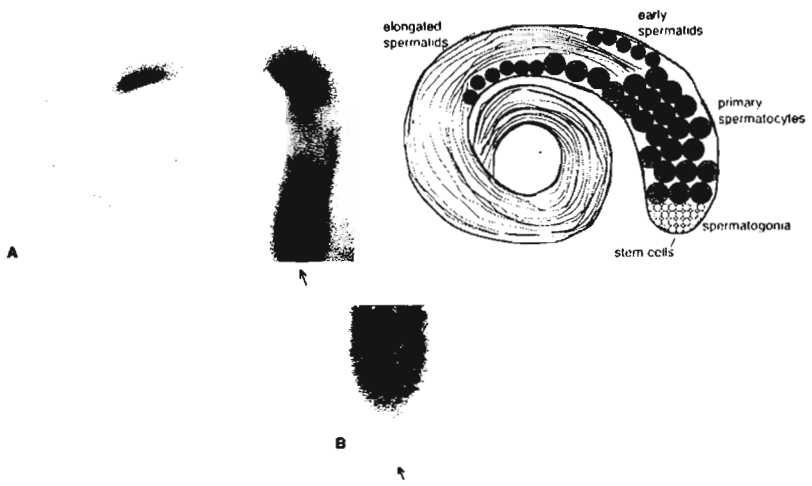

Figure 2. **A,B** Distribution of the *Trf* mRNA in developing germ cells of the adult testis. **A,** Whole testis, in same orientation as the drawing to the right. The apical end is left out of frame in order to include all of the remainder of the tissue. **B,** Higher magnification photo of the apical end of the testis shown in panel **A**.

To determine the temporal and spatial regulation of expression of *Trf* cluster genes during spermatogenesis, a cDNA derived from the *Trf* message was labeled with digoxigenin and hybridized to a whole mount preparation of a wild type testis. As shown in Figure 2, the probe hybridized to RNA in the cytoplasm of the enlarged primary spermatocytes, and this hybridization signal was much stronger than any produced by control probes made of non-*Drosophila* sequence (data not shown). The apical tip of the testis tube, which is the location of the germline stem cells and

the spermatogonial cells going through mitotic divisions, is not stained and there is clearly no signal in the elongated spermatids at the basal end of the testis. Similar results were obtained when cDNAs derived from the mRNAs encoded by the other two genes of the *Trf* cluster were used as probes (data not shown).

Since transcription of the *Trf* cluster in the wild type is restricted to the stage which precedes meiosis, one would expect that the male-sterile effect of the P[lacW]*Trf* transposon insertion would be caused by a defect in the entry into meiosis, meiosis itself, elongation of the haploid spermatids, individualization of the spermatids from the cyst or lack of motility of the mature sperm. Examination of the reproductive tracts of adult males homozygous for P[lacW]*Trf* reveals that no sperm are present in the seminal vesicles. The preelongation spermatogenic stages, such as the early round spermatid, the metaphase I spermatocyte with condensed chromosomes preparing to undergo meiosis, and the enlarged primary spermatocyte all appear unaffected by P[lacW]*Trf* (data not shown).

Since no defects in sperm maturation are visible until long after the time of transcription of the *Trf* cluster, it seems likely that P[lacW]*Trf* is a hypomorph and that stronger *Trf* alleles might show premeiotic defects. The method to test this hypothesis involves inducing deletion of the P-lacW transposon at its insertion site at *Trf* via the introduction of the gene coding for the transposase enzyme (Johnson-Schlitz and Engels, 1993). Flies carrying a copy of the 2nd chromosome which have had a deletion of the transposon are identified by scoring for loss of eye pigment produced by the *white* minigene within P-lacW, and stocks are then established with each mutant chromosome over a balancer. These alleles are designated [w^{-}]*Trf*, although as explained above this is only for convenience and does not imply that associated phenotypes are necessarily due to alterations in expression of *Trf* rather than the other two genes of the cluster. The reproductive tracts of males homozygous for these [w^{-}]*Trf* chromosomes are then examined for defects in spermatogenesis which cause male-sterility. Any new mutant which is male-sterile is then tested for lack of complementation of P[lacW]*Trf* to ensure that the mutation causing sterility is in fact at *Trf* and not somewhere else on the 2nd chromosome. A new lethal is tested for lack of complementation of the $C6^{R20}$ lethal deletion described in Crowley *et al.* (1993), and shown in Figure 1. Mutations which fail to complement the previously characterized *Trf* alleles are designated Trf^{s} for a male sterile or Trf^{l} for a lethal.

Sixty-nine [w^{-}]*Trf* alleles have been generated; one of these is homozygous lethal, 16 cause male-sterility when homozygous and the remainder have no known phenotype. Some of the male-sterile alleles do in fact show more severe spermatogenic defects than P[lacW]*Trf*, in particular Trf^{s10} and Trf^{s11} show an alteration in the distribution of the stages of spermatogenesis. The number, distribution and structure of the premeiotic enlarged primary spermatocytes in testes of Trf^{s11} and control flies was studied by introducing a gene fusion coding for the EXU/GFP fusion protein which provides a fluorescent marker in the cytoplasm of these cells. The *exu* gene, originally identified and studied due to its importance in oogenesis, also has a critical function in the male germ line. Eight of the nine *exu* alleles which have been tested cause sterility in males when homozygous, and immunohistochemistry experiments have shown EXU to be first expressed in the enlarged primary spermatocytes of the germ line and not in the somatic cells of the testis. The *exu/Gfp* fusion contains all the *cis*-regulatory sequence required for proper transcription regulation so that the EXU/GFP fusion protein is expressed in the same pattern as endogenous EXU in the testis, being localized to the cytoplasm of the spermatocytes, and in fact can carry out the function of the endogenous protein as well (Wang and Hazelrigg, 1994).

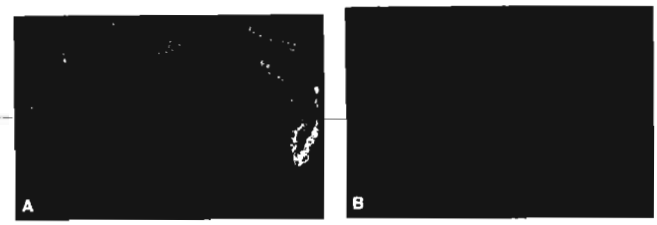

Figure 3. **A,B** Reduction in number of enlarged primary spermatocytes in the adult testis shown by fluorescence of the EXU/GFP marker. **A,** *w exu/Gfp*; **B,** *w exu/Gfp*; Trf^{s11}. The primary spermatocytes are 25 μm diameter spherical cells with a 12 μm diameter nucleus; only the cytoplasm is labeled with EXU/GFP resulting in a donut-like appearance in these photos. The apical end of the testis is in the lower right in each panel, and the variation in shape of the testes is due to the pliability of the tissue and not part of a mutant phenotype.

Confocal imaging of the fluorescently labeled live cells in whole testes reveals that Trf^{s11} reduces the number of enlarged primary spermatocytes to approximately 10% the wild type level (Figure 3), resulting in a cluster of these cells close to the apical end of the testis, but not extending nearly as far towards the basal end as in wild type. The size and shape of these spermatocytes, and the expression of one marker gene, *i.e. exu*, have not been disrupted by this mutation. The testes of the Trf^{s10} allele show a similar phenotype, although not quite as extreme as Trf^{s11} (data not shown).

The reduced number of enlarged primary spermatocytes in [w^{-}]*Trf* testes could be the result of a reduced number of mitotic divisions in the spermatogonial cells which arise from stem cell division, failure of the gonial cells to enlarge after the last (*i.e.,* fourth) mitosis, death of some spermatocytes early in this 90 h stage, or an early entry into meiosis of all the enlarged primary spermatocytes. To address the first possible explanation, a chromosome carrying a reporter construct

known to label the mitotic male germ cells was introduced into the $Trf^{\delta 11}$ genome, and testes from these flies stained to detect reporter expression. As shown in Figure 4, equal numbers of gonial cells are found at the apical end of control and $Trf^{\delta 11}$ testes, indicating that this mutation is not inhibiting mitosis during spermatogenesis.

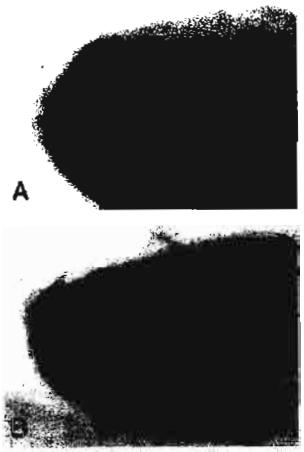

Figure 4. **A,B** Staining of mitotic cells in $Trf^{\delta 11}$ testis. **A,** Apical end of testis from a $S_3$46 adult, fixed and stained for reporter expression as described in Materials and Methods. **B,** Apical end of testis from a $S_3$46; $Trf^{\delta 11}$ adult. $S_3$46 is an X chromosome with an enhancer-trap P element insertion known to give expression in the spermatogonial cells (Gonczy *et al.,* 1992). The reporter gene codes for a fusion protein containing a portion of the P element transposase linked to beta-galactosidase, which results in the enzyme activity being localized to the nucleus of cells in which the reporter is transcribed (Bier *et al.,* 1989).

If the observation made with the enhancer-trap marker of the spermatogonial cells, that $Trf^{\delta 11}$ does not reduce the number of mitotic divisions during sperm development, is correct, then it is expected that each cyst of primary spermatocytes should contain 16 cells which have resulted from the four mitoses after stem cell division. To address this issue, individual cysts of enlarged primary spermatocytes in mutant and control testes were examined under higher magnification than was used previously for examining the entire testis. Again, the cytoplasm of these cells is labeled with the fluorescent EXU/GFP fusion protein; the results are shown in Figure 5. The fluorescence throughout the cytoplasm in these cells provides a clear outline of the nucleus, and by counting nuclei in the images, it is clear that the cysts in the mutant testis contain 16 cells just like the control. This observation indicates that $Trf^{\delta 11}$ does not inhibit the mitotic divisions, consistent with the conclusion drawn from the staining of mitotic cells. These mutant spermatocytes do appear to go through meiosis since orcein-staining of condensed chromosomes in metaphase I nuclei shows the typical set of bivalents, and the spermatids eventually elongate in bundles as in a wild type testis (data not shown).

The loss of eye pigment in the [w^r]*Trf* mutants indicates that at least a portion of the *white* gene has been eliminated from the P-lacW transposon, but provides no further information regarding the nature of these mutations. To establish exactly how much of the transposon and flanking genomic DNA are missing in each [w^r]*Trf* stock, genomic Southern blots were performed as described in Materials and Methods, the results of which are summarized in Figure 1. The original allele P[lacW]*Trf*, has an insertion which is close to the transcribed regions of three genes, but does not interupt any of the transcribed sequence. $Trf^{\delta 10}$ removes 3 kb from the center of P-lacW, while $Trf^{\delta 11}$ eliminates the same 3 kb missing in $Trf^{\delta 10}$ plus some adjacent sequence to give a 4 kb deletion overall. Neither deletion extends beyond the ends of P-lacW, so none of the flanking genomic DNA has been lost. The P[lacW]*Trf* male-sterile phenotype is probably due to alteration in *cis*-acting transcription regulatory sequence at the *Trf* site, and this effect becomes more severe when a portion of the transposon is removed.

The importance of the *Trf* cluster in *Drosophila* sperm development first became clear with the observation that males homozygous for a transposon insertion at this site are unable to produce functional sperm, *i.e.,* no motile sperm are present in the seminal vesicles of these animals. More detailed cytological studies and the generation and examination of more *Trf* mutant alleles traces the function of this gene cluster back to the premeiotic stages of spermatogenesis. As is often the case, a defect in production of a particular type of differentiated cell is due to events which have occured in precursor cells, and it is in these precursor cells where many important developmental decisions are made. In the *Drosophila* male germ line, regulated steps include: stem cell division which determines the rate at which cells enter the sperm development pathway, spermatogonial mitosis which affects the number of primary spermatocytes generated subsequent to each stem cell division, the halt of the cell cycle for 90 h in the primary spermatocyte to allow for the dramatic increase in cell volume, the entry into meiosis, the two meiotic divisions and the beginning of the elongation process in the postmeiotic early round spermatid. The first *Trf* allele, P[lacW]*Trf*, appears to prevent elongated spermatids from entering the seminal vesicles and acquiring motility without affecting regulation of the progression through the developmental stages, while the transposon-deletion alleles whose generation is described in this work are clearly affecting a regulatory decision before meiosis.

The observation that a *Trf* mutation reduces the total number of enlarged primary spermatocytes per testis without reducing the number of spermatogonial cells or primary spermatocytes per cyst, or affecting the production of elongated spermatid bundles, suggests that in this mutant the amount of time the male germ cells spend in the enlarged primary spermatocyte stage has been reduced. Apparently these mutant spermatocytes are entering meiosis before the typical 90 h timepoint after the last mitotic division.

How might alterations in the expression of the *Trf* cluster genes affect the regulation of the primary spermatocyte stage? To address this question the arrangement of the genes of the *Trf* Cluster and the structure and implied function of the encoded proteins must be considered. The insertion in P[lacW]*Trf* lies on one side of the *Trf* cluster, and the cluster is arranged with *Trf* in the middle, being transcribed away from the insertion site, while the most distal gene which produces an 8 kb mRNA, is transcribed towards the insertion. The sequence of the distal gene contains a coding sequence (CDS) whose hypothetical translation shows similarity to several proteins of the mammalian immune system: alpha$_2$Macroglobulin, a serum proteinase-inhibitor, and the complement proteins. The *Drosophila* protein is named Macroglobulin Complement-Related (MCR) and the gene *Mcr*. The transposon-insertion lies 2.8 kb downstream of the 3' end of *Mcr*, so the size of the mRNA transcribed from this gene assures that the transcription initiation site and promoter lie at least 10.8 kb away from the site of mutation in the various *Trf* alleles. This means that effects on *Mcr* expression are the least likely explanation for the observed phenotypes in the *Trf* mutants described in this work, which points to altered regulation of *Trf* and/or the gene most proximal to the insertion site, *Trf-proximal* (*Trfp*), as the probable explanation for the defective male germ cells. Since the TRF protein is a transcription factor, altered expression of *Trf* would probably result in some sort of effect on transcription in spermatocytes, while *Trfp* harbors a CDS for a protein of 252 residues which does not resemble any yet characterized, so it is impossible to predict the consequences of changes in its expression.

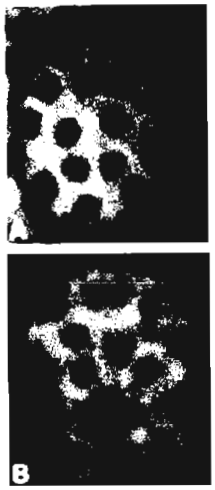

Figure 5. **A,B** Cysts of enlarged primary spermatocytes visualized with the EXU/GFP fluorescent fusion protein. **A,** A cyst from a *w exu/Gfp* testis. **B,** A cyst from a *w exu/Gfp* ; *Trf*^{s11} testis.

The phenotypes created by the *Trf* mutations discussed here are most likely due to a *cis*-regulatory transcription mechanism which is somehow different in these mutants than in wild type. There are several possible explanations for how the P-lacW insertion at the *Trf* site might alter transcription control of the three genes coded there, including: destruction of an endogenous enhancer or repressor by the insertion, displacement of a regulatory sequence (by 10.5 kb) to a point where it can no longer function properly, or fortuitous regulatory action of a sequence within the transposon. Some of the sequence within P-lacW is *Drosophila* sequence, but none of it is normally found at the *Trf* locus. This last possible mechanism for the *Trf* mutation effects is particularly relevant due to the observation that deletions internal to the transposon, *i.e.*, the [w^{r}]*Trf* alleles, result in appearance of a much more dramatic phenotype in the male germ line than was present in P[lacW]*Trf*. The finding that deletions of less than 50% of the transposon sequence generate a new phenotype, hints at the possibility that a sequence within the P-element construct is counteracting the effect of a second sequence within the element (not deleted in the [w^{r}]*Trf* alleles), or the disruption or displacement of an endogenous *cis*-acting sequence described above. Perhaps two effects on transcription of the *Trf* cluster, antagonistic to one another, occur in P[lacW]*Trf*, and when the sequence for one of these effects is removed by deletion as in [w^{r}]*Trf*, only one of the altered regulatory mechanisms remains and its effect is now much more pronounced.

Quantitative Northern blots of testis RNA with cDNA probes for each of the three genes will eventually show if in fact these mutations do affect transcription at this site; however, signals have not been obtained for any of the three genes in preparations from 50 pairs of testes although strong signals for the *exuperantia* transcript (Crowley and Hazelrigg, 1995) were detected on these filters. Apparently the *Trf* cluster RNAs are expressed at a very low level in this tissue and higher specific activity probes or more tissue will be needed to produce detectable signals.

Acknowledgments: I would like to thank Sheng Wang and Tulle Hazelrigg for providing the chromosome carrying *exu/Gfp*, and Stephen Dinardo for the S$_3$46 chromosome carrying the spermatogonial marker. Also, thanks to Bethany Slater for introducing the S$_3$46 chromosome into the *Trf*^{s11} stock.

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Breitmeyer, C., and G. Hocutt. Arizona State University, Tempe, AZ 85287. Alternative feeding sites in desert *Drosophila*: fly-ant interactions.

Host plant specificity has long been the cornerstone of our understanding of the basic ecology of the cactophilic *Drosophila* of the Sonoran Desert (Heed, 1978). Sonoran Desert *Drosophila* use specific host cacti necroses as sites for feeding and breeding. Oviposition and larval development take place within the necrotic tissue while the adults feed on the surface. In the case of one of these species, *D. pachea* (Patterson and Wheeler, 1942), a unique sterol found in the decaying tissue of its host *Lophocereus schottii* (senita), is actually required for successful larval development (Heed and Kircher, 1965).

One assumption made by many investigators is that the necrotic cactus tissue and its microbial flora are the sole nutritional source for the adult flies. However, this necrotic tissue contains many physiologically taxing secondary compounds and, therefore, may not provide an optimum source of nutrition. A source of free sugars, such as those produced by extrafloral nectaries would provide a much richer energy source as well as free amino acids at a lower metabolic cost (Baker *et al.,* 1978). Data on spatial resource availability indicates that rot distribution for these cactophilic species is patchy and for some species suitable substrates are rare (Breitmeyer and Markow, 1998). Sonoran Desert *Drosophila* have the ability to disperse over 2 km in a 24 hour period (Johnston and Heed, 1976; Breitmeyer, unpub.). Flights of this distance would require a significant expenditure of energy. Ganter, Starmer, Lachance and Phaff (1986) hypothesized that non-cactus food sources may be used by dispersing flies. Until now there have been no previous reports of cactophilic *Drosophila* utilizing alternative feeding sites. Here we present observational data indicating that alternative food sources are available to, and are utilized by, at least some of these cactophilic *Drosophila* species.

We observed flies of two species, *D. pachea* and *D. arizonae* (Ruiz, Heed and Wasserman, 1990), feeding at the extra floral nectaries of senita cacti during two separate periods in October 1995 and May 1996 near San Carlos, Sonora, Mexico. Observations were made at each cactus for approximately 10 minutes. Flies were concentrated on the terminal end of a single cactus arm in groups of 5-10 individuals, and these clusters were always associated with a necrosis found on another arm of the same cactus. The observations of *D. arizonae* occurred where its preferred host, *Stenocereus alamosensis*, is absent from the local area. *Drosophila arizonae* is the sister species of the Sonoran desert endemic *D. mojavensis*, both of which are considered generalists. Observed along with the feeding *Drosophila* were large ants of the Genus *Pseudomyrex*. Ants in this genus have been reported to have an association with both barrel and prickly pear cactus nectaries (Pickett and Clark, 1979; Ruffner and Clark, 1986). These ants associated with the extrafloral nectaries of senita were observed to prey upon the *Drosophila* and appeared to be tending the extra-floral nectaries, perhaps feeding on them or promoting nectar flow to bait in more *Drosophila* prey. This behavior is an ant actively chasing a fly found near the nectaries.

With the exception of one cactus, *D. arizonae* were more numerous than *D. pachea* (Table 1). While these numbers are not large they are comparable to the numbers of *D. pachea* aspirated from cactus rots during the same time period. Population sizes of *D. pachea* have been estimated by mark-recapture to be as low as 15-20 individuals (Breitmeyer and Markow, 1998). The individuals observed feeding at the nectaries may account for 5 –20 percent of the *D. pachea* population at a given plant. Additional *Drosophila* have been observed feeding at the nectaries since the initial discovery, but their numbers have not been quantified. When flies were observed at nectaries they were never more than 30 cm away, unless being pursued by an ant. The *Drosophila* did not resort to aerial escape immediately and pursuits lasted 15-30 seconds.

Ant behavior was distributed evenly over the three categories (Table 2). Table 2 does not reflect the amount of time spent in each activity. Although no direct measurements of time spent in each behavior were made, approximately 90 percent of ant behavior was split between tending and guarding with short bouts of pursuit. The prescence of a fly near the nectary did not always illicit a pursuit response. A few *Drosophila* were able to feed successfully and escape unmolested. Two ants were observed with flies in their mouthparts and were scored as pursuing.

Discussion: Because of its ability to detoxify Senita sterols and its unique dependence on one of them for development, *D. pachea* is considered the extreme specialist of the four Sonoran desert endemic *Drosophila*. These data represent the first confirmed use of an alternative feeding site for *D. pachea*, demonstrating that at least one species of cactophilic *Drosophila* is not strictly dependent on the host plant for all its nutritional requirements. The absence of the preferred host of *D. arizonae* from the local area of our observations indicates that this species may use extrafloral nectaries during dispersal. It is reasonable to expect that *D. mojavensis*, the sister species, has these same resources

Table 1. Observations of *Drosophila pachea* and *Drosophila arizonae* feeding at the terminal extrafloral nectaries of six senita cacti.

Senita #	*D. pachea*	*D. arizonae*
1	4	2
2	2	7
3	4	6
4	1	5
5	2	3
6	2	3

Table 2. Number of ants observed and their primary behaviors at extrafloral nectaries of senita cacti. Individual ants were classified as tending or guarding based upon the amount of time spent engaging in this activity, although they may have engaged in both behaviors.

Senita #	Number of Ants	Tending	Guarding	Number of Pursuits
1	2	2	0	0
2	1	1	0	2
3	2	0	2	0
4	2	0	2	2
5	1	1	0	1
6	2	1	1	0

available to it during dispersal. In addition, pollenia have been observed in the mouthparts of *D. nigrospiracula* (Polak and Markow 1998). Members of our lab have observed what appears to be juice from saguaro and/or *Opuntia* fruit in the abdomens of *D. mojavensis*, *D. nigrospiracula*, and *D. mettleri*. These findings raise questions regarding our knowledge of the basic ecology of the Sonoran Desert *Drosophila*. Breitmeyer and Markow (1998) demonstrated that there is an annual lack of utilization of host plants by flies in August. One hypothesis, suggested by the observations reported here, is that the *Drosophila* are able to utilize alternative food sources and, thus, survive whatever conditions make the host plants unsuitable during the summer months. It should be noted that the observation of these flies at the nectaries occurred during a period when there were less than the usual number of rots available. Perhaps, these flies are not consistently observed feeding at the nectaries at other times due to a high risk of predation. During periods when resources are depleted *Drosophila pachea* may be forced to feed at these alternative sites.

These findings also have implications for dispersal in these flies. The ability to use alternative food sources would greatly enhance the chances of these flies to survive long enough to locate suitable necroses for mating and oviposition. The other cactophilic *Drosophila* endemic to the Sonoran Desert often face large distances separating suitable hosts. Understanding the role that alternative hosts play in aiding dispersal will allow us to answer questions about gene flow in all four of these species. In addition, the use of alternative feeding sites may be one explanation for the apparent disappearance of these flies from July through early October. Our findings do not challenge the concept of host cactus specificity but rather the degree of dependence of each *Drosophila* species on actual necroses for their nutritional requirements. Further investigation into the feeding behavior and physiology of all of the Sonoran Desert *Drosophila* should be pursued. In addition, these cactus-fly-ant interactions merit further investigation.

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Ivanov, Yu.N. Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Sciences, Novosibirsk, 630090, Russia. FAX: (3832) 35 65 58. E-mail: ivanov@bionet.nsk.ru. Estimation of the number of spontaneous dominant lethal mutations in the genome of the fruit fly *Drosophila melanogaster* by the sex ratio.

The most noticeable consequences of induced mutagenesis in males of *D. melanogaster* are 1) an increased death rate of zygotes in their progeny and 2) a shift of the sex ratio among surviving flies in the direction of prevalence of males (Hadorn, 1961). The two phenomena may serve for estimation of the number of dominant lethal mutations (DLM) in the genome. The average number of spontaneous DLM in the whole genome (autosomes + X-chromosome) calculated by us by zygotes' survival is 0.0223 ± 0.0062 (Ivanov, 1998). However, the reliability of any measurement is determined by its replicability. That is why, apart from indicating the error of the measured quantity, which is although an indispensable yet a conditional formality, one has to try to check whether the given result can be obtained by another independent method. In the given work a method of quantitative estimation of DLM in *D. melanogaster* by sex ratio is described, and results of its application obtained in two similar experiments are presented, which has served both for checking and specifying the mean number of DLM in the genome calculated earlier by zygotes' survival. The importance of DLM studies for understanding of the time course and role of mutagenesis in nature has also been demonstrated.

Let us define the sex ratio r as a ratio of the number of females to the number of males among the surviving flies. When the males are treated with a mutagen (irradiation or chemical agents), the X spermia that give origin to females and contribute to the zygote more genetic material than the Y spermia (giving rise to males), do contribute at the same time more lethal injuries, which accounts for the elevated death rate of females. Y spermia contain a heterochromatin Y chromosome whose even complete loss does not affect the zygotes' viability; that is why injuries of the Y chromosome do not bring lethal factors into the zygote, and males have a lower mortality from DLM than females do. This is valid both for induced and for spontaneous mutagenesis, because in both cases a males zygote obtains from the father on the average only (1 - s)th part of the injuries obtained from the father by a female, if s designates the fraction of X chromosome genes in the whole genome. That is why the progressive decrease of the sex ratio accompanying the increase of DLM frequency or mutagen dose seems surprising at the first glance and requires a quantitative analysis for clear understanding. As it often happens, considering the extreme cases makes things clearer. A qualitative explanation of the phenomenon consists in the fact that at extremely high mutagen doses the females may practically disappear, while the males still remain, and then the r will really decrease to zero. Consequently as the number of DLM increases, the sex ratio r must drop from approximately 1 (in spontaneous mutagenesis) to zero, which is really the case (Hadorn, 1961). A quantitative analysis of the phenomenon is made herein below. The law according to which r decreases will be established as a by-product of the present work, i.e. analytical dependencies of the sex ratio r on the number Ã of DLM in the genome and on the dose D of irradiation of the male parents - $r(\tilde{A})$ and $r(D)$ - will be obtained.

Let n_f be the number of females, and n_m the number of males among surviving flies, A - the average number of DLM in the whole genome (autosomes + X chromosome), a - the mean number of recessive lethal (RLM) and visible (VM) mutations arising in the X chromosome, and u - the incidence of RLM and VM in the X chromosome due to spontaneous mutagenesis. Let the symbols with a tilda be respective values when the male parents are irradiated, as, e.g., r and $\tilde{r}$ are sex ratios without and with irradiation of male parents, respectively. Let N_f be the initial number of females in the culture, i.e. their number at the egg stage at the moment of fecundation; R_f be the accidental mortality of females, i.e. their fraction dead from any other factors except DLM; N_m and R_m be the initial number of males in the culture and their accidental mortality, respectively; $r_o = N_f / N_m$ be the initial sex ratio; $s = 0.19$ be the fraction of genes of the X chromosome in the whole genome (Ivanov, 1998); and e be the base of natural logarithms.

Let us deduce some relations from which, by means of substituting empirical values, we shall find the average number of DLM in the whole genome.

Let us find the sex ratio $r = n_f : n_m$ among the surviving flies in the culture originating from non-irradiated male parents. The fraction of survivors among the daughters of non-irradiated male parents is equal to the product of the probability of a female not dying from spontaneous DLM by the probability of her not dying from accidental causes. The former probability is e^{-2A} where $2A$ is the mean number of spontaneous DLM in the female's zygote. The latter probability is $1 - R_f$. Hence the fraction of surviving daughters is equal to $e^{-2A}(1 - R_f)$, and their number among imagines in the culture originating from non-irradiated male parents is $n_f = N_f e^{-2A}(1 - R_f)$.

Similarly, the fraction of survivors among the sons of non-irradiated male parents is equal to the product of the same probabilities for a male, the former of which is now equal to $e^{-(2-s)A}$, and the latter $1 - R_m$. Hence the number of surviving males among imagines in the culture without irradiation is $n_m = N_m e^{-(2-s)A}(1 - R_m)$.

The sought sex ratio among the surviving flies in the culture without irradiation is

$$r = \frac{n_f}{n_m} = \frac{N_f\left(1-R_f\right)}{N_m\left(1-R_m\right)}e^{-sA} = r_o\frac{1-R_f}{1-R_m}e^{-sA}. \qquad (1)$$

The sex ratio $\tilde{r} = \tilde{n}_f : \tilde{n}_m$ among surviving imagines in the culture originating from irradiated male parents is found in a similar way. Now the number of surviving daughters is $\tilde{n}_f = N_f e^{-\left(A+\tilde{A}\right)}\left(1-R_f\right)$, since the probability of a female not dying from DLM is already calculated from their mean number in the female zygote, which is $A + \tilde{A}$. The number of surviving sons in the culture is now $\tilde{n}_m = N_m e^{-\left[A+(1-s)\tilde{A}\right]}\left(1-R_m\right)$, where the probability of a male not dying from DLM is calculated from their mean number in the male zygote which is $A + (1 - s)\tilde{A}$.

The sought sex ratio among the surviving flies in the culture from irradiated male parents is

$$\tilde{r} = \frac{\tilde{n}_f}{\tilde{n}_m} = \frac{N_f\left(1-R_f\right)}{N_m\left(1-R_m\right)}e^{-s\tilde{A}} = r_o\frac{1-R_f}{1-R_m}e^{-s\tilde{A}}. \qquad (2)$$

Assuming the initial sex ratio r_0 and the sexes' mortality R_f and R_m to be equal in all the cultures, for which appropriate conditions have to be observed, one may exclude these unknown quantities from the equations. By means of term-by-term divisions of equations (1) and (2), we will obtain an equation with only two unknown quantities A and $\tilde{A}$:

$$\frac{r}{\tilde{r}} = e^{s\left(\tilde{A}-A\right)}. \qquad (3)$$

The incidence u of spontaneous RLM and VM in the X chromosome is measured as the probability of at least one such mutation arising in it and is equal to the difference between unit and the probability of no such mutation arising in the X chromosome. The latter probability is found from Poisson distribution with the parameter a and is equal to e^{-a}, whence

$$u = 1 - e^{-a}. \qquad (4)$$

In quite the same way the incidence $\tilde{u}$ of RLM and VM in the X chromosome of irradiated male parents is found:

$$\tilde{u} = 1 - e^{-\tilde{a}}. \qquad (5)$$

The number of DLM in the genome is proportional to that of RLM and VM arising in the X chromosome. Therefore we have a proportion

$$\frac{A}{\tilde{A}} = \frac{a}{\tilde{a}}. \qquad (6)$$

Equations (3) - (6) form a system with unknown quantities A, $\tilde{A}$, a, and $\tilde{a}$ whose solution will give us expressions for A and $\tilde{A}$ through $s = 0.19$ and empirically found r, $\tilde{r}$, u, and $\tilde{u}$:

$$A = \frac{\ln\frac{\tilde{r}}{r}}{s\left[1-\frac{\ln\left(1-\tilde{u}\right)}{\ln\left(1-u\right)}\right]}; \quad \tilde{A} = \frac{\ln\frac{r}{\tilde{r}}}{s\left[1-\frac{\ln\left(1-u\right)}{\ln\left(1-\tilde{u}\right)}\right]}. \qquad (7)$$

Due to independence of the empirical quantities each of which is measured in a separate experiment, the error of each of the calculated quantities A and $\tilde{A}$ is found from the dispersion of respective function of independent variables which (dispersion, not the function) can be easily calculated by arguments' dispersions.

The conditions for which the calculations have been done and the final expressions of (7) determine completely the organization of the experiment in which the mean number of DLM in the genome is found from the sex ratio. Two experiments were made: at γ-irradiation doses of 1500 and 2500 r. Each experiment was made as follows. Two groups of males from the Canton-S population were taken: 1) non-irradiated and 2) irradiated with a preset dose of γ-rays. Both were crossed with wild type (+) females from the Canton-S population and simultaneously, in the same tubes, with M5 (Basc) females. In the progeny of (+) females the sex ratio was estimated among the surviving flies: r in variant (1), without irradiation, and $\tilde{r}$ in variant (2), with irradiation. In crosses with M5 females estimated was the incidence of RLM and VM in the X chromosome of spermia: u in variant (1) and $\tilde{u}$ in variant (2). The general scheme of the experiment is shown diagrammatically in Figure 1.

Intact and irradiated males were taken in a number of 300 each; 4 - 6 males, 2 (+) females and 2 - 5 M5 females were placed in each tube containing currant medium in which crossing was performed. They were kept together for 24 hours whereafter (+) and M5 females were transferred to tubes with enriched medium and placed separately, two (+) females or three M5 females in each tube. In order to increase the number of cultures in which the sex ratio was counted, the tubes with currant medium where the flies had mated also served for counting, since the females had already laid

rather many eggs. The incidence of sex-linked mutations in the offspring of M5 females was estimated on the following conditions. Taken into account were only those F_2 cultures in which there were no less than 10 pupae. The RLM included both lethal and semilethal, i.e. mutations that reduced the number of males to 0 - 20% of the expected one. The expected number of non-M5 males was taken as 1/3 of all the other flies in the given F_2 culture.

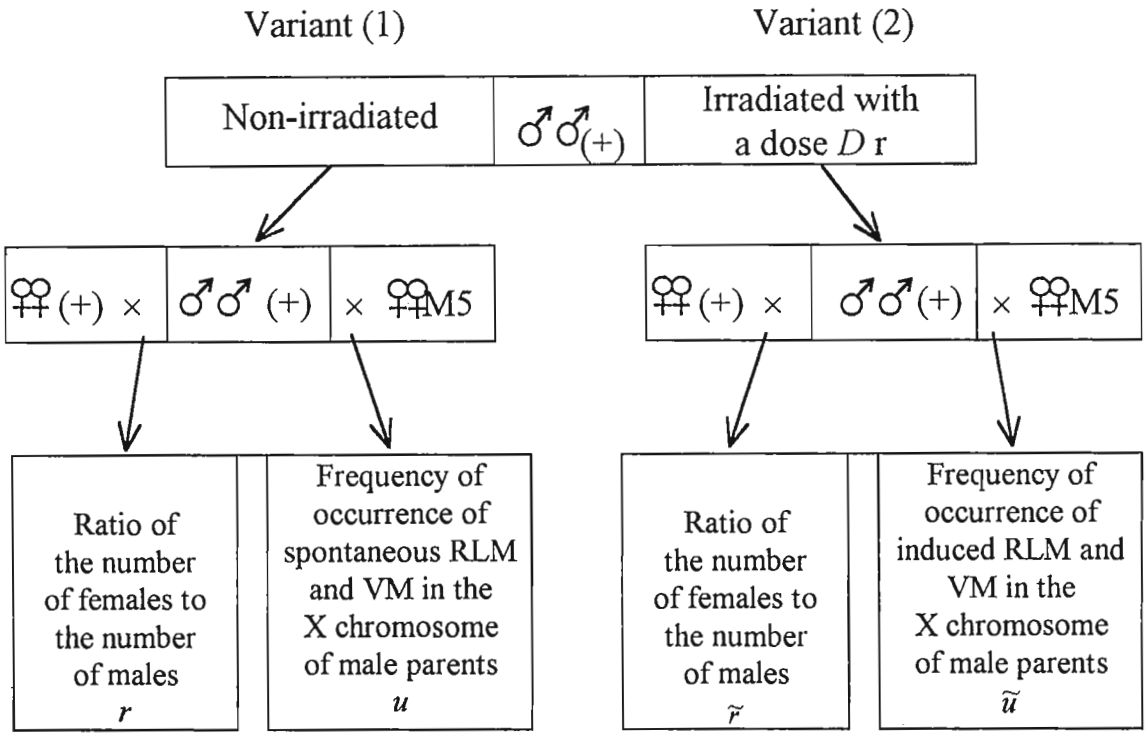

Figure 1. A scheme of experiment on estimation of the number of spontaneous genic ELM in the genome of *D. melanogaster* by sex ratio.

The results, according to ordinal numbers of the experiments, are presented in Tables 1 and 2. In the 1st experiment at an irradiation dose of 1500 r there was no significant shift in the sex ratio, and the error of the mean number of spontaneous DLM in the genome turned out to be the same as the number itself (0.0147 ± 0.0148). In the 2nd experiment at a dose of 2500 r the shift in the sex ratio was larger and became significant at $\alpha = 0.05$, while the error of the mean number of DLM in the genome decreased (0.0178 ± 0.0106). In Table 3, expansion of the dispersion of A value in each experiment into contributions corresponding to dispersions of separate independent variables from which A was calculated is presented. As the dose increased (experiment 2), the contribution of the dispersions of sex ratios r and $\tilde{r}$ to the error of the A value decreased noticeably. Whereas in the 1st experiment their total contribution was as high as 90%, in the 2nd experiment it decreased to 64%. At the same time, the dispersion S_A^2 also decreased 2-fold. It seems that one could, without any risk, increase the dose to 5000 r, and the accuracy of the experiment would have been still higher.

The new values of the mean number of DLM in the genome - 0.0147 and 0.0178 - do not disprove the former value of 0.0223 calculated by the zygotes' survival, especially if one takes into account the considerable errors that decrease their weight. All the three values of A may be used for its specification, averaging them with inverted weights of their dispersions according to the formula

$$A = \frac{\sum_i \frac{A_i}{s_i^2}}{\sum_i \frac{1}{s_i^2}} \pm \sqrt{\frac{1}{\sum_i \frac{1}{s_i^2}}}, \qquad (8)$$

where A_i is the value of A in the i-th experiment, and s_i^2 is the estimate of dispersion of A_i.

Table 4 contains the results of estimation of the mean number A of spontaneous DLM in the genome in all the three experiments and an averaged estimate of the A value obtained from formula (8).

Table 1. Estimation of the number of DLM in the whole genome (autosomes + X chromosome) by sex ratio at a γ-irradiation dose of 1500 r (experiment 1)

Experiment conditions	Experimental data: Number of females	Number of males	Mean value of $r = n_f / n_m$ in culture	Number of cultures	Mutability in X chromosome of male parents (%) u	Gamete sample size	Calculated values: Mean number of RLM and VM in X chromosome a	Mean number of DLM in the genome A
With irradiation of male parents	8204	8343	1.0169 ± 0.0222	120	3.553 ± 0.672	760	0.03617	0.1723 ± 0.1640
Without irradiation	10392	10390	1.0478 ± 0.0191	119	0.309 ± 0.073	5828	0.00309	0.0147 ± 0.0148

Table 2. Estimation of number of DLM in the whole genome (autosomes + X chromosome) by sex ratio at a γ-irradiation dose of 2500 r (experiment 2)

Experiment conditions	Experimental data: Number of females	Number of males	Mean value of $r = n_f / n_m$ in culture	Number of cultures	Mutability in X chromosome of male parents (%) u	Gamete sample size	Calculated values: Mean number of RLM and VM in X chromosome a	Mean number of DLM in the genome A
With irradiation of male parents	4845	5104	0.9957 ± 0.0231	122	7.023 ± 1.480	299	0.07282	0.3759 ± 0.1790
Without irradiation	7199	7119	1.0658 ± 0.0241	122	0.345 ± 0.089	4353	0.00345	0.0178 ± 0.0106

Table 3. Contribution of dispersions of $\tilde{r}, r, \tilde{u}$, and u to the dispersion $s_{\tilde{A}}^2$ of the mean number of DLM in the genome in 2 experiments

Year and γ-ray dose	Contribution to $S_{\tilde{A}}^2$	$S_{\tilde{r}}^2$	S_r^2	$S_{\tilde{u}}^2$	S_u^2	$S_{\tilde{A}}^2 \times 10^6$
1979	Absolute x 10^6	115.7	80.7	9.7	14.4	220.5
1500 r	%	53	37	4	6	100
1982	Absolute x 10^6	36.9	35.0	16.7	23.3	111.9
2500 r	%	33	31	15	21	100

Table 4. Results of estimation of the number of spontaneous DLM in the genome of *D. melanogaster* in 3 experiments

Estimation method	Mean number of DLM in the genome A	Dispersion $S_{\tilde{A}}^2$
By zygotes' survival	0.0223 ± 0.0062	38.1×10^{-6}
By sex ratio, dose 1500 r	0.0147 ± 0.0148	220.5×10^{-6}
By sex ratio, dose 2500 r	0.0178 ± 0.0106	111.9×10^{-6}
Average	0.0204 ± 0.0050	25.2×10^{-6}

Table 5. Dependence of the number of DLM in the genome of *D. melanogaster* on the γ-ray dose in 3 experiments

Experiment and DLM recording method	Dose (r) D	Mean number of DLM in the genome $\tilde{A}$	Dispersion $S_{\tilde{A}}^2$
All the experiments	0	0.0204 ± 0.0050	25.2×10^{-6}
1976, by zygotes' survival	1500	0.3559 ± 0.0467	2184.5×10^{-6}
1979, by sex ratio	1500	0.1723 ± 0.1640	26886×10^{-6}
1982, by sex ratio	2500	0.3759 ± 0.1790	32037×10^{-6}

The number of DLM in irradiated genome was counted in all experiments, i.e. for various irradiation doses. The results are presented in Table 5 as a dependence of the mean number $\tilde{A}$ of DLM in the complete genome on the dose D of γ-rays in roentgens. According to data of all the three experiments, by the method of least squares with weights, an approximation of this dependence was obtained:

$$\tilde{A}(D) = 0.02048 + 0.0002035D. \quad (9)$$

It permits calculating, by the found $\tilde{A}$ value, the mortality of zygotes from DLM at the given irradiation dose for all the cases when 1) both sexes, 2) only males, or 3) only females are irradiated. It is quite easy to obtain respective formulae.

Let us come back to analysis of the shift in the sex ratio accompanying the increase of the number of DLM or of the mutagen dose. The law according to which this shift occurs has in fact been found by us herein above. Assuming in equation (2) $r_0 \dfrac{1-R_f}{1-R_m} = K$, we have $\tilde{r} = Ke^{-s\tilde{A}}$, whence, designating $\tilde{r}$ as $r(\tilde{A})$, we obtain a decreasing exponential relation

$$r(\tilde{A}) = Ke^{-s\tilde{A}}, \quad (10)$$

which tends asymptotically to zero.

The dependence of the number of DLM on the irradiation dose is a linear function

$$\tilde{A} = A + kD, \quad (11)$$

where k is a coefficient of proportionality or increment of the mean number of DLM in the genome per 1 r irradiation. By substituting the expression (11) into equation (10) we obtain:

$$r(D) = Ke^{-s(A+kD)} = Ke^{-sA} \cdot e^{-skD}.$$

Designating here $Ke^{-sA} = \rho$ and $sk = \alpha$, we obtain again a decreasing exponential function

$$r(D) = \rho e^{-\alpha D},$$

where ρ is the sex ratio without irradiation of male parents. One has to note that the sex ratio does not depend on the irradiation of females; therefore, as it is easy to see in deducing equation (2), the dependence of r on $\tilde{A}$ or D is irrespective of whether we irradiate only males or also females.

We found dependencies of the sex ratio r on the mean number $\tilde{A}$ of DLM in the genome and on the irradiation dose D in a most general form where K, ρ, and α are positive constants whose values in each concrete case should be selected by the least squares method. So, having treated the data of Catcheside and Lea (1945) (Hadorn, 1961) on dependence of r on the X-rays dose by the least squares method, we found it as a function $r(D) = 0.9973e^{-0.00004466D}$. The constant s which for *D. melanogaster* is equal to 0.19 (Ivanov, 1998a), in other species has different values, and then it also requires an experimental estimation. In the absence of sex chromosomes in the genome or when these are indistinguishable from autosomes, which in this context is the same, the fraction of X chromosome in the genome is $s = 0$; therefore the relation (10) degenerates into a constant $r(\tilde{A}) = K$, i.e. r ceases to depend on the number $\tilde{A}$ of DLM in the genome and cannot serve for its estimation. In this way, the presence of heterochromosomes and large X chromosome in *D. melanogaster* is very favourable to measurement of the number of DLM in the genome of this species, which we used in the present work.

Estimation of the number of DLM in the genome acquires a special importance in connection with the fact that mutagenesis plays a regulatory role in the ecosystem: there are data on dependence of mutability in *D. melanogaster* and probably other species on the population density, so that at a higher density it is also higher, and vice versa. A heightened mutability brings about an increased mortality and thereby limits the species' population density, when this is advantageous for the ecosystem (Ivanov and Ivannikov, 1997). Those who do not know the role of DLM in spontaneous mutagenesis usually doubt that the mutagenesis can bring about a high and, what is the most important, an immediate death of zygotes. It seems that mutations must first be accumulated and only after this will they become homozygotized and entail a considerable increase of mortality. However, in fact even at a low level of mutability, when, e.g., in X chromosome RLM and VM arise at a rate of $u = 0.3\%$, DLM bring about death of over 6% of zygotes.

In order to get convinced thereof, one has to know the dependence of zygotes' mortality on DLM in the population on the incidence of RLM and VM in the X chromosome which can be found very simply from decomposition of the process of mutagenesis into the main mutation types (Ivanov, 1991; Ivanov, 1998a). According to our results, the spontaneous mutagenesis in *D. melanogaster* may be decomposed into the following types of mutations and their proportions: DLM make up 68%, RLM 29%, and VM 3% of all the spontaneous mutations arising in the genome and recorded in usual experiments on measurement of mutability. Those mutations whose detection requires special method, e.g. mutations of sterility, inversions, translocation, duplication, etc., are detected by no less than an order more seldom than VM - the rarest of the main mutation types; they may therefore be ignored in this case. The zygotes' mortality from DLM in the population at a primary sex ratio of 1:1 is $S(A) = 1 - e^{-(2-s/2)A}$, or, at $s = 0.19$,

$$S(A) = 1 - e^{-1.905A}. \qquad (12)$$

Designating the incidence of RLM and VM in the X chromosome as u_1, we may write an expression for the incidence of these mutations in the whole genome as $u_1 / 0.19 = 5.26u_1$. From the decomposition of the spontaneous mutagenesis given here, a proportion $A / 5.26u_1 = 68 / 32$ follows, whence $A = 11.2u_1$. Substituting this expression of A through u_1 into formula (12), we obtain the dependence of the mortality caused by DLM on the incidence of RLM and VM in the X chromosome:

$$S(u_1) = 1 - e^{-21.3u_1}.$$

Expanding the exponent into a series and limiting ourselves to its first three terms, we obtain an approximation

$$S(u_1) = 21.3u_1 - 226.8u_1^2, \qquad (13)$$

which demonstrates that the zygotes' death from DLM is practically directly proportional to the mutability u_1 in the X chromosome, for u_1^2 is a sufficiently small number.

In a similar way we obtain the expression of the mortality from DLM in the population through the incidence u_2 of RLM and VM in chromosome 2. The fraction of chromosome 2 genes in the whole genome is 0.36 (Ivanov, 1998b). The incidence of RLM and VM in the whole genome is $u^2 / 0.36 = 2.78u_2$. From the proportion $A / 2.78u_2 = 68 / 32$ we obtain $A = 5.90u_2$. Substituting this expression into (12), we obtain a function

$$S(u_2) = 1 - e^{-11.2u_2},$$

whose approximation gives a practically directly proportional relation

$$S(u_2) = 11.2u_2 - 62.7u_2^2. \qquad (14)$$

Variations of mutability in the population are larger than those expected from the actual change of abiotic agents of spontaneous mutagenesis. They cannot be accounted for either by the change of cosmic rays flow, or by solar activity, or by chemical factors. If all these factors were responsible for the strong increases of mutability in populations of *D. melanogaster*, the same would simultaneously occur in other species, however, there are no confirmations thereof. According to data borrowed from C. Stern's textbook (1960), the dose that doubles the incidence of mutation in *D. melanogaster* is about 50 r, and a fly receives for the 1 month of its life, due to natural radioactivity background, a dose of no more that 0.01 r, so that the gametes produced by it get on the average 0.005 r. Let us calculate the fraction α of spontaneous mutability u which is referred to as the natural radioactivity background $D_\alpha = 0.005$ r, if the doubling dose is $D_1 = 50$ r. The incidence of mutations from the radiation background D_α is αu , and at the doubling dose D_1 the mutability is increased by a quantity u equal to itself. Due to the fact that the mutability increment is directly proportional to the dose increase, we have $\alpha u / u = D_\alpha / D_1$, whence $\alpha = D_\alpha / D_1$, which at our figures gives $\alpha = 0.0001$. In fact, this fraction is still smaller, since the calculation is made for an acute doubling dose, while the chronic doubling dose is higher than the acute one by about 4 times (Stern, 1960). Besides, the acute DLM-doubling dose calculated by our data from equation (9) is 100 r. It is clear that at such a small contribution of the natural radiation background to the spontaneous mutagenesis, even highly repeated changes of the cosmic rays level and of other natural radiation sources cannot have brought about the observed changes of mutability in nature. Hence a conclusion of biotic nature of the main factors of spontaneous mutability.

Numerous measurements of the frequency of occurrence of RLM show that it varies in the X chromosome from 0.05 to 1.1%, and in chromosome 2 from 0.3 to 1.27% (Dubinin, 1966). In our 45 measurements of the RLM and VM incidence in the X chromosome in natural populations and in Canton-S (1970 - 1993), it varied from 0 to 1.3%. The highest values of mutability exceed its usual level by 3 - 5 times. From the data presented here, by formulae (13) and (14), it is possible to estimate the highest values of zygotes' mortality from DLM in populations, i.e. those obtained from the highest values of mutability $\hat{u}_1 = 1.3\%$ in the X chromosome and $\hat{u}_2 = 1.27\%$ in chromosome 2: $S(\hat{u}_1) = 23.9\%$ and $S(\hat{u}_2) = 13.2\%$.

These not at all small values of mortality from DLM point to a considerable scope of its fluctuations and to these fluctuations being a most important consequence of mutability change in the population. If the mutability plays the role of a regulator of the species number, then its time course must be more or less correlated with that of population number, and therefore the results of measurement of mutability may not be extrapolated from some species to other. The mutability of each species has an independent time course of its own and, according to all data, is determined by biotic factors. Just like the selection, it has nothing to do with the biogenesis, but plays a regulatory role in the ecosystem. The selection as a repressive, and the mutability as a destructive, principles cannot be sources of transmutation (speciation), or else ecosystems could not have existed, but both are regulators of the biotic circulation. The mutation process, evolutionary by its nature, i.e. random, chaotic, and destructive, serves nevertheless the high goal of maintenance of order, constancy, and system in nature: it causes death for the sake of life.

Due to the fact that mutability must depend on the phase at which the population is during the fluctuations of the numbers so that at large numbers the mutability is higher and vice versa, otherwise it would not have been a regulatory factor, specification of the absolute number of DLM in the genome has by itself no great meaning. However, in connection with establishment of the regulatory role of DLM in populations, specification of their relative number in the genome among other main types of spontaneous mutations conserves its importance. It is especially important to study DLM in other species than *D. melanogaster* with different, contrasting, karyotype structures. According to our concept (Ivanov, and Ivannikov, 1997; Ivanov, 1998b), a non-adaptive karyotype structure, and namely concentration of the genome in a small number of large chromosome arms, a large fraction of non-coding DNA regions in the genome, terminal positions of euchromatin in the arms, etc., i.e. everything that increases the probability of disruptions of the chromosome thread or enhances their damaging effect, makes the genome vulnerable to chromosomal DLM and suggests that they play an important role in control of the species abundance. Nevertheless, however verisimilar the theoretical statements could be, they require factual corroboration. That is why a comparison of the relative numbers of DLM in the genome in species having karyotypes with sharply different numbers and sizes of chromosome arms is very interesting from the viewpoint of verification and specification of the ecological role of the mutation process as one of factors limiting the species population numbers.

To summarize, one may make the following conclusions:

1. A method of estimation of the mean number of spontaneous dominant lethal mutations (DLM) in the genome of *D. melanogaster* by the sex ratio is described, and its results obtained in two experiments at γ-irradiation doses of 1500 and 2500 r are presented: they are 0.0147 ± 0.0148 and 0.0178 ± 0.0106, respectively.

2. Due to the fact that these results do not differ from the value of 0.0223 ± 0.0062 obtained earlier by an independent method based on estimation of zygotes' survival, a generalized estimate of the mean number of DLM in the genome for three experiments equal to 0.0204 ± 0.0050 is presented.

3. It is demonstrated that the law according to which the sex ratio of the surviving offspring of irradiated males changes as the number of DLM or the irradiation dose increases is described by an exponential function of $y = ae^{-kx}$ type where a and k are positive constants.

4. It is shown that the contribution of the natural radiation background to spontaneous mutagenesis is too negligible for even multiple changes of the background being able to influence noticeably the mutability in nature; the idea that the time course of mutability in populations is determined by biotic factors, and most probably by the time course of the species abundance, is thereby confirmed.

5. Dependencies of zygotes' mortality from DLM in *D. melanogaster* population on the frequency of occurrence of recessive lethal and visible mutations in X chromosome and chromosome 2 are deduced, and it is demonstrated that the observed increases of mutability in populations of this fly can increase the zygotes' mortality to more than 20% by means of DLM. Due to this, DLM may be a very efficient factor limiting the species' population density.

6. The importance of a comparative study of the number of DLM, and especially their fraction in the spontaneous mutagenesis in species with karyotypes sharply differing in the number and sizes of chromosome arms, for verification and specification of our view on the role of mutations in the species' abundance control, is discussed.

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Ivanov, Yu.N. Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Sciences, Novosibirsk, 630090, Russia. FAX: (3832) 35 65 58. E-mail: ivanov@bionet.nsk.ru. Estimation of the number of genic dominant lethal mutations in the genome of the fruit fly *Drosophila melanogaster* using ethylmethane sulphonate.

A dominant lethal mutation (DLM) is any newly arisen mutation resulting in an immediate death of zygote in the very first generation. The studies carried out by Muller, Pontecorvo, Haldane and Lea, Demerec and Kaufmann, Catcheside and Lea, and especially McClintock's cytological data have shown that the mortality from DLM affects usually early stages of development (in *Drosophila* – the egg, larva, and rarely pupa) and that a considerable part of DLM represent breakages of chromosome arms with loss of their terminal acentric fragments, i.e. terminal deletions, in the course of subsequent divisions; therein, incorrect healing of the chromosome also happens when its sister chromatids join with their broken ends, due to which the cell division results in formation of a chromosomal bridge which is then broken at a random point, so that the daughter cells obtain unbalanced gene assortments (Hadorn, 1961). DLM have been studied mainly in induced mutagenesis where they are abundant, while the spontaneous mutagenesis remains so far not studied both with respect to the number of DLM and with respect to their nature. Difficulties arise due to the fact that the zygotes' mortality from DLM is directly indistinguishable from accidental death caused by other genetic agents and adverse environmental factors. This requires a rather long special experience. The interest for DLM seems to have abated also due to the fact that they are obviously deprived of any biogenetic meaning. The chromosomal mechanism of DLM has fascinated the audience so much that even doubts have been expressed as to whether single gene mutations could be dominant lethal factors. What fraction of all DLM is made up by the point, or genic DLM, remains yet a problem. In the present work a method for solving this problem on the fruit fly *D. melanogaster* using ethylmethane sulphonate (EMS) is proposed. This supermutagen is remarkable for the fact that, being nontoxic, it induces genic mutations without influencing the frequency of chromosome aberrations. It is just on these properties that the performance of the experiment described here is based. Besides, we also used the result of measuring the mean number of spontaneous DLM in the genome of *D. melanogaster* obtained by us earlier (Ivanov, 1998).

The experiment was as follows. Males of Canton-S population were divided into two groups: 1) untreated and 2) EMS-treated. The treatment was carried out as follows. A pinch of sucrose and 0.05 ml of an almost 100% EMS

solution were added to 20 ml of distilled water, stirred for better dissolvement of EMS in water, and several pieces of filter paper were soaked in the obtained solution in a hermetically closed bowl for 24 hrs. The EMS-solution-soaked pieces of filter paper were placed into several flasks, three in each; into each of the same flasks 100 ether-anesthetized males were placed in paper bags, kept there at 25°C for 24 hrs in a thermostate, whereupon crosses began to be carried out. Males of each group were crossed simultaneously, i.e. in the same flasks, with wild type (+) females from the Canton-S population and with M5 (Basc) females. In the progeny of (+) females, the survival of zygotes at the stages from egg to imago was studied, and so was the ratio of number of females to that of males among the surviving flies. The cultures in dismountable flasks where the zygote survival and the sex ratio were studied were kept under strictly equal conditions at 27°C in order that the accidental death rates were the same. In the progeny of M5 females, the incidence of recessive lethals (RLM) and visible mutations (VM) in the X chromosome of males with which they mated was measured by the well known technique. The general scheme of the work is shown in the Figure. Details of the experiment are described in our previous work, and the difference from it consists only in the fact that EMS, and not irradiation was used here as mutagen (Ivanov, 1998).

Let Q be the proportion of zygotes that survived at stages from egg to imago, r be the ratio of the number of females to that of males among the survived flies, u – incidence of RLM and VM in the X chromosome of male parents, A – the mean number of DLM in the whole genome (autosomes + X chromosome), A_1 – the mean number of genic DLM in the whole genome, and a – the mean number of RLM and VM arising in the X chromosome without EMS treatment of male parents; $\tilde{Q}$, $\tilde{r}$, $\tilde{u}$, $\tilde{A}$, $\tilde{A}_1$, and $\tilde{a}$ – the same quantities when the male parents are treated with EMS, respectively; R – the proportion of zygotes dying from accidental causes, R_f and R_m – accidental mortality of females and males, respectively; s – proportion of the genes of X chromosome in the genome.

Let us deduce some relations from which we will calculate the quantities we need, such as the mean number $\tilde{A}$ of induced DLM in the whole genome under the influence of EMS, the mean number A_1 of spontaneous genic DLM in the whole genome, etc.

The frequency Q of zygotes' survival without EMS treatment of male parents is equal to the product of the probability of no spontaneous DLM getting into the zygote by the probability of the zygote not dying from accidental

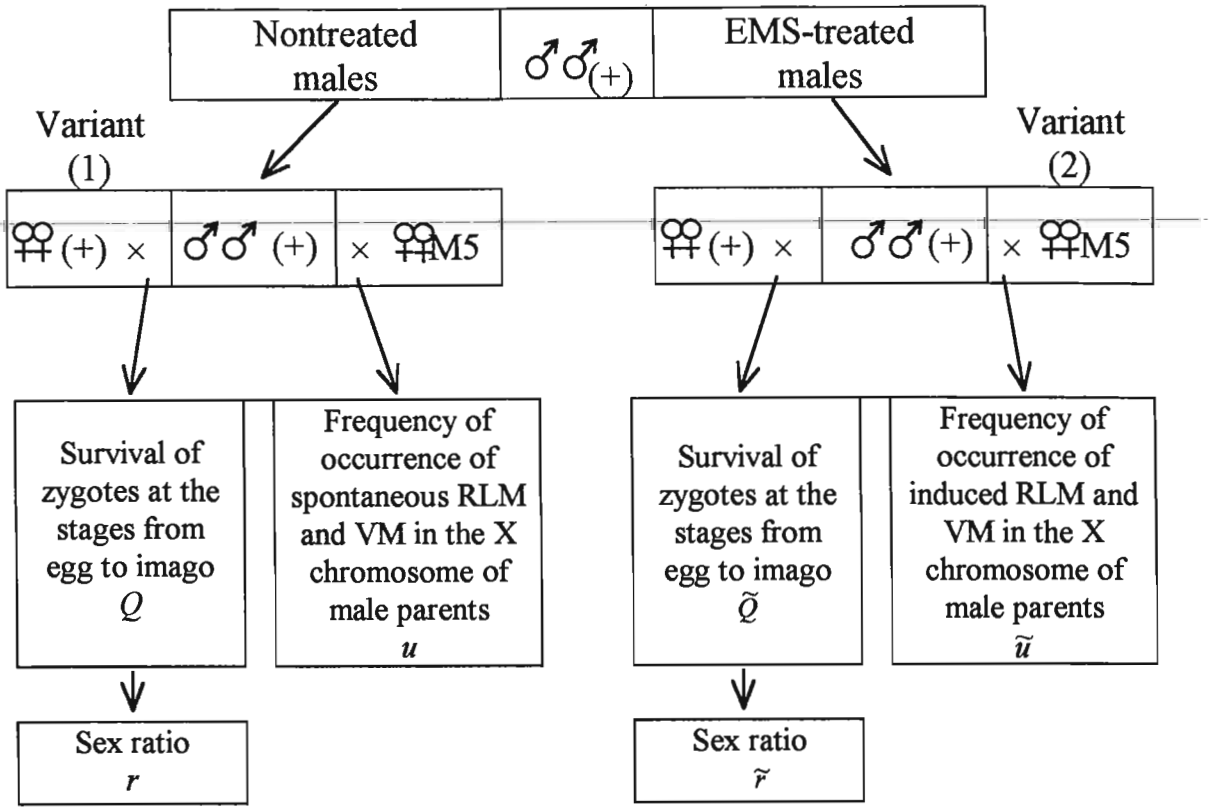

Figure 1. A scheme of experiment on estimation of the number of spontaneous genic ELM in the genome *of D. melanogaster.*

factors. If the mean number of DLM getting into the zygote is designated as X, then the former probability is e^{-X}. The number of whole genomes in the female's zygote is 2, and that in the male's zygote is 2 - s, because the male has, instead of another X chromosome, a genetically empty Y chromosome. Then an average, intersexual zygote, the sex ratio being 1:1, contains $(2-s/2)$ whole genomes, and the mean number of DLM in it is $X = A(2-s/2)$, whence the former probability is $e^{-X} = e^{-A(2-s/2)}$. The latter probability is $1 - R$. Hence

$$Q = e^{-A(2-s/2)}(1-R). \quad (1)$$

The frequency $\tilde{Q}$ of zygotes' survival when male parents are treated with EMS is found in a similar way. One of the genomes of the zygote now is treated with the mutagen, therefore the mean number of DLM in the zygote is $\frac{\left(A+\tilde{A}\right)+\left[A+\tilde{A}(1-s)\right]}{2} = A+\tilde{A}(1-s/2)$, where the first item of the numerator $(A+\tilde{A})$ is the mean number of DLM in the female zygote, and the second one $\left[A+\tilde{A}(1-s)\right]$ is the mean number of DLM in the male zygote. The probability of there being no DLM in an average zygote is $e^{-\left[A+\tilde{A}(1-s/2)\right]}$, and the sought expression is

$$\tilde{Q} = e^{-\left[A+\tilde{A}(1-s/2)\right]}(1-R). \quad (2)$$

Let us find the expression for the sex ratio $r = n_f : n_m$ in culture among the survived imagines without treatment of male parents with the mutagen. The number of female imagines is $n_f = N_f e^{-2A}\left(1-R_f\right)$, where N_f is the initial number of females, i.e. their number at the egg stage, and the product $e^{-2A}\left(1-R_f\right)$ is the fraction of the surviving females. The number of male imagines is $n_m = N_m e^{-A(2-s)}\left(1-R_m\right)$, where N_m is the initial number of males and $e^{-A(2-s)}\left(1-R_m\right)$ is the fraction of the surviving males. Hence

$$r = \frac{N_f e^{-2A}\left(1-R_f\right)}{N_m e^{-A(2-s)}\left(1-R_m\right)} = r_o \frac{1-R_f}{1-R_m} e^{-As},$$

where $r_0 = N_f : N_m$ is the initial sex ratio. In the same way the expression for the sex ratio $\tilde{r} = \tilde{n}_f : \tilde{n}_m$ in culture among imagines is found when the male parents are treated with EMS. Then the number of female imagines is $\tilde{n}_f = N_f e^{-\left(A+\tilde{A}\right)}\left(1-R_f\right)$, and the number of male imagines is $\tilde{n}_m = N_m e^{-\left[A+\tilde{A}(1-s)\right]}\left(1-R_m\right)$. Hence

$$\tilde{r} = \frac{N_f\left(1-R_f\right)}{N_m\left(1-R_m\right)} e^{-\tilde{A}s} = r_o \frac{1-R_f}{1-R_m} e^{-\tilde{A}s}.$$

The initial sex ratio r_0 and respective mortalities are assumed to be constant in all cultures, therefore the final expressions for r and $\tilde{r}$ through r_0 are applicable to sets of all the cultures from intact and EMS-treated males, respectively. Dividing r by $\tilde{r}$, we obtain an expression free from unknown r_0, R_f, and R_m:

$$\frac{r}{\tilde{r}} = e^{s\left(\tilde{A}-A\right)}. \quad (3)$$

The frequency u of the incidence of RLM and VM in the X chromosome of male parents is measured as the probability of the incidence of at least one such mutation in the X chromosome and is equal to the difference between unit and the probability of arising no such mutation. The latter probability is found from the Poisson distribution with a parameter a and is equal to e^{-a}, whence

$$u = 1 - e^{-a}. \quad (4)$$

Analogously, the incidence of the mutations in the X chromosome of EMS-treated male parents is

$$\tilde{u} = 1 - e^{-\tilde{a}}. \quad (5)$$

The assumption that the number of genic DLM in the genome is proportional to that of RLM and VM arising in the X chromosome will be written as a proportion

$$\frac{A_1}{\tilde{A}_1} = \frac{a}{\tilde{a}}. \quad (6)$$

From equation (1) and (2), by dividing one by another, we find that the mean number of DLM in the whole genome with EMS treatment is

$$\tilde{A} = A + \frac{1}{1-s/2} \ln \frac{Q}{\tilde{Q}}. \quad (7)$$

Here and herein below, $A = 0.0223 \pm 0.0062$ is the mean number of spontaneous DLM in the whole genome found by us in a special experiment (Ivanov, 1998).

Another independent estimate of $\tilde{A}$ is obtained from the data on the sex ratio, and namely from the equation (3):

$$\tilde{A} = A + \frac{1}{s}\ln\frac{r}{\tilde{r}}. \qquad (8)$$

For calculation of the mean number A_1 of spontaneous genic DLM in the genome let us transform expressions (1) and (2). Let us designate the mean number of chromosomal DLM in the whole genome as A_2, so that $A = A_1 + A_2$, and assume that $A_2 = \tilde{A}_2$, i.e. that the effect of EMS does not change the number of chromosomal DLM. Hence $\tilde{A} = \tilde{A}_1 + A_2$. It is obvious that if the genic DLM coincide with chromosomal ones, they are not recorded as genic, i.e. the sets of genic and chromosomal DLM do not intersect. Then equations (1) and (2) assume the form of

$$Q = e^{-(2-s/2)(A_1-A_2)}(1-R) \text{ and } \tilde{Q} = e^{-\left[A_1+(1-s/2)\tilde{A}_1+(2-s/2)A_2\right]}(1-R).$$

Supplementing the new expressions for Q and $\tilde{Q}$ with equations (4) - (6), and solving this system, we will obtain expressions for A_1 and $\tilde{A}_1$ through empiric values:

$$A_1 = \frac{\ln\frac{\tilde{Q}}{Q}}{(1-s/2)\left[1-\frac{\ln(1-\tilde{u})}{\ln(1-u)}\right]};\quad \tilde{A}_1 = \frac{\ln\frac{Q}{\tilde{Q}}}{(1-s/2)\left[1-\frac{\ln(1-u)}{\ln(1-\tilde{u})}\right]}. \qquad (9)$$

Since $\tilde{A} - A = \left(\tilde{A}_1 + A_2\right) - \left(A_1 + A_2\right) = \tilde{A}_1 + A_1$, equation (3) may be written as

$$\frac{r}{\tilde{r}}e^{s\left(\tilde{A}_1 - A_1\right)}.$$

Supplementing this with equations (4) – (6) and solving this system, we will obtain other, independent expressions for A_1 and A_1 deduced from the sex ratio:

$$A_1 = \frac{\ln\frac{\tilde{r}}{r}}{s\left[1-\frac{\ln(1-\tilde{u})}{\ln(1-u)}\right]};\quad \tilde{A}_1 = \frac{\ln\frac{r}{\tilde{r}}}{s\left[1-\frac{\ln(1-u)}{\ln(1-\tilde{u})}\right]}. \qquad (10)$$

Assuming that s = 0.19 and using the experimental data, in particular the earlier known value of A = 0.0223, we can now make calculations for all the deduced formulae (7) – (10). The error of any calculated quantity can be found from the dispersion of respective function of several independent variables, which can be easily calculated from the arguments' dispersions.

The results of the experiment are presented in Table 1. In section (a) experimentally measured and , in (b) calculated values and their standard deviations (errors) are given. Noteworthy is the difference between estimates of the number of DLM in the genome found by zygotes' survival from formulae (7) and (9), and by sex ratio from formulae (8) and (10). Tables 2 and 3 show decomposition of dispersion of the mean number A_1 of genic DLM in the genome into contributions corresponding to dispersions of single independent variables from which the A_1 value is calculated. From the Tables one can see that the estimate of dispersion of the A_1 value calculated by the sex ratio ($s^2_{A_1} = 1093.3\cdot10^{-8}$) exceeds by 2 orders the estimate of dispersion of this quantity calculated by zygotes' survival ($s^2_{A_1} = 973.8\cdot10^{-10}$) and that this is accounted for by the strong variability of the sex ratio. That is why the estimates obtained from the zygotes' survival are more efficient, and it is just these that were taken as the basis, since they are closer to real ones. The estimates obtained from the sex ratio are of some interest only because this method is checked here: in spite of its apparent simplicity, it requires rather large samples. In order to diminish the error of the number A_1 of genic DLM in the genome, we measured the spontaneous mutability in the X chromosome with a higher accuracy than it was possible by one measurement in the experiment with EMS carried out in 1977. Due to uniformity of data, we merged the results of 16 measurements of mutability in the X chromosome of males from the Canton-S population for the period of June 1973 to October 1981, increasing the size of the gamete sample to 17243 from 1637 in 1977.

Table 1. Estimation of the number of genic DLM in the whole genome (autosomes + X chromosome) of *Drosophila melanogaster* by the effect of EMS.

a) Experimental data

Experiment conditions	Mutability in X chromosome of male parents (%) u	Gamete sample size	Mean value of the fraction of survived zygotes in cultures Q	Number of cultures for estimation of survival	Mean value of the ratio of the number of females to that of males in cultures r	Number of cultures for estimation of sex ratio
With EMS treatment of male parents	34.971	346	0.7355 ± 0.0255	26	0.9852 ± 0.0678	26
Without treatment	0.203	17243	0.9414 ± 0.0075	24	1.1242 ± 0.1239	26

b) Calculated values

Experiment conditions	Mean number of RLM and VM in the X chromosome a	Estimation of the mean number of A of all DLM in the genome		Estimation of the mean number A_1 of genic DLM in the genome	
		by zygotes' survival	by sex ratio	by zygotes' survival	by sex ratio
With EMS treatment of male parents	0.43034	0.2949 ± 0.0398	0.717 ± 0.684	0.274 ± 0.040	0.698 ± 0.687
Without treatment	0.00203	—	—	0.00129 ± 0.00031	0.00330 ± 0.00331

Table 2. Contribution of dispersions of $\tilde{Q}$, Q, $\tilde{u}$, and u to dispersion $s^2_{A_1}$ of the mean number of genic DLM in the genome.

Contribution to $s^2_{A_1}$	$s^2_{\tilde{Q}}$	s^2_{Q}	$s^2_{\tilde{u}}$	s^2_{u}	$s^2_{A_1}$
Absolute x 10^{10}	331.1	17.6	142.2	482.9	973.8
%	34	2	15	49	100

Table 3. Contribution of dispersions of $\tilde{r}$, r, $\tilde{u}$, and u to dispersion $s^2_{A_1}$ of the mean number of genic DLM in the genome.

Contribution to $s^2_{A_1}$	$s^2_{\tilde{r}}$	s^2_{r}	$s^2_{\tilde{u}}$	s^2_{u}	$s^2_{A_1}$
Absolute x 10^{10}	295.0	757.8	9.2	31.3	1093.3
%	27	69	1	3	100

Table 4. Relative numbers of genic and chromosomal DLM in *D. melanogaster* in spontaneous mutagenesis and under the influence of EMS in the given experiment.

Experiment conditions	Mean number of DLM in the genome	Genic A_1	Chromosomal A_2	Total DLM A
Without EMS treatment	Absolute	0.0013	0.0210	0.0223
	%	6	94	100
With EMS treatment of males	Absolute	0.2740	0.0209	0.2949
	%	93	7	100

Data on the relative numbers of genic and chromosomal DLM in spontaneous mutagenesis and after EMS treatment of flies in our experiment are presented in Table 4. The mean number of chromosomal DLM in the genome was obtained as the difference $A_2 = A - A_1$ (or $\tilde{A}_2 = \tilde{A} - \tilde{A}_1$). Genic DLM made up about 6% of all spontaneous DLM, whereas with EMS treatment the proportion of genic DLM increased to 93% of the total number of DLM.

The hypothesis that EMS does not affect the chromosome aberrations, leaving their incidence at the spontaneous level, seems too strong. Let us consider the question of how the result of our calculations would change if the EMS effect

in our experiment increased the number of chromosomal DLM by k times, so that the number of induced chromosomal DLM would be equal to $\tilde{A}_2 = kA_2$, where $k \geq 1$. Let us calculate the A_1 value for this general case. We have equations $A_1+A_2 = A$ and $\tilde{A}_1 + kA_2 = \tilde{A}$. From the data of our experiment (Table 1) we have: $\tilde{A}_1 : A_1 = a : \tilde{a} = 211.8$, whence $\tilde{A}_1 = 211.8A_1$; $\tilde{A} = 0.2949$, and $A = 0.0223$. Substituting these numbers in our equations, we obtain a system of equations with unknown A_1 and A_2:

$$A_1 + A_2 = 0.0223;$$
$$211.8A_1 + kA_2 = 0.2949,$$

solving which we find:

$$A_1 = \frac{0.0223k - 0.2949}{k - 211.8}.$$

Considering A_1 as a function of k, we see that A_1 (1) = 0.00129 and that the derivative of this function

$$\frac{dA_1}{dk} = -\frac{4.428}{(k-211.8)^2} < 0$$

is negative at all values of k, i.e. A_1 (k) is a decreasing function of k. Hence it follows that at k = 1 we found the highest value for A_1 , and in this case the true A_1 value must be lower, while that for A_2 , on the contrary, higher than in our calculation.

However, the value of A_1 = 0.00129 evokes little doubt, since it finds its confirmation from population genetics, and if so, EMS retains its reputation as a mutagen which brings about only genic mutations without any disruption of the chromosome thread. In studies of *D. melanogaster* populations, at least at moderate latitudes, there exists a problem of elimination of lethal chromosomes 2 connected with the fact that the usual frequency of spontaneous incidence of RLM in chromosome 2 supplies the population with by an order more lethals than they are eliminated in compounds l_i / l_j due to identity, or allelism, when the lethal is homozygotized (Dubinin, 1966). The fraction of lethal chromosomes 2 is maintained relatively constant in the population, and therefore it is inevitable to hypothesize that autosomal RLM possess a certain level of dominance and are eliminated mainly due to penetrance in heterozygotes. Thereby the nature of genic DLM is confirmed: these are usual newly arisen RLM with a certain penetrance in heterozygotes. According to the experiment conditions they cannot be preexistent, because in this case the mortality caused by them would be equal in variants (1) and (2), and would be excluded as accidental.

The body of mathematics of the selection theory of Fisher, Haldane, and Wright permits estimating, with a certain degree of inevitable idealization, the mortality from genic DLM in a *D. melanogaster* population, and determining therefrom their number in the genome. Let the population be panmictic, i.e. zygotes be formed in it according to the Hardy – Weinberg rule; then the frequency of lethal chromosomes 2 in it will be q = 0.16; the incidence of spontaneous RLM in chromosome 2 will be u = 0.008; and the probability of allelism (loci identity) of lethals in compounds will be I = 0.004. The coefficient of selection against heterozygotes with respect to lethal chromosome 2 will be expressed through these quantities by an equation

$$s = 1 - \frac{q - u - p + \sqrt{(1-u)^2 - 4p(q-u)I}}{2q(1-I)},$$

where $p = 1 - q$. After substitution of numbers we obtain s = 0.0494. Let us now estimate the mortality from lethal chromosomes 2 on a set of zygotes not affected by chromosomal DLM. The total mortality of the zygotes from lethals in chromosome 2 is

$$S(l) = 2pqs + q^2\left[1 - (1-s)^2(1-I)\right].$$

It can be rather simply divided into 2 items: 1) mortality from preexistent lethals both in homozygotes and heterozygotes:

$$S(l_o) = \frac{q-u}{1-u}\left\{2ps + \frac{q-u}{1-u}I + q(1-I)\left[1-(1-s)^2\right]\right\},$$

and 2) mortality from newly arisen RLM acting mainly due to the dominant effect in heterozygotes and rarely due to allelism with preexistent lethals in compounds:

$$S(l_n) = \frac{pu}{1-u}\left\{2ps + \left(q + \frac{q-u}{1-u}\right)I + q(1-I)\left[1-(1-s)^2\right]\right\}.$$

The latter is just the mortality from genic DLM in chromosome 2. Substituting our numbers, we obtain:
$S(l) = 15.84 \bullet 10^{-3}$ (100%); $S(l_o) = 15.16 \bullet 10^{-3}$ (96%), and $S(l_n) = 0.68 \bullet 10^{-3}$ (4%).

A strict extrapolation of zygotes' mortality from genic DLM of chromosome 2 to the whole genome is made difficult by the fact that the pair of sex chromosomes has its peculiarities which we have to ignore for want of necessary data; that is why our results become rather rough. Let us assume sex chromosomes being similar to autosomes and

assume the proportion of genes of chromosome 2 in the whole genome as equal to 0.36. This fraction is obtained as the fraction of euchromatin of chromosome 2 in the euchromatin of the whole genome according to the data borrowed from compendia made by Zakharov (1979), Korochkina (1977), and Zhimulev (1993). Then the zygotes' mortality from genic DLM arising in the whole genome is equal to 0.00068:0.36 = 0.00189. The zygotes' mortality from total DLM amounts to 0.0415 (Ivanov, 1998), therefore the fraction of genic DLM among them is 0.00189 : 0.0415 = 0.0455, whence the average number of genic DLM in the whole genome is approximately equal to A_1 = 0.0455 x 0.0223 = 0.00101. Our independent estimates of the average number of genic DLM in the whole genome 1) 0.0013 and 2) 0.0010 are rather close to each other and confirm each other.

Extrapolation of data on the number of DLM from *D. melanogaster* to species of other taxa will undoubtedly come across limitations due to the difference in their karyotypes with respect to the number of chromosome arms. As the number of arms increases, their affection by DLM decreases, since the genetic content of arms thereby diminishes, due to which their breakages accompanied by formation of terminal deletions become less dangerous. In larger chromosomes, the harmful effect of even very small terminal deletion may be enhanced to lethality due to growing together of sister chromatids and to the chromosome bridge which is in this case formed in cell division, is broken in a random site and forms unbalanced gene assortments in daughter cells. The chromosomes broken at bridges may again have a defective healing, i.e. growing together of sister chromatids, which results in a new bridge at the next cell division, etc. When chromosomes are small, the bridges will not be as harmful, which is especially well seen on the chromosomes that can be lost completely without any lethal consequences. If one ignores such an influence of chromosome bridges and makes some other simplifying assumptions, then, at such idealization, the dependence of the mean number A_2 of chromosomal DLM in the genome on the number f of chromosome arms is a function $A_2 = k(1-\alpha f)$, where k and α are positive constants, α being the mean size of the maximal non-lethal terminal arm deletion in the genome expressed as a genome fraction, so that $1 - \alpha f$ is the genome fraction vulnerably to chromosomal DLM, i.e. its part whose breakages result in deletions of lethal size. As f increases due to karyotype fragmentation into progressively smaller chromosomes on the condition that the largest chromosome arms in the genome do not remain fixed, but decrease infinitely in size, the number A_2 of chromosomal DLM decreases and becomes equal to zero as soon as the relation $\alpha f \geq 1$ is attained. Species with large numbers of chromosomes in which even the largest chromosome arms are very small and are close by their fraction in the genome to the α value may be free from chromosomal DLM and have a low DLM level in general, because the number of genic DLM, apparently similar in various species, is rather negligible. On the contrary, species of the genus *Drosophila* and those karyotypically similar to them, which have few chromosomes and whose chromosome arms are large as compared to α, must have many chromosomal DLM and therefore DLM in general. As contrary to the few-chromosome karyotype of *Drosophila*, one may refer to that of grayling *Thymallus thymallus* which contains 100 – 106 , and on the average 102 chromosomes and 170 chromosome arms (Severin, 1979). DLM in grayling do not seem to play any essential role, since the genetic content of each of so many chromosome arms is negligible, their breakages are not dangerous and are not DLM, which is probably confirmed by the variation of the number of chromosomes in the karyotype. The mutagenesis as regulator in the ecosystem must be especially efficient when it limits the population number of few-chromosome species like *Drosophila*, and it is probably not without reason that the first factual data on the regulatory action of mutations on the population density were obtained just on *D. melanogaster* (Ivanov and Ivannikov, 1997).

To summarize, the use of EMS for induction of mutations throws some light on the nature of DLM. It is believed that EMS causes mainly genic mutations and much less often chromosome breaks. If all the spontaneous mutations were genic, then EMS treatment would increase their number as efficiently as that of genic mutation in the X chromosome. However, the effect of EMS in the experiment was accompanied by an extraordinary increase in the number of RLM and VM in the X chromosome and by a comparatively small increase in the number of DLM – by 212 and 13.3 times, respectively. Therefore the overwhelming majority of spontaneous DLM represent chromosome aberrations, i.e., as it has been established cytologically (Hadorn, 1961), losses of chromosome fragments during the division of the developing zygote and chromosome bridges. Calculation of the mean number of genic DLM in the whole genome (autosomes + X chromosome), on assumption that EMS does not bring about chromosome breaks, gives a value of $A_1 = 0.00129 \pm 0.00031$, which is about 6% of the whole number of spontaneous DLM in the genome measured by us earlier as $A = 0.0223 \pm 0.0062$, whereas the chromosomal DLM make up the remaining 94% of this quantity. Data on *D. melanogaster* population genetics show that a considerable part of autosomal RLM are eliminated in heterozygotes. Such elimination, if it concerns newly arisen mutations, is a phenomenon recorded as DLM. Zygotes' mortality from newly arisen RLM, i.e. from genic DLM, can be measured, whence another, independent estimate of the mean number of genic DLM in the whole genome $A_1 \approx 0.0010$ rather close to the former, is obtained. It has been demonstrated that species must differ in the number of DLM in their genomes, and an idealized dependence of the number of chromosomal DLM in the genome on the number of chromosome arms in the species' karyotype has been considered.

Conclusions

1. A genetic method of studying the nature of spontaneous dominant lethal mutations (DLM) and estimating the relative number of their types in the genome of the fruit fly *D. melanogaster* with the help of ethylmethane sulphonate (EMS) is described.
2. DLM are divided into 1) chromosomal and 2) genic. The mean number of genic DLM in the whole genome (autosomes + X chromosome) measured in an experiment with EMS, on assumption that it causes only genic mutations and does not break chromosomes, is 0.00129 ± 0.00031, i.e. about 6% of all the DLM. The rest 94% are chromosomal DLM.
3. In classical works it has been established that chromosomal DLM are chromosome breaks with loss of their acentric fragments and formation of chromosome bridges between daughter cells in the course of subsequent divisions of the developing zygote. The present work evolves the idea that genic DLM are ordinary genic mutations with some penetrance of lethal effect in heterozygote which are recorded as DLM only when they cause zygotes' death immediately at their origin, i.e. in the 1st generation.
4. Estimation of the mean number of genic DLM in the genome obtained from the mortality from newly arising recessive lethals in the population of *D. melanogaster* under usual conditions gives a value of 0.0010 which is rather close to the empirical one.
5. Limitations to extrapolation of the results of measurement of the number of DLM in the genome of *D. melanogaster* to species of other taxa are discussed, and an idealized dependence of the number of chromosomal DLM in the genome on the number of chromosomal arms the species' karyotype is presented.

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Estimation of the number of spontaneous dominant lethal mutations in the genome of *Drosophila melanogaster.*

A dominant lethal mutation (DLM) is any newly arisen mutation that causes death of the zygote immediately, in the 1st generation (Hadorn, 1961). Any, including recessive lethal (RLM) or visible (VM) mutation, due to its negative influence on the viability, may cause immediate death of the zygote, and in this case it is recorded as DLM. However, if it survives at first and causes death afterwards, in subsequent generations, it may not be considered as DLM, although it manifests its lethal effect in heterozygote: the death caused by it will be attributed to chance mortality. It is in this way that DLM are understood, which will be discussed in the present paper.

The difficulty in estimating the number of DLM consists in the fact that it is impossible to separate DLM-induced death of zygotes from occasional death: it can be done only by means of a special organization of the experiment. The present work contains a description of such an experiment and of its result in solution of this problem on the fruit fly *D. melanogaster.*

In order to remove all the obstacles brought in by accidental death, the following method was used. Under equal conditions, in the Canton-S population, the total death rate of zygotes at the stages from the egg to imago was counted 1) without irradiation and 2) with γ-irradiation of male parents, so that one genome of each zygote was irradiated in order to heighten the DLM frequency. For estimation of the degree of its heightening, in the same male parents the frequency of occurrence of mutations in the X chromosome was determined by the M5 (Basc) method 1) without irradiation and 2) with irradiation. The number of DLM in irradiated genome increased just like that of RLM and VM in the X chromosome, while the accidental death rate in variants (1) and (2) remained equal, which permitted excluding it from respective equations.

Let Q be the proportion of zygotes that have survived from the egg to imago stage, u be the frequency of arising of RLM and VM in the X chromosome, A be the mean number of DLM in the whole genome (autosomes + X chromosome), and a be the mean number of RLM and VM arising in the X chromosome without irradiation; $\tilde{Q}$, $\tilde{u}$, $\tilde{A}$, and $\tilde{a}$ be the same quantities under γ-irradiation of male parents; R be the accidental death rate (caused by lethal genetic factors apart from DLM or by adverse environmental factors); s be the proportion of genes of the X chromosome in the whole genome, and e be the base of natural logarithms.

Then we obtain the following relations. The frequency Q of survival of non-irradiated zygotes is equal to the product of the probability of no DLM occurring in the zygote by the probability of the zygote not dying from chance causes. The former probability is equal to e^{-X} where X is the mean number of DLM in the zygote, i.e. a Poisson distribution parameter that the number of DLM in the zygote obeys. The number of whole genomes in a female's zygote is 2, and in a male's zygote 2 - s., since the male contains, instead of the second X chromosome, genetically empty Y chromosome. Then an average, intersexual, zygote, the sex ratio being 1:1, contains $2-s/2$ whole genomes, and the mean number of DLM in it is $X = A(2-s/2)$. Hence, the former probability is $e^{-X} = e^{-A(2-s/2)}$. The latter probability is 1 - R. Therefore

$$Q = e^{-A(2-s/2)}(1-R). \tag{1}$$

In a similar way the expression for the frequency $\tilde{Q}$ of survival of zygotes of γ-irradiated male parents, when one of the zygote's genomes turns out to be irradiated, is found. However, this time the mean number of DLM in an average zygote will be $\frac{\left(A+\tilde{A}\right)+\left[A+\tilde{A}(1-s)\right]}{2} = A+\tilde{A}(1-s/2)$, where the first item in the numerator, $\left(A+\tilde{A}\right)$, is the number of DLM in the female's zygote, and the second one, $\left[A+A(1-s)\right]$, is the number of DLM in the male's zygote. The probability of there being no DLM in an average zygote is $e^{-\left[A+\tilde{A}(1-s/2)\right]}$, and the sought expression will be

$$\tilde{Q} = e^{-\left[A+\tilde{A}(1-s/2)\right]}(1-R). \tag{2}$$

The frequency u of mutation occurrence in the X chromosome is measured as the probability of there being at least one mutation in it and is equal to the difference between unit and the probability of there occurring no such mutation. The latter probability is found from Poisson distribution with parameter a and is equal to e^{-a}, whence

$$u = 1-e^{-a}. \tag{3}$$

Similarly, the frequency of occurrence of mutations in the X chromosome when male parents are irradiated is

$$\tilde{u} = 1-e^{-\tilde{a}} \tag{4}$$

where ã is a parameter of Poisson distribution for the number of mutations arising in the X chromosome under irradiation.

Another equation is assumption that at our rather low irradiation dose the number of DLM in the genome is proportional to the number of mutations arising in the X chromosome:

$$A : \tilde{A} = a : \tilde{a}. \tag{5}$$

Equations (1) – (5) form a system with unknown A, $\tilde{A}$, a, $\tilde{a}$, and R, by whose solution we find the expression for the mean number of spontaneous DLM in a whole genome:

$$A = \frac{\ln\frac{\tilde{Q}}{Q}}{(1-s/2)\left[1-\frac{\ln(1-\tilde{u})}{\ln(1-u)}\right]}. \tag{6}$$

Due to independence of the quantities $\tilde{Q}$, Q, $\tilde{u}$, and u which are obtained in independent experiments, the error of the A value is found by a simple formula which, for the sake of brevity, may be given in a general form as an estimate of dispersion of a function of several variables:

$$D\left[f(x_1, x_2, \ldots, x_n)\right] = \sum_{i=1}^{n}\left(\frac{\partial f}{\partial x_1}\right)^2 s_i^2, \tag{7}$$

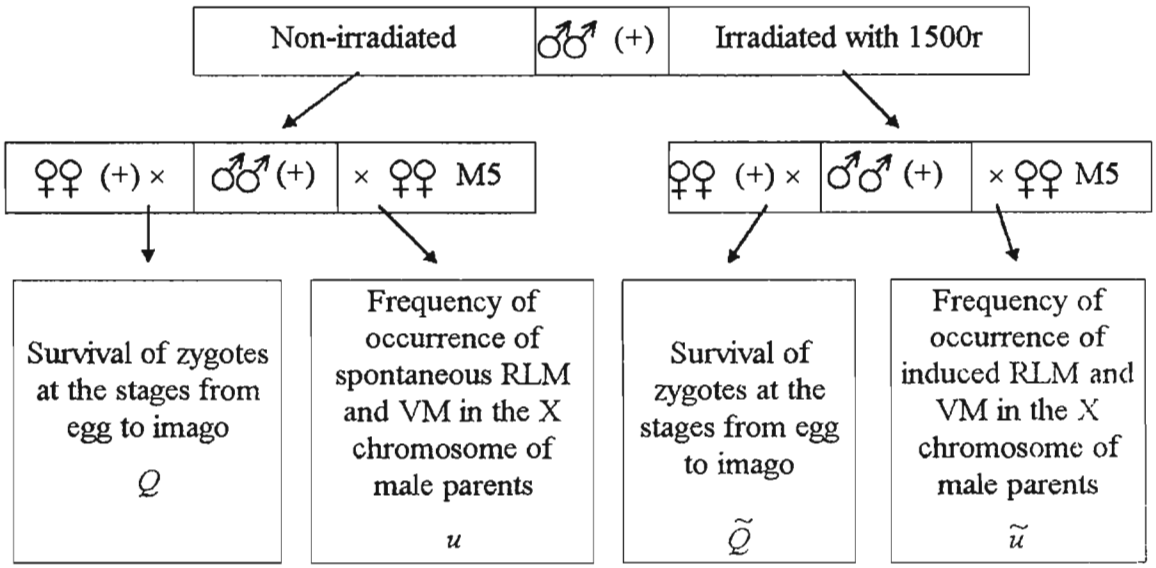

Figure 1. Scheme of the experiment on quantitative estimation of spontaneous DLM in the genome of *D. melanogaster.*

where $\frac{\partial f}{\partial x_i}$ is the partial derivative of the function f with respect to x_i at the point $(\hat{x}_1, \hat{x}_2, ..., \hat{x}_n)$, $\hat{x}_i$ is the empirical value of x_i, and s_i^2 is the estimate of dispersion of the $\hat{x}_i$ value.

In the experiment, two groups of males from the laboratory Canton-S population – 1) non-irradiated and 2) γ-irradiated with a dose of 1500 r - were used. Both of them were crossed with wild type (+) females from the Canton-S population and simultaneously, in the same tubes, with females of the strain M5 (Basc). In the progeny of (+) females, the survival of zygotes from the egg to the imago stage was estimated: Q in variant (1) and $\tilde{Q}$ in variant (2). In crosses with M5 females, the frequency of occurrence of X-linked mutations in spermia was estimated: u in variant (1) and $\tilde{u}$ in variant (2). The general scheme of the experiment is diagrammatically presented in Figure 1.

100 irradiated and 100 non-irradiated males were taken. Every 4 males were placed into a tube with 6 (+) females and 4 M5 females and kept for 1 – 2 days for mating. Every 6 (+) females were placed into dismountable flasks fixed with adhesive tape, in which eggs and imagos were counted. The counting of laid eggs was performed in 7 – 20 hours (till larvae hatching). The survived imagos were counted in 9 days for several days until depupation of all the developed flies. In variants (1) and (2) there were 23 and 25 dismountable flasks, respectively. M5 females were placed in 4s into simple tubes containing medium, and the experiment on estimation of the X chromosome mutability of males with which they were mated was carried out with them in the usual manner. Considered as RLM were both lethals and semilethals. Considered as lethals and semilethals were mutations that reduced the number of (+) males in F_2 to 0 – 5 and 5 – 20% of the expected one, respectively. Assumed as the expected number of (+) males was 1/3 of all F_2 flies with other phenotypes. The tubes in which the initial crosses were carried out and the dismountable flasks were kept at 27°C until the complete development of flies, and the rest of crosses for estimation of X-linked mutability were carried out at room temperature.

The results of the experiment are presented in Table 1. In section (a) the measured, and in (b) the calculated values are given. When survival at the stages of egg to imago was estimated, it turned out that the proportion of survived zygotes Q or $\tilde{Q}$ depended on the number of flies developed in the flask. At large numbers of flies, i.e. at a high population density, the survival was lower, and *vice versa*. Linear approximations of this dependence in our variants are

1) $Q(x) = 0.9664 - 9.988 \cdot 10^{-4} x$ and 2) $\tilde{Q}(x) = 0.7208 - 7.991 \cdot 10^{-4} x$.

Table 1. Estimation of the number of DLM in the whole genome (autosomes + X chromosome) by the zygotes' survival.

a) Empirical data

Conditions of experiment	Number of eggs N	Number of imagos n	Survival n/N	Survival under equalized population density Q	Mutability in the X chromosome of male parents (%) u	Gamete sample size
With irradiation of male parent	3198	2038	0.6373	0.6377 ± 0.0205	3.197	1220
Without irradiation	3593	2925	0.8141	0.8625 ± 0.0197	0.203	17243

b) Calculated data

Conditions of experiment	The mean number of RLM and VM in the X chromosome a	The mean number of DLM in the genome A	Accidental death R	Zygotes' death from DLM
With irradiation of male parent	0.03249	0.3559 ± 0.0467	0.1002	0.2913
Without irradiation	0.00203	0.0223 ± 0.0062	0.1002	0.0415

The mean number of imagos in a flask in variants (1) and (2) was 127.2 and 81.5, respectively. Such a difference in population density brought about also a difference in chance mortality R. However, this obstacle is easily removed by reducing the zygotes' survival in the two variants to the same flies' population density in the culture. If, for a higher accuracy, the mean number of flies for both variants is assumed to be $\frac{1}{2}(127.2+81.5) \approx 104$, and the values $Q(104)$ and $\tilde{Q}(104)$ are calculated, they will correspond already to conditions of equal population densities under which the accidental mortality R will also be equal. It is just these values that are presented in Table 1(a) as those correcting the survival value of n/N, where N is the total number of eggs and n is the number of developed flies in all the cultures of the variant.

Now let us estimate the needed fraction s composed of the genes of the X chromosome in the whole genome. The length of the X chromosome amounts to 70.4 map units or, in the cells of salivary glands, 220 μ. The length of the whole genome is 287.7 map units, or in the cells of salivary glands, 1180 μ (Lindsley and Grell, 1968). Hence the X chromosome makes up 70.4 : 287.7 = 24% or 220 :1180 = 19% of the whole genome. "Cytologically chromosome 2 is longer than the X chromosome by 2.5 times. N.I. Shapiro and R.I. Serebrovskaya (1934) in experiments with X-rays demonstrated that the frequency of induced mutations in chromosome 2 was also by 2.5 times higher than in the X chromosome" (Dubinin, 1967). Assuming that large chromosomes 2 and 3 have equal lengths, and therefore taken together exceed by 5 times the X chromosome, we obtain the proportion of the latter in the genome $s = 1/6$. Probably the most correct estimate of fraction of the genes of X chromosome in the genome will be the proportion of its euchromatin in that of the whole genome. According to our calculations based on the data borrowed from the reviews of Korochkina (1977) and Zhimulev (1993), the length of euchromatin of the X chromosome amounts to about 1.0 μ, that of euchromatin of the whole genome about 5.2 μ, whence $s = 0.19$. If a chromosome DNA thread is measured not by the length but by the mass (Kavenoff and Zimm, 1973), which seems to be more accurate, then the X chromosome euchromatin mass amounts to $14.3 \cdot 10^9$ daltons, and the mass of euchromatin of the whole genome does so to $74.95 \cdot 10^9$ Daltons, whence the same estimate, $s = 0.19$, is obtained. The calculated values in Table 1(b) were obtained for $s = 0.19$.

In Table 2, the relative role of the quantities included in formula (6) in the error of the mean number A of spontaneous DLM in the genome is shown. The contribution of this variable to the estimate of the function dispersion is found as the ratio of respective item in the right-hand part of the formula (7) to the total sum. It became clear that the largest contribution to the estimation of dispersion of A was made by the dispersion of the u value, i.e. variance of the spontaneous mutation rate in the X chromosome.

In order to diminish the A error, we measured the u value with a higher accuracy already after the completion of the experiment performed in 1976. The spontaneous mutation rate in the males' X chromosome in our Canton-S population practically had not changed for several years. and this permitted merging the data of its measurements obtained from June 1973 to October 1981 (totally 16 samples containing 17243 gametes). In Table 2, already more

accurate data are presented, but the contribution of s_u^2 to s_A^2 remains nevertheless the largest (42%). In this way, development of dispersion of the function under calculation into its components corresponding to independent variable is a very useful method. It shows the researcher the critical points in the experiment and permits diminishing the error in its replication, increasing the accuracy where this increase gives the highest effect.

The mean number of spontaneous RLM and VM arising in the whole genome, calculated from their mean number in the X chromosome $a = 0.00203$ and its fraction in the genome $s = 0.19$, is equal to 0.0107. Totally, together with DLM, on the average about 0.0107 + 0.0223 = 0.0330 mutations arise, and the proportion of DLM among them is 68%. In this way, the DLM are the most numerous class of mutations in spontaneous mutagenesis whose significance has not so far been understood quite well. It becomes clear that mutations play a regulatory role in the ecosystem, and DLM must have here a decisive importance as a factor of mortality (Ivanov and Ivannikov, 1997).

Table 2. Contribution of dispersions of $\tilde{Q}$, Q, $\tilde{u}$, and u to dispersion s_A^2 of the mean number of DLM in the genome.

Contribution to s_A^2	$s_{\tilde{Q}}^2$	s_Q^2	$s_{\tilde{u}}^2$	s_u^2	s_A^2
Absolute x 10^6	5.4	2.8	13.9	16.0	38.1
IN %	14	7	37	42	100

DLM are the most important factors of embryonic death in induced mutagenesis (Hadorn, 1961), and therefore the frequency of zygote death from spontaneous DLM is undoubtedly of a special interest. As we saw, the average number of spontaneous DLM in an average zygote at an equal frequency of sexes in the population is $A(2-s/2)$, and the fraction of zygotes having no DLM is $e^{-A(2-s/2)}$. Then the fraction of zygotes that died from DLM is $1-e^{-A(2-s/2)}$, which at $A = 0.0223$ and $s = 0.19$ yields a value of 0.0415, i.e. about 4%.

Extrapolating the results obtained on our Canton-S population to other *D. melanogaster* populations, one may conclude that:

1) the mean number of spontaneous dominant lethal mutations (DLM) in the whole genome (autosomes + X chromosome) is (223 ± 62). 10^{-4}, which makes up about 2/3 of all the mutations arising in the genome.
2) the frequency of zygote death from spontaneous DLM is about 4%.

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Technique Notes

Chan, H.Y. Edwin and Cahir J. O'Kane. Department of Genetics, University of Cambridge, Downing Street, Cambridge CB2 3EH, England. An easy and direct method for sequencing of high molecular weight cloned DNA.

Abstract: This report describes a novel technique for direct sequencing of high molecular weight DNA clones. *Drosophila P1* genomic DNA template is first digested with a restriction enzyme. The restriction digestion mix is then used directly for DNA sequencing. Using this method, we have successfully located the positions of two introns in a *Drosophila* gene, *drongo*.

Introduction: Genomic DNA sequence contains much regulatory information (*e.g.*, promoters, enhancers) which are essential for gene expression. It also differs from complementary DNA (cDNA) sequence by the presence of introns, the splicing pattern of which can vary between tissues. Because of the importance of genomic sequence in gene regulation, it is useful to be able to sequence genomic DNA at specific locations.

Recently, *Drosophila* genomic DNA has been cloned using the *P1* system (Smoller *et al.*, 1991), and a complete *P1* genomic framework map is now being constructed (Berkeley *Drosophila* Genome Project, personal communication). The average insert size of a *P1* genomic clone is between 75 and 100 kb system (Smoller *et al.*, 1991), some 20 times larger than typical clones in purpose-designed sequencing vectors. It is therefore of use to be able to sequence such large clones directly without the time-consuming step of subcloning.

Materials and Methods: A single colony of bacteriophage *P1* clone DS08554 was used to inoculate 500 ml of LB medium containing 25 µg/ml kanamycin and 1 mM IPTG. Purification of *P1* genomic DNA was performed using the QIAGEN plasmid maxi kit (Qiagen). Five micrograms of DS08554 *P1* genomic DNA were digested with *Eco*RI (Promega) (Sambrook *et al.*, 1989). The amount of genomic DNA used for restriction digestion depends on the size of the final restriction fragment template. The predicted sizes of template fragments used from DS08554 for the first and second intron/exon boundary determination were 1 kb and 5 kb, respectively (Schneuwly *et al.*, 1989) implying template quantities of approximately 60 ng and 300 ng accordingly. After restriction digestion, DNA was used directly as a sequencing template without further purification. Sequencing reactions were performed using an ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer) and oligonucleotides which had been synthesized using an ABI 391 synthesizer. Cycle sequencing reactions were carried out using a PREM™ thermocycler and the conditions were as follows: 10 seconds at 96°C, 5 seconds at 50°C, 4 minutes at 60°C for 25 cycles. Sequences were then determined on an Applied Biosystems 373 ABI sequencer. Raw sequence output was analyzed using SeqEd™ version 1.0.3 software. Sequencing reactions were performed in duplicate and no ambiguities or conflicting base assignments were found in the first 200 base pairs of sequence, demonstrating that DNA sequences obtained were reliable and reproducible.

Results and Discussion: *P1* clone DS08554 (2) contains a gene called *drongo* (Harris, 1994) which lies in a region for which an extensive restriction map is available (Schneuwly *et al.*, 1989). In an attempt to obtain genomic sequence from the *drongo* locus, DS08554 was digested with *Eco*RI. The digestion mix was then used for sequencing without further purification. Standard DNA sequencing reactions were performed according to the manufacturer's instructions (Perkin Elmer). By comparing the genomic sequence obtained from the DS08554 genomic digest with the *drongo* cDNA sequence, we have mapped the positions of two introns in *drongo* (Figure 1). No signal was detected from undigested *P1* genomic DNA template, suggesting that restriction digestion of *P1* template is crucial for this technique (data not shown). Reproducible results were also observed using this technique on *P1* and cosmid templates from a completely different genomic region (Samantha Loh, personal communication).

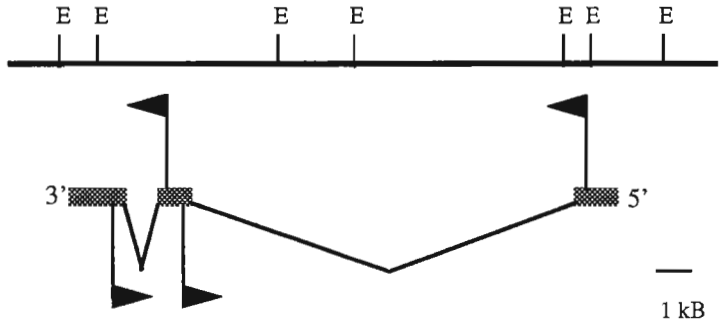
This method can also be potentially employed to sequence any cloned large DNA molecules (*e.g.*, *P1* genomic clones from any of the model organism genome projects in which they are used). When such clones exist, this technique

Figure 1 (see next page). a.) Genomic map of the *drongo* locus. The positions of the first and second intron and DNA sequencing primers are shown. The three exons are shown as shaded boxes joined by two introns. Vertical lines show the positions of sequencing primers, arrowheads show their 5' - 3' directions.

b.) Sequences of the 5' and 3' splice junctions of both introns. Exon sequences are shown in upper cases; intron sequences in lower cases; splice junctions are underlined.

c.) Chromatogram showing the 5' splice junction of the second intron of *drongo* using *EcoRI* digested DS08554 *P1* DNA template.

a.



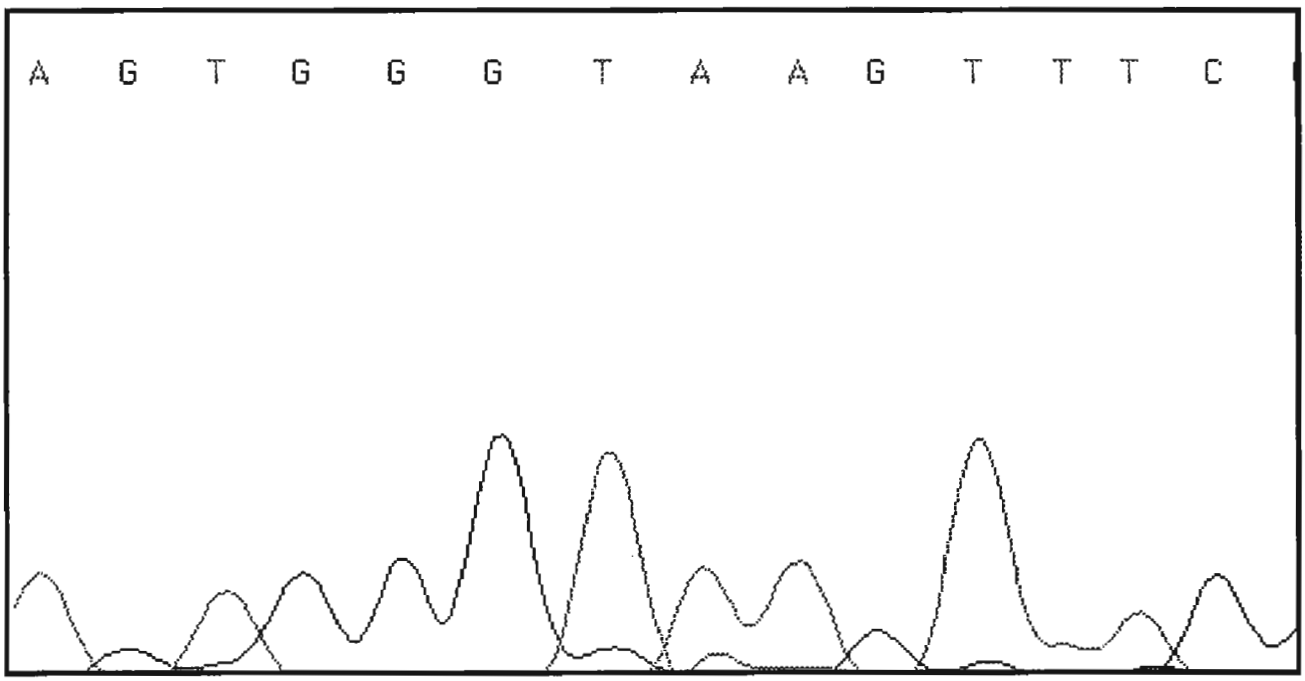
intron II intron I

b.

intron I 5' GTC CT gtaagtatt...cttttccag G CGC GGT 3'

intron II 5' GTG G gtaagtttc...atgttgcag GT GCT CCC 3'

c.



is much simpler and faster than the RAGE method which requires restriction digestion and polyadenylation of uncloned genomic DNA and multiple rounds of PCR (Cormack and Somssich, 1997).

Acknowledgments: We thank Dr. Stephen J. Harris for the *drongo* cDNA. H.Y.E.C. has been supported by scholarships from the Cambridge Commonwealth Trust, The Chinese University of Hong Kong Chung Chi College C.F. Hu Scholarship for Overseas Studies, and the Croucher Foundation.

References: Cormack, R.S. and I.E. Somssich 1997, Gene 194:273-276; Encyclopaedia of *Drosophila* Release 3.1., 1996, Berkeley *Drosophila* Genome Project; Harris, S., 1994, The characterization of two enhancer trap lines expressed in the embryonic nervous system of *Drosophila melanogaster*. Ph.D. thesis, University of Warwick; Sambrook, J., T. Maniatis, and E.F. Fritsch. 1989, Molecular Cloning. Cold Spring Harbour Laboratory Press, New York, Second Edition; Schneuwly, S., R.D. Shortridge, D.C. Larrivee, T. Ono, M. Ozaki and W.L. Pak 1989, Proc. Natl. Acad. Sci. USA 86:5390-5394; Smoller, D.A., D. Petrov and D.L. Hartl 1991, Chromosoma 100:487-494.

Reichhart, J.M., and D. Ferrandon. UPR CNRS 9022, Institut de Biologie Moléculaire et Cellulaire, 15 rue René Descartes, 67084 Strasbourg Cedex, France. (email: reichhart@ibmc.u-strasbg.fr). Green balancers.

We have used the S65T green fluorescent protein (GFP; Chalfie *et al.*, 1994; Heim *et al.*, 1995) as a vital reporter to introduce a dominant innocuous marker onto the balancers of the three major chromosomes of *D. melanogaster*.

Construction: The drosomycin promoter contained in pJM802 (Ferrandon *et al.*, 1998) was replaced by the distal actin 5C promoter as an *Eco*RI-*Nhe*I fragment originating from pPac (Krasnow *et al.*, 1989) in which an *Nhe*I linker was inserted into the polylinker. The P element mediated transformation plasmid derived from pCaSpeR contained the actin 5C promoter, followed by the S65T version of the GFP and the drosomycin terminator. The nucleotide sequence of the transformation vector is available upon request. Transgenic fly lines were established as described (Driever *et al.*, 1990). One of the P element insertions obtained was remobilized using Delta(2-3) source of transposase. Insertions in FM7 (FM7i; Heitzler, 1997), CyO, and TM3 balancer chromosomes were selected. The following stocks were sent to the Bloomington stock center:

FM7i-pAct-GFP:

C(1)DX, f/FM7, y[93j],sc[8],w,oc,ptg,B,P[w^{+mC} act::GFP = pActGFP]

CyO-pAct-GFP:

w; ln(2LR)noc[4L],Sco[rv9R],b / In(2LR)O,Cy,dp[lvI],pr,cn[1],P[w^{+mC} act::GFP = pActGFP]

TM3-pAct-GFP:

w; Sb[1] / In(3LR)TM3,ri,p^{p},sep,l(3)89Aa,bx34e,Ser,P[w^{+mC} act::GFP = pActGFP]

Expression Pattern: Since their cuticle is transparent, third instar larvae carrying the marked balancers are easy to score under the fluorescent dissecting microscope. The main GFP expression pattern consists of a strong fluorescence in the salivary duct, the copper cells, the proventriculus and the visceral musculature of the midgut. A weaker signal can be detected in imaginal disks. In first instar larvae, the fluorescence appears to be restricted to the midgut (Burn *et al.*, 1989).

Adult flies carrying GFP balancers can be recognized by a deep pseudopupil type of expression in the eye, a mild fluorescence in the proboscis and a strong signal in the abdomen. Upon dissection, it appears that the abdominal fluorescence is due to:

– GFP expression in the reproductive tract of the male;

– GFP expression in ovaries (yolk of mature stages and musculature of the ovary sheath) and in the seminal receptacle in females.

In many animals, the visceral musculature of the midgut is also fluorescent.

In the embryo, there is a strong maternal contribution which masks the zygotic expression until about stage 15 of development, when a weak signal can be detected in the midgut, as in first instar larvae. In the absence of this maternal contribution, the expression of GFP can first be detected around 12 h after laying.

Selected pictures showing these expression patterns can be viewed at http://ibmc.u-strasbg.fr/upr9022/GreenBalancers.html

In conclusion, these "green balancers" constitute a highly useful tool to score living larvae, pupae, and adult flies, especially when working with mutations on the second chromosome.

References: Burn, T.C., J.O. Vigoreaux, and S.L. Tobin (1989), Dev. Biol. 131: 345-355; Chalfie, M., Y. Tu, G. Euskirchen, W.W. Ward, and D.C. Prasher 1994, Science 263: 802-805; Driever, W., V. Siegel, and C. Nüsslein-Volhard 1990, Development 109: 811-820; Ferrandon, D., A.C. Jung, M. Criqui, B. Lemaitre, S. Uttenweiler-Joseph, L. Michaut, J. Reichhart, and J.A. Hoffmann 1998, Embo J. 17: 1217-1227; Heim, R., A.B. Cubitt, and R.Y. Tsien 1995,

Nature 373: 663-664; Heitzler, P., 1997, Dros. Inf. Serv. 80: 103; Krasnow, M.A., E.E. Saffman, K. Kornfeld, and D.S. Hogness 1989, Cell 57: 1031-1043.

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No effect of marking flies either by nail polish on scutellum or by wing clipping on mating success in *Drosophila ananassae*.

In different species of *Drosophila*, males and females of different strains are marked for identification in mating preference tests. For marking the flies, different methods have been used by various investigators. These methods are:

a) wing-clipping -- margin of one wing is clipped in one of the strains (Ehrman, 1966, 1968)

b) placing a small drop of quickly drying enamel paint on mesonotum just anterior to the scutellum (Arita and Kaneshiro, 1979)

c) placing a small drop of nail polish on scutellum (Singh and Chatterjee, 1985)

d) placing a small mark of ink on both wings (Zouros and D'Entremont, 1980)

e) flies had been coloured with either pink or blue fluorescent dust (Markow, 1980) and

f) flies were fed red and green coloured food (Wu *et al.*, 1995).

In these studies, no effect of marking was found on the performance of flies or the outcome of mating preference test, because similar results have been found when the strains marked and unmarked are alternated in successive replicates.

Rare-male mating advantage which is an example of frequency-dependent selection, has so far been reported in nine species of *Drosophila* (Singh and Sisodia, 1997). *Drosophila ananassae* is a cosmopolitan and domestic species. This species occupies unique status in the whole of the genus *Drosophila* due to certain peculiarities in its genetic behaviour (Singh, 1985). Extensive work on population and behaviour genetics of *D. ananassae* has been carried out by Singh and others (for references see Singh, 1996). Rare-male mating advantage has also been reported in *D. ananassae* (Singh, and Chatterjee, 1989). It has been suggested by Bryant *et al.* (1980) that rare-male mating advantage is induced by wing-clipping in housefly and thus it is nearly an artifact resulting from alternately marking the rare and the common strains. On the other hand, Knoppien (1984) questioned the arguments given by Bryant *et al.* (1980) and proposed that any artificial rare-male mating advantage caused by wing-clipping is less important than suggested by Bryant *et al.* (1980). Further, Markow (1980) has clearly demonstrated that rare-male effect is not induced by marking with fluorescent dust in *D. melanogster*. In view of this, we have planned experiments to test the effect of marking on rare-male mating advantage in *D. ananassae*. Further, the phenomenon of rare-male mating advantage will be investigated in detail in *D. ananassae* by employing different wild type and mutant strains and inversion karyotypes as well as different experimental techniques. Before starting the detailed experiments, we have carried out preliminary experiments to test the effect of marking on mating success in *D. ananassae* and the results are reported in this note.

A wild type laboratory stock of *D. ananassae* (Bombay strain) established from a large number of flies collected from Bombay in 1985 was used. Virgin females and males were collected from this stock and aged for seven days.

Two marking procedures were used and for each procedure "male-choice" and "female-choice" techniques were employed:

A. Nail polish marking on scutellum

In "female-choice" experiments, males were marked by placing a small drop of quick drying nail polish on scutellum. Marking was done on lightly etherized flies 24 hr before the experiment. Twenty unmarked females with 10 marked and 10 unmarked males were introduced into an Elens-Wattiaux mating chamber and thus 20 pairs of flies were tested and the sex ratio was 1:1. Flies were observed for 60 minutes. When a pair commenced mating it was aspirated out and the type of male mated was recorded. In total five replicates were run.

In "male-choice" experiments, 20 unmarked males with 10 marked and 10 unmarked females were introduced into the mating chamber and were observed for 60 minutes. When a pair commenced mating it was aspirated out and the type of female mated was recorded. In total five replicates were run.

B. Marking by clipping the margin of wing of one side

Flies were lightly etherized and a small part of the distal tip of the right wing was clipped.

In "female-choice" experiments, 20 unmarked females with 10 marked and 10 unmarked males were introduced into the mating chamber. After commencement of mating, mated pair was aspirated out and the type of male mated was recorded. Observation continued for 60 minutes. In total five replicates were run.

In "male-choice" experiments, 20 unmarked males with 10 marked and 10 unmarked females were introduced into the mating chamber and were observed for 60 minutes. When a pair commenced mating it was aspirated out and the type of female mated was recorded. In total five replicates were run.

All the experiments were conducted in a room maintained at 24°C approximately under normal light conditions from 7 to 11 a.m.

Number of matings in female- and male-choice experiments involving marked and unmarked flies are presented in Table 1. It is evident from the results that marked and unmarked males as well as marked and unmarked females are equally successful in mating as there is no significant difference in the number of matings between marked and unmarked flies in all the comparisons ($P > 0.05$). Further, marking by both the methods (placing a small drop of nail polish on the scutellum and wing-clipping) produced identical results. Thus, it is concluded that marking the flies either by placing a drop of nail polish on scutellum or by wing-clipping has no effect on mating success in *D. ananassae*.

Table 1. Number of matings in female- and male-choice experiments involving marked and unmarked flies

	Female-choice experiment			Male-choice experiment		
	Mated males			Mated females		
Replicates	Unmarked	Marked	Total	Unmarked	Marked	Total
A. Nail polish marking on scutellum						
1	11	6	17	10	9	19
2	9	7	16	10	10	20
3	9	10	19	7	9	16
4	9	8	17	10	7	17
5	9	5	14	8	7	15
Total	47	36	83	45	42	87
χ^2 for 1:1 ratio 1.44		P> 0.05			.10	P > 0.05
B. Wing Clipping						
1	8	6	14	8	9	17
2	8	6	14	9	8	17
3	9	8	17	8	8	16
4	7	6	13	8	9	17
5	8	9	17	9	9	18
Total	40	35	75	42	43	85
χ^2 for 1:1 ratio 0.33		P > 0.05			0.01	P> 0.05

References: Arita, L.H., and K.Y. Kaneshiro 1979, Proc. Hawaiian Entomol. Soc. 13: 31-34; Bryant, E.H., A. Kence, and K.T. Kimball 1980, Genetics 96: 975-993; Ehrman, L., 1996, Anim. Behav. 14: 332-339; Ehrman, L., 1968, Genet. Res. 11: 135-140; Knoppien, P., 1984, Am. Nat. 123: 862-866; Markow, T.A., 1980, Behav. Genet. 10: 553-556; Markow, T.A., 1991, Evolution 45: 1525-1529; Singh, B.N., 1985, Nucleus 28: 169-176; Singh, B.N., 1996, Genetica 97: 321-329; Singh, B.N., and S. Chatterjee 1985, Can. J. Genet. Cytol. 27: 405-409; Singh, B.N., and S. Chatterjee 1989, Genet. Sel. Evol. 21: 447-455; Singh, B.N., and S. Sisodia 1997, Genetika 29: 41-48; Wu, C.-I., H. Hollocher, D.J. Begun, C.F. Aquadro, Y. Xu, and M.-L. Wu 1995, Proc. Natl. Acad. Sci. USA 92: 2519-2523; Zouros, E., and C.J. D'Entremont 1980, Evolution 34: 421-430.

de Melo Sene[1], Fabio, and Maura Helena Manfrin[2].
[1] Depto. Genética – FMRP-USP. Av. Bandeirantes 3900 CEP 14049-900. [2] Depto. Biologia – FFCLRP – USP. Av. Bandeirantes 3900 CEP 14040-901, Brasil. A chamber to record the courtship sound in *Drosophila*.

In the genus *Drosophia* the most conspicuous element of the male's courtship is the wing vibration which emits an acoustic stimulus. This stimulus is species specific being a character that has been used to identify species in the genus (Spieth, 1974), and there is variation among populations and subspecies (Miller *et al.*, 1975; Manfrin *et al.*, 1997). This makes the courtship sound an important character for behavioral, evolutionary and molecular studies. In order to obtain the sound pattern, it is necessary to record it with appropriate equipment, to observe courtship behavior, and to eliminate background noise such as female wing vibration and flies movement. We erected a mating chamber that can be easily handled and allows the observation of the fly couple. The chamber, presented in Figure 1, is composed by an acrylic block 3×3×1 cm. In the central part of its inferior base there is a concave chamber with a diameter of 1.5 cm and a height of 1.7 cm in its deepest part. This interior opening is sealed by a nylon nest allowing the sound produced during courtship to reach the microphone. In its superior face there is an inclined opening 0.6 cm wide which falls laterally in the enclosed space of the chamber. After the introduction of the flies through this opening in the chamber, we close it by plugging it with a cap. Because of its characteristics, this chamber is easily installed, fitting nicely on the exposed membrane of the microphone. In addition, it allows the flies movement in its interior and the observation of their behavior during courtship.

References: Manfrin *et al.*, 1997, Rev. Bras. Biol. 57:349-355; Miller, *et al.*, 1975, Evolution 29:531-544; Spieth, H.T., 1974, Ann. Rev. Entomol. 19:385-405.

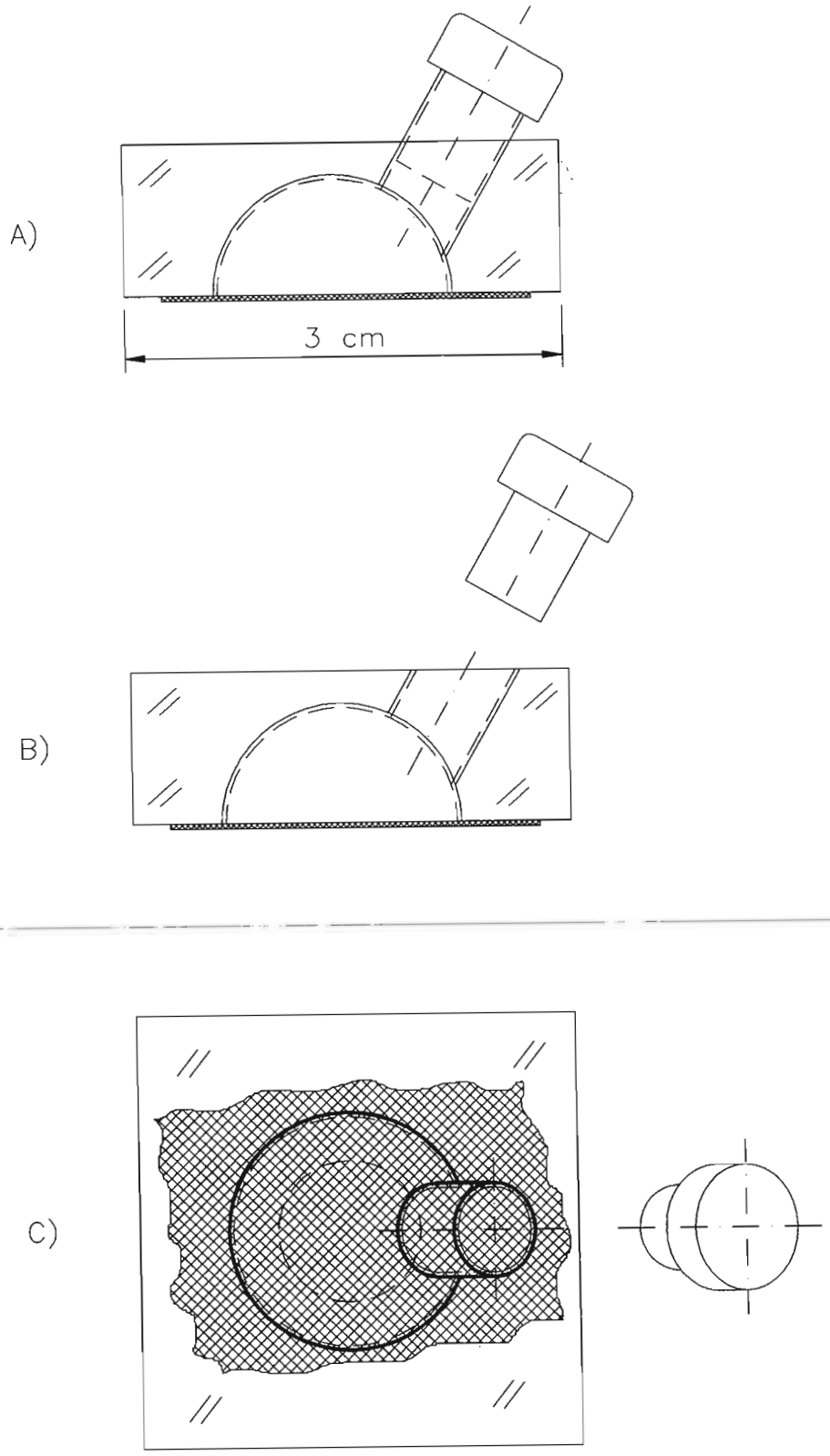

Figure 1. Chamber to record the courtship sound in *Drosophila*. A) Lateral view showing the concave chamber. B) Lateral view showing the opening in the chamber and the cap. C) Superior view showing the inferior base sealed by a nylon nest.

Robert Farkaš[1], Gabriela Šuťáková[2], Peter Daniš[1] and Lucia Medveďová[1]. [1]Institute of Experimental Endocrinology, Slovak Academy of Sciences, Vlárska 3, 833 06 Bratislava, Slovakia; and [2]Institute of Experimental Phytopathology and Entomology, Slovak Academy of Sciences, Nám. hrdinov 7, 900 28 Ivanka pri Dunaji, Slovakia. Useful Antifungal Effects Of Borate In *Drosophila* Cultures.

The problem of *Drosophila* culture infection by molds is as old as culturing fruit flies (Frobisher, 1926; Glass, 1934, 1936; Crew, 1936; Shipman, 1936; Cross, 1939; Gowen, 1943). Despite numerous studies on molds and their elimination from *Drosophila* cultures, they can become a serious problem because universal and efficient treatment is still missing. One of the earliest chemical treatments comes from Mittler (1947) who applied propionic acid to fly food to retard mold growth. It is used also presently in combination with phosphoric acid (Ashburner and Thompson, 1978; Ransom, 1982) and often potentiated with methylparaben (Shorrocks, 1972; Ashburner, 1989) also known as Nipagin or Tegosept. However, application of these compounds, even at increased concentration of methylparaben or propionic/phosphoric acid mix, seems to be unsuccessful to resistant fungi like green coloured *Penicillium sp.* or black *Aspergillus sp.*

On the other hand, most of the antimetabolites and inhibitors (nystatin, benzalkonium chloride, *o*-hydroxybiphenyl, Benomyl, carbendazime, Difocol, Tetradifon) described to have fungicidal or preventive effects in fly cultures (McDonough, 1956; Lamb and Lilly, 1973, 1980; Sparrow and Warr, 1984; Warn and Geeson, 1988; Ashburner, 1989) were either ineffective against these two species or at the concentrations effective against molds they were deleterious to fly stock as well. Although one possibility lies in trials of various combinations of the above mentioned drugs, we turned our attention to borate, an old and traditional medical disinfectant composed of boric acid and sodium tetraborate. After first and random trials, we could see successful prevention of mold growth in cultures transferred from infected vials which led to a series of tests to find optimal concentrations. Conclusively, we found that a solution of boric acid and sodium tetraborate (stock of 5% boric acid and 1% sodium tetraborate has pH 6.5 - 7.0) at final concentration around 0.005% and 0.001%, respectively, were effective in systematic treatment against growth and spreading mold infection. This solution was successfully used in agar-cornmeal-molasses-yeast medium as well as in commercial instant fly food Formula 4-24 (Carolina Biological Supply Company). This is a concentration we would recommend for initial treatment, although some higher doses can be used as well. Figure 1 illustrates fungicidal activity of a wide range of tested concentrations; borate solution below 0.001% boric acid and 0.0002% sodium tetraborate are ineffective. Higher concentrations of borate (0.05% boric acid and 0.01% sodium tetraborate) were too toxic to flies (Figure 2) albeit still effective against molds.

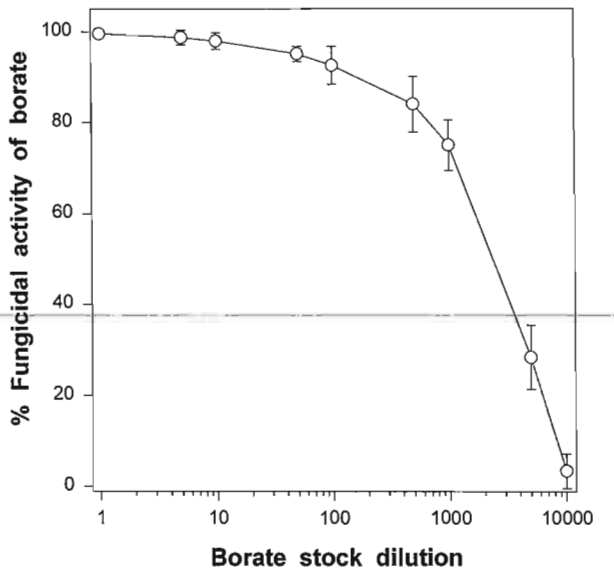

Figure 1 Efficiency of borate against molds expressed in percentage of non-infected vials. Various dilutions from undiluted up to 1:10,000 dilution, of the 5% boric acid and 1% sodium tetraborate, were tested for their ability to prevent/inhibit growth of fungi in new vials with flies transferred from infected cultures.

Systematic treatment with borate means continuous care of rescued stocks by transferring them to vials with fresh borate-containing food every 2-3 days. Developing larvae are picked from the food by clean forceps and transferred to vials with borate food; no adult flies are added to minimize contamination. Larvae were allowed to complete development, and if no molds were observed, newly emerged adults were transferred to vials with half-concentrated borate (0.0025% boric acid and 0.0005% sodium tetraborate). When the next generation with no fungal contamination was obtained, the culture was considered healthy and included among other stocks. Here it needs to be emphasized that handling and maintaining mold infected cultures has to be performed in a place separated from healthy stocks (quarantine). All plastic vials were discarded after use, whereas glass vials were baked in a hot oven (200°C) for 4 hr before reuse. Above procedure described here can appear more laborious than simple chemical treatment with other anti-

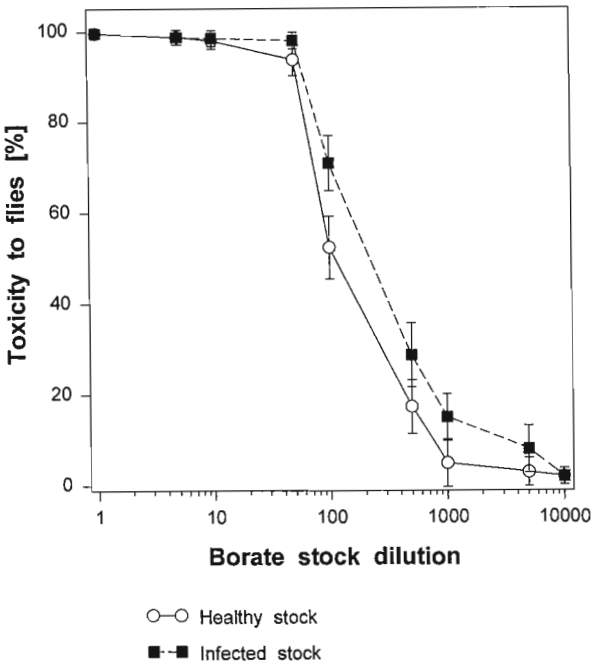

Figure 2 Toxicity of ingested borate to flies. Borate enters the larval body by ingestion, and the toxicity of various dilutions from undiluted up to 1:10,000 dilution, of 5% boric acid and 1% sodium tetraborate, was expressed as percentage of the vials giving progeny or not giving progeny after adult flies from infected cultures were transferred to them (black squares). For comparison, general toxicity of borate was also evaluated for flies from healthy cultures (white circles). The graph shows that flies from infected cultures are more sensitive to borate than healthy ones.

fungal agents, nevertheless it is giving satisfactory results in one generation time. This is apparently important for rescuing more difficult stocks.

Even though borate is generally known to be toxic to animals including insects (Appel, 1992; Heindel *et al.*, 1992; Ku *et al.*, 1993; Cochran, 1995), the concentrations used here in fly food to retard mold growth are deeply below toxicologically effective doses and are also ineffective in producing *eyeless* phenocopies described by Rapoport (1939, 1947) and Goldschmidt and Piternick (1957). The mechanism of borate action is not known yet, but it is assumed that it is capable of binding to serine and histidine in proteins thereby affecting their activity (Tsilikounas *et al.*, 1992; Mazurkiewicz *et al.*, 1993; Ikeda *et al.*, 1995). This might also be the way borate acts against molds. Borate drops are a widely spread ophthalmological disinfectant, and unbufferred 3 - 10% boric acid is often used for deep wound disinfections (Blech *et al.*, 1990; Borrelly *et al.*, 1991) and against various fungal infections in humans and animals (Meers and Chow, 1990; Shubair and Larsen, 1990; Jovanovic *et al.*, 1991; Nyirjesy *et al.*, 1995). Despite the fact that borate as any other chemical treatment can slightly affect duration of development, we believe that application of borate solution can serve as another alternative in elimination of fungal infections in *Drosophila* cultures.

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Lin, J. Department of Biological Sciences, Northwestern State University, Natchitoches, Louisiana, U.S.A. A polytene chromosome arms spreader.

To get a good spread of the larval salivary gland polytene chromosome arms by squashing method (Ashburner, 1989) is often beyond our reasonable control. There are many factors influencing the outcome of a spread which include the age of the larvae, strain of the insect, amount of fat bodies associated with the glands, the composition and temperature of the isolation solution, and most important of all, the strength and direction of tapping onto the coverslip of the preparation.

This paper describes an alternative method. The distinctive feature of this method is to offer a way to hold the coverslip and tossing the chromosomes up and down for the arms to be spread by capillary force. Again, the success of getting good spreads depends on the strength and frequency of tossing. I obtained good spreads more frequently with this device than with the conventional method.

The spreader I designed is made of small pieces of 1/4" and 1/16" thick plexiglass. The thickness is not critical. It can also be constructed of small pieces of wood. A 10 cc or 12 cc plastic syringe, a rubber cap of a Vacutainer blood collecting tube, a large paper clip, a used 2" × 2 1/2" photographic film or cut out from a plastic folder cover, and a small amount of superglue are the additional materials for the construction.

The different parts of the spreader in Figure 1 are prepared as follows:

a. Syringe plunger: A hole is drilled at the upper inner corner of one of the four fins of the syringe plunger with a needle flamed on a burner. The size of hole should only be slightly larger than the diameter of the wire of a large paper clip.

b. Plunger holder: A large paper clip is bent straight with fingers. A 1 1/2" section is cut out with a wire cutter, and one end is slipped in with a 3/8" length of insulation sleeve cut out from an electric wire. After threading the paper clip wire through the hole in the syringe plunger, another piece of insulation sleeve is slipped into the other end of the paper clip wire. The insulation sleeves serve the function of limiting the side travel of the plunger holder. The ends of the plunger holder are bent up as shown.

c. Body of the syringe: If the syringe you use has a colar outside the needle connecting end, you should cut it off with a sharp single side razor blade.

d. Syringe guide. A clear plastic film of 2" in length and 2 1/2" in width is wrapped around the syringe body. The overlapping ends are glued with small amount of superglue. After the syringe stand is constructed, the syringe guide will be glued to parts e and f at the edges of the large central holes.

e. Upper end of the syringe stand: A 1 1/2" × 3" × 1/4" plexiglass piece is marked in the center with a ring with a diamond pencil. The diameter of the ring should be about the same as the diameter of the syringe guide. Use flamed hot needle drill holes along the ring and remove the central piece. Smooth the central hole with a round file. Be sure the syringe guide can snuggly fit through the hole.

f. Cental board of the syringe stand: A 1 1/2" × 3" × 1/8" plexiglass is drilled with a central hole same as "e" above. The four corners of the board are notched (1/4" × 1/2" pieces) to fit to the four pillars of the stand.

g. Pillars of the stand: four 1/4" × 1/2" × 2" plexiglass pieces are cut and all sides smoothed with sand papers.

h. Bottom board of the stand: A 1 1/2" × 3" × 1/4" plexiglass without central hole or corners removed.

i. Vacutainer cap: The rubber cap of a Vacutainer blood collecting tube with a diameter of the large end slightly smaller than the diameter of the syringe body is bored with a small hole in the center with a cork borer. The syringe tip is poked through the hole in the rubber cap. The top of the rubber cap is glued to the end of the syringe body with superglue.

j. Coverslip: It is good to use siliconized round coverslip of 8 mm in diameter, which is close to the diameter of the small end of the rubber cap. The coverslip of 20 mm in diameter as shown in the photo is just for showing how the coverslip is sucked up at the end of the rubber cap, it can not be visible in the photo if a 8 mm diameter coverslip is used.

After all parts are prepared, the plexiglass parts and the syringe guide are glued together with superglue.

In practice, a microscope slide loaded with two to three pairs of salivary glands dissected from the third instar larvae is added with two drops of polytene chromosome pre-treatment solution (Kalish and Whitmore, 1986). The rubber cap of the syringe, which has been cleaned with 70% ethanol and dried, is pressed against the coverslip on a flat surface. With the plunger holder moved to the side and the plunger inserted all the way into the syringe, the plunger is pulled up quickly. Dropping of the plunger holder to the top of the syringe body to hold the plunger is automatic. The coverslip is held snuggly by vacuum with the center of the slip slightly bent in. The glands on the slide are then placed on the base of the syringe stand under the syringe guide from the side of the syringe stand (shown by arrow in Figure 1). The syringe assembly is inserted into the syringe guide down to the glands. Move the syringe assembly up and down gently for five to six times and exam the specimen under a phase microscope. Repeat the process if not enough spreading of the arms of

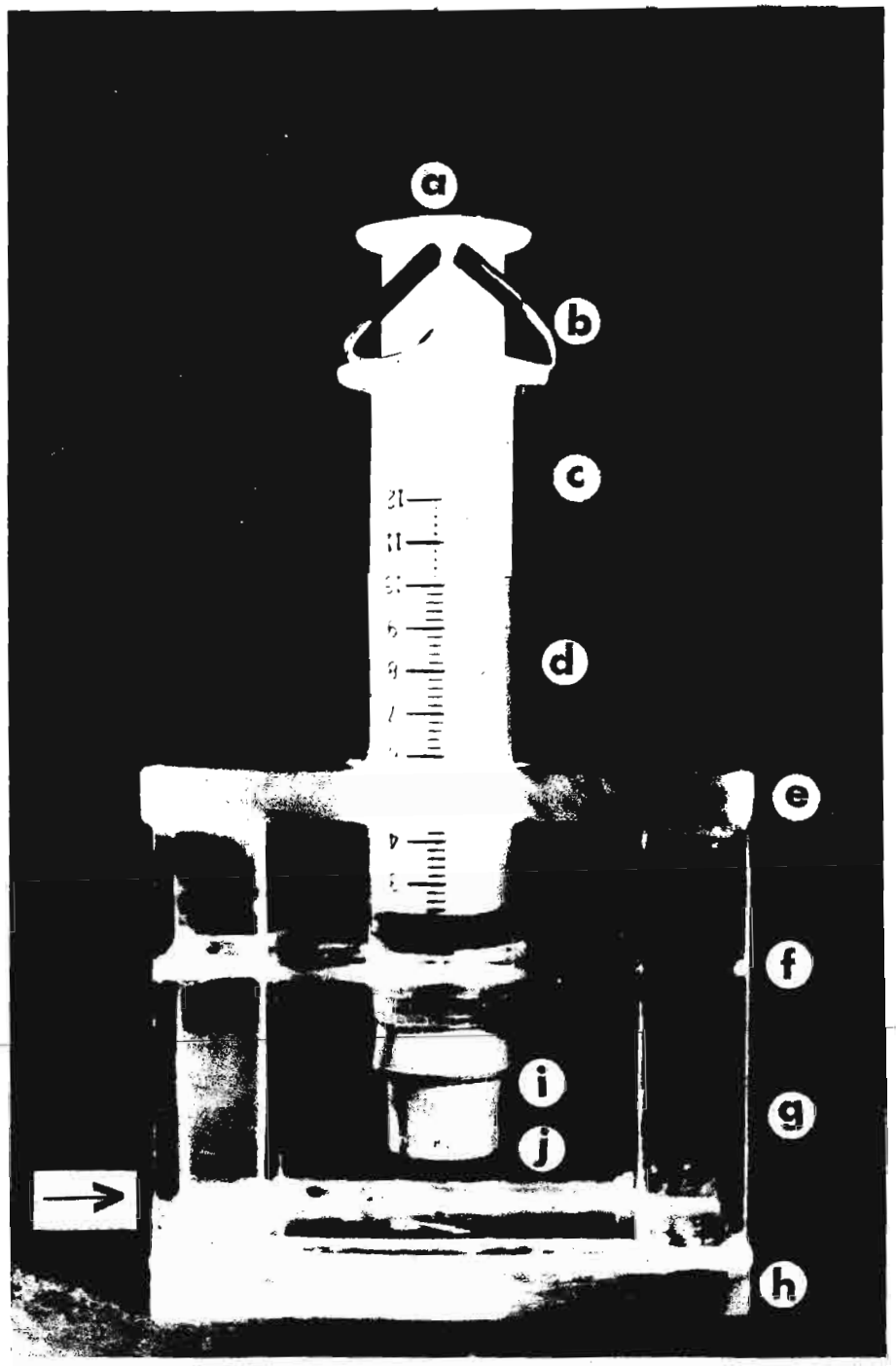

Figure 1. A polytene chromosome arms spreader.

chromosomes is obtained. If the spreading is satisfactory, drop the coverslip onto the microscope slide by moving the plunger holder to the side to break the vaccum. Further processing of the glands, such as removal of coverslip by freezing with liquid nitrogen and staining with special stains, will depend on the purpose of the investigation.

Acknowledgment: I want to thank Dr. Ken Williams for critical reading of the manuscript.

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Ashburner, M., 1989, Drosophila: *A Laboratory Handbook.* Cold Spring Harbor Laboratory Press; Kalisch, W.-E., and T. Whitmore 1986, Dros. Inf. Serv. 63:142-146.

Mutation Notes - Drosophila melanogaster

Report of I.F. Zhimulev, E.I. Volkova, V.F. Semeshin, and E.S. Belyaeva. Institute of Cytology and Genetics, Novosibirsk, 630090, Russia

New cytological data on some chromosome rearrangements in *D. melanogaster*.

Aberration	Data in FlyBase	New Cytology
Df(2R)E3363	47A; 47F	47A3; 47D12
Df(2R)Stan1	46D7-9; 47F15-16	44A1-2; 47F
Df(2R)Stan2	46F1-2; 47D1-2	46F1; 47B9
Df(2R)X-58-5	58B1-2; 59A1	58B3; 58F8
Df(2R)X58-6	58A3-B2; 58E3-10	58A3-4; 58E1-4
Df(2R)X58-7	58A1-2; 58E4-10	58B1-2; 58E1-4
Df(2R)X58-8	58A1-2; 58F3-5	58B3; 59A1
Df(2R)X58-11	58A3-4; 58E3-7	58B1-2; 58E1-4
Df(3L)Aprt 14	-	62B3; 62E1-2
Df(3L)Aprt 27	62B5-7; 62D6-E1	62B6; 62D7
Df(3L)Aprt 32	62A2-B1; 62E6-F1	62B1-2; 62E3
Df(3L)Aprt 65	62B8-9; 62D6-E4	62B3; 62D4
Df(3L)Aprt 72	62B2-4; 62D6-E4	62B6; 62E7
Df(3L)Aprt 105	62B2-4; 62D6-E1	62B3; 62E1-2
Df(3L)Aprt 112	62A10-B1; 62D6-E1	62B2; 62E1-2
Df(3L)Aprt 113	62B8-9; 62D6-E2	62B11; 62E2
Df(3L)Aprt 123	-	62B4-5; 62E1-2
Df(3L)Aprt 124	62B8-9; 62D6-E4	62B9; 62E1-2
Df(3L)Aprt 144	-	62C1-2
Df(3L)Aprt 148	62A7-B1; 62E6-F1	62B11; 62E1-2
Df(3L)Aprt 156	62B2-4; 62E6-F1	62B6; 62E9
Df(3L)Aprt 166	-	62B9; 62E1-2
Df(3L)Aprt 175	62B8-9; 62D6-E4	62B6; 62E1-2
Df(3L)Aprt 177	62B2-4; 62D6-E1	62B9; 62E2
Df(3L)Aprt 185	-	T(2;3)62B9; 49D
Df(3L)BK10	71E1-2; 71F4-5	71C3; 71E5
Df(3L) brm11	71F1-4; 72D1-10	72A3-4; 72C1
Df(3L) D-5rv14	70C2-3; 72A	70D-E1-2;71C1-2 + In(3L) 71C3; 72D1-2
Df(3L)GN19	63E6-9; 64B2-4	63F3; 64A12
Df(3L)GN34	63F7; 64A7-B2	63E6; 64A10
Df(3L)GN50	63E1-2; 64B17	63E2-3; 64B17
Df(3L)HR119	63C6; 63E	63C2; 63F7
Df(3L)R-G7	62B8-9; 62F2-5	62B9; 62E7
Df(3L) th102	71F3-5; 72D12	72A2; 72E12

Report of R.L. Williamson, B. Sun, P. Xu, and P.M. Salvaterra. Division of Neurosciences, Beckman Research Institute, City of Hope, Duarte, California 91010 U.S.A.

Correction on the cytogenetic location of *Nervana 1* and *2*.

We wish to correct a mistake that was made on the cytological location of *Nervana 1* and *2* (*Nrv1 and 2*) as reported by two of us (Sun and Salvaterra, 1995). These genes are homologous to Na^+, K^+-ATPase b subunits from other species (Sun and Salvaterra,1995). Figure 1 shows salivary polytene chromosomes of a *Df(2L)J-H/+* larva. *Df(2L)J-H* is deficient for 27C2-9;28B3-4 (Tiong and Nash, 1990). A section of Y chromosome spans the deficient segment and is not in polytene form and therefore appears as a gap in this region. Using the deficiency as a landmark and looking to the left, one sees 27B4-C1, then a barely visible 27B3 and then a dark line marking the position of the *Nrv 1* and *2* RNA probes hybridizing in the vicinity of 27B1-2. In other preparations, we have also seen that these probes hybridize to the left of the deficiency, $Df(2L)spd^{J2}$ (27C1-2;28A) (Neumann and Cohen, 1996).

Salivary gland preparations followed the method of Lin (1993) except that the slides used were precoated with polylysine from Sigma and then coated again with a brief dip in standard subbing solution of 1% gelatin 0.1% chromium potassium sulfate. Preparations were squashed between two taped bundles of slides. Pressure was applied with a vice grip as suggested by Hepperle (1995). Hybridization was carried out with digoxigenin-labeled *Nrv1* and *2* RNA probes, and visualized by incubation with alkaline phosphatase conjugated anti-digoxigenine antibody followed by development with bromchloroindolphenylphosphate/nitroblue tetrazolium solution.

Southern blots of *Sal*I digests of the Berkeley Genome Project P1 clones DS02944 from region 27A1-27B2 and DS00391 from region 27B1-27C1 were probed with three different genomic subclones: one from the 3' region of *Nrv1* and the others from either the 5' or 3' region of *Nrv2*. The 3' *Nrv1* and the 3' *Nrv2* probes exposed the same pattern of fragments, but the 5' *Nrv2* probe hybridized to DS00391 but not to DS02944. Both P1 clones share the 27B1-2 cytology, but the fact that part of *Nrv2* is missing from the DS02944 clone indicates that DS02944 terminates in that same region also. This would place *Nrv1* and *2* in 27B2.

Using the Berkeley Drosophila Genome Project BLAST search engine, we found that a 341bp shared sequence tagged site or (STS) (Dm80A2S) from the European Mapping Project (EMP) matches part of our *Nrv1* genomic DNA sequence. The STS has a primary *in situ* localization at 27B-C and a secondary site at 15D. The same search revealed a comment from Michael Ashburner to the EMP (1996) which noted the identity of the STS sequence as part of *Nervana* and its conflict with the published localization at 92C-D which we now recognize as incorrect. We did not detect a secondary site of hybridization.

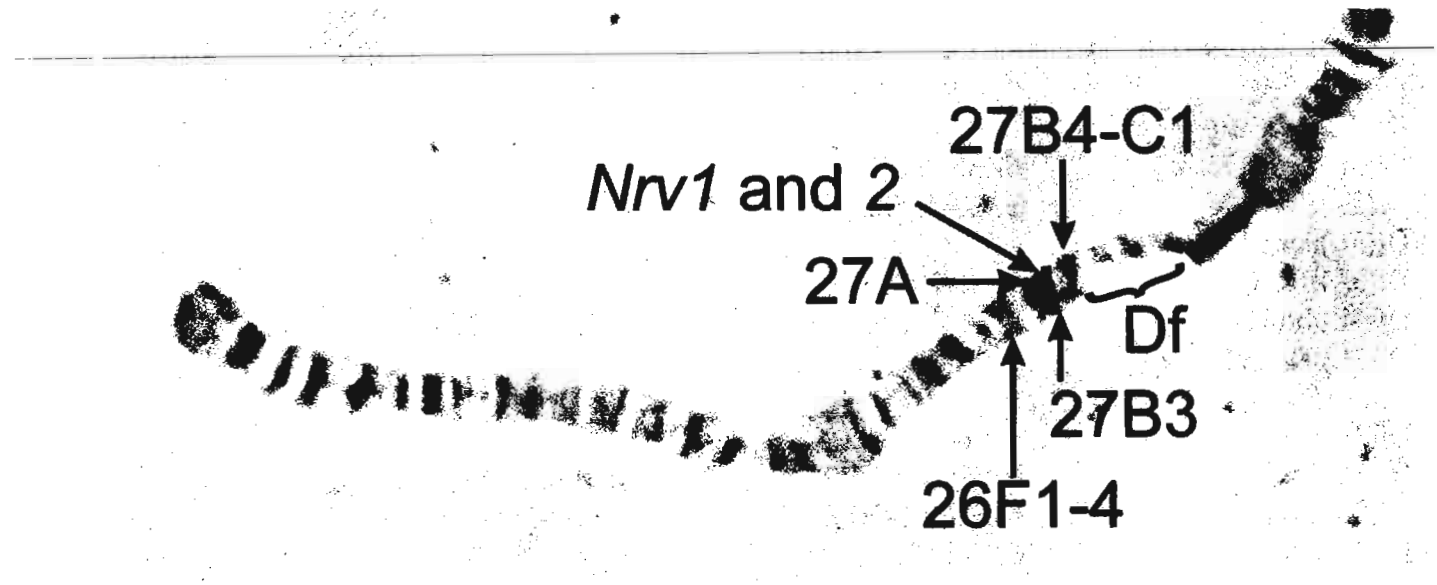

Figure 1. *In situ* hybridization of *Df(2L)J-H/+* polytene chromosomes marked with RNA probes to *Nrv1* and *2*. The heterozygous deficient region is indicated by Df. Cytogenetic identity is based upon comparisons with the maps of Bridges (1938, 1942) and Lefevre (1978) which were compiled by Lindsley and Zimm (1992). Photographic maps from FlyBase were also used.

Using the combined evidence we are assigning *Nervana 1* and *2* to 27B1-2.

Acknowledgments: We wish to thank Robert Barber and Kazuo Ikeda for help and advice with photography, Linda Iverson for helpful discussions, David Nash for sending us the *Df(2L)J-H* stock, the Bloomington Stock Center for providing *Df(2L)spd*^{i2} and to Lawrence Zipursky for P1 clones from the Berkeley Genome Project whose members we also thank.

References: Bridges, C.B., 1935, J. Hered. 26: 60-64; Bridges, P.N., 1942, J. Hered. 33: 403-408; Hepperle, B., 1995, Dros. Inf. Serv. 76: 175-176; Lefevre, G., 1976, *The Genetics and Biology of* Drosophila (Ashburner, M., and E. Novitski, eds.) Academic Press 1a: 31-66; Lin, J. K., 1993, Dros. Inf. Serv. 72: 73-77; Lindsley, D.L., and G.G. Zimm 1992, *The Genome of* Drosophila melanogaster. Academic Press; Neumann, C.J., and S.M. Cohen 1996, Genetics 142: 1147-1155; Sun, B., and P.M. Salvaterra 1995, Proc. Natl. Acad. Sci. USA 92: 5396-5400; Tiong, S.Y.K., and D. Nash 1990, Genetics 124: 889-897.

Mutation Notes - Other Species

Report of M.S. Krishna and S.N. Hegde. Drosophila Stock Centre, Department of Studies in Zoology, University of Mysore, Manasagangothri, Mysore 570 006, India.

A spontaneous mutation in *Drosophila malerkotliana.*

Drosophila malerkotliana is a member of the *bipectinata* complex of the *ananassae* subgroup of the *melanogaster* species group. No spontaneous or induced mutation has been described in this species so far. In the present study we report a spontaneous autosomal recessive mutation in *D. malerkotliana.*

In our laboratory we detected several females and males with spread wings in one stock which originated from a naturally inseminated isofemale line obtained from Janshi, India, in 1993. These spread-winged flies were aspirated out and maintained in vials containing wheat cream agar medium. These flies were used for making crosses. The crosses between spread-winged males and females produced spread-winged offspring indicating that the culture was pure for spread-wings.

Table 1. The normal, reciprocal and test crosses between normal and spread winged flies in *Drosophila malerkotliana.*

Class	Normal cross			Reciprocal cross			Test cross		
	Number observed (a)	Number expected (mn)	χ^2	Number observed (a)	Number expected (mn)	χ^2	Number observed (a)	Number expected (mn)	χ^2
Wild	246.00	241.50	0.04	221.00	214.50	0.10	86.00	83.50	0.04
Spread wing	76.00	80.50	0.12	65.00	71.50	0.30	81.00	83.50	0.04
Total	322.00	322.00	0.16	286.00	286.00	0.40	167.00	167.00	0.08

P_{value} = insignificant at 0.05 level.

The pattern of inheritance of spread-wings was studied by crossing spread-winged males with wild type females. In the reciprocal cross wild males and virgin mutants were used. In the F1 all the flies of both the crosses irrespective of sex were normal. This suggests the spread-winged mutant is an autosomal recessive gene. In the F2 progeny both wild and mutant flies appeared in a 3:1 ratio (Table 1). Thus these results suggest that the *spread winged* mutant (*spw*) is inherited as a normal Mendelian trait. The test cross results confirm the monofactorial inheritance of the *spread winged* gene. This could be the first report of mutation in *D. malerkotliana.*

Acknowledgments: The authors are grateful to the Chairman, Department of Studies in Zoology, University of Mysore for providing facilities.

Report of E. Solé and F. Mestres. Dept. Genètica, Fac. Biologia, Universitat de Barcelona, 08071 Barcelona, Spain.

One-winged flies obtained again in *D. subobscura.*

While carrying out lethal allelism in the population of Centralia (Washington) one-winged flies arose in one of the crosses. Two females and one male were found. Both females presented only the right wing and the male the left

wing. This trait has been observed in other experiments in which the *Va/Ba* balanced lethal strain was involved (Mestres and Busquets, 1991; Orengo and Mestres, 1993; Orengo *et al.*, 1997). The present finding seems to confirm that factors producing this phenotype are located in the *Va/Ba* strain.

References: Mestres, F., and D. Busquets 1991, Dros. Inf. Serv. 70: 145-146; Orengo, D. J., and F. Mestres 1993, Rev. Brasil. Genet. 16: 471-475; Orengo, D. J., E. Hauschteck-Jungen and F. Mestres 1997, Bras. J. Genet. 20: 359-361.

Announcements

Availability of Cheap Glass Vials

Information provided by Robert Farkas, Institute of Experimental Endocrinology, Slovak Academy of Sciences, Vlarska 3, 833 06 Bratislava, Kramare, Slovakia. email: ueenfark@savba.savba.sk

Cheap glass vials for flies can be obtained from:

Sklarny Kavalier (Glassworks Kavalier) Co.
Zavod 03
41752 Hostomice
Czech Republic
Fax: (00420417) 926-888

They manufacture vials of various sizes according to customer's request; mostly 22-30 mm wide in diameter and 90-100 mm high. Customer may provide own technical picture of the vial. To my knowledge minimum order was 10,000 pcs, and the price according to my calculation was around $ 0.10 per vial, but I do not know price change for overseas operations. Besides my friends and I from Czech Academy of Sciences, recently my colleagues from Heidelberg, Germany and Drosophila Stock Center in Szeged, Hungary ordered large numbers of fly vials from this source, and they have been satisfied.

Orcein

Information provided by Robert Farkas, see address above.

Orcein for microscopy, which for a long time was on the market only as a synthetic compound, was not perfectly suited for staining polytene chromosomes. It has become available recently as natural orcein from Polysciences Inc., Warrington, PA 18976-2590, phone: 800-523-2575 under catalog number 19936. This price is higher than for the synthetic one; again, according to my calculations (because I bought it from Polysciences GmbH in Germany and paid in German marks) is about $70 for 5 g.

Conference Programs

In an effort to provide as diverse a source of information on *Drosophila* genetic research as possible, Drosophila Information Service will print programs for research conferences whenever space allows. The editor invites conference organizers or participants to submit copies of meeting programs and a brief description of the theme, location, and time the conference was held. DIS will endeavor to publish the names of authors, the affiliation of the senior author or corresponding presenter, and the title of the talks or posters. This can then be used by readers of DIS to locate individuals pursuing problems of common interest, locate possible postdoctoral researchers, and find sources of materials or information that may not have yet been published in other journals. Even if space does not permit publishing all proceedings, we hope that a listing of regional and national conferences will be useful.

39th Annual *Drosophila* Research Conference
25 to 29 March 1998, Washington, D.C., U.S.A.

The 39th Annual *Drosophila* Research Conference was held at the Omni Shoreham Hotel in Washington, D.C., and the 1998 Program Chairs were Kristin White (Massachusetts General Hospital), Laurel A. Raftery (Massachusetts General Hospital), and Terry L. Orr-Weaver (Whitehead Institute). The conference was sponsored by the Genetics Society of America, 9650 Rockville Pike, Bethesda, Maryland 20814-3998. Large numbers of slide presentations and posters were presented. The plenary session lectures are listed here, grouped by topic as in the conference program.

Gelbart, William (Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138). FlyBase: A New Look.

Green, M.M. (Section of Molecular and Cellular Biology, University of California, Davis, CA 95616). Historical Perspective.

Schedl, Paul (Department of Molecular Biology, Princeton University, Princeton, NJ 08544). Sex lethal gene function.

Sokolowski, Marla (Department of Biology, York University, North York, Ontario, Canada). Fine fly dining: A role for cGMP dependent protein kinase.

Hariharan, Iswar (Massachusetts General Hospital Cancer Center, Charleston, MA 02129). Negative regulators of cell proliferation.

Cagan, Ross (Department of Molecular Biology and Pharmacology, Washington University Medical School, St. Louis, MO 63110). Life, death, and patterning the fly eye.

Zusman, Susan (Department of Biology, University of Rochester, Rochester, NY 14627). Diverse requirements for *Drosophila* integrins and integrin functional domains.

Beachy, Phil (Department of Molecular Biology and Genetics, Johns Hopkins University Medical Center, Baltimore, MD 21205). Hedgehog protein biogenesis and signaling.

Hoffmann, Jules (Institut de Biologie Moléculaire et Cellulaire, CNRS, Strasbourg, France). The immune response of *Drosophila*.

Klämbt, Christian (Institute of Neurobiology, University of Münster, Münster, Germany). Glia development in the embryonic CNS of *Drosophila*.

Feder, Martin (Department of Organismal Biology and Anatomy, University of Chicago, Chicago, IL 60637). From benchtop to treeside: Functional consequences of transgenic and natural variation in genes encoding the heat-shock protein Hsp70.

Fischer, Janice (Department of Zoology and Institute for Cellular and Molecular Biology, University of Texas at Austin, Austin, TX 78712). Ubiquitin and cell communication.

Sullivan, Bill (Department of Biology, University of California, Santa Cruz, CA 95064). Coupling the cell cycle to development in the early embryo.

Rubin, Gerald (Department of Molecular and Cell Biology, HHMI, University of California, Berkeley, CA 94720-3200). The *Drosophila* Genome Project.

Announcements

Request for Materials

Request from Bruno Contreras Moreira, Centro de Biologia Molecular, C-X 504, Universidad Autonoma de Madrid, Cantoblanco, 28049 Madrid, Spain. (email: bcontreras@trasto.cbm.uam.es)

I am mapping the 3L arm and I need the following stocks or information about any P-inserted stocks in the 76-77 cytological region. Thank you for any help you can provide.

P{UChs:neo}l(3)27, mwh red e/TM3, ry[RK] Sb e (cytological insertion 76A)
w[1118]; P{w[+] = lacW}Y0397, ry[506] (c.i.76A-B)
TE{w[a] rst[+]}4; y w[-] rst[-]/y[+]Y (c.i.76E-F)
TE{w[+] rst[+]}44; y w[-] rst[-]/y[+]Y (c.i.77A)
TE{w[+] rst[+]}181; y w[-] rst[-]/y[+]Y (77A)
w[1118]; P{w[+] = lacW}Y460, ry[506] (c.i.77B-C)

Call for Papers

Submissions to Drosophila Information Service are welcome at any time. Typically, we would like to have submissions no later than 1 May to insure their inclusion in the regular annual issue. Submissions in Microsoft Word, which is now the program we use for our page setup, are especially helpful. Submissions by email are also possible, but if they are sent as attached files, we have greatest success using MS Word or ASCII format. In all instances, especially where tables are concerned, it is useful to have a paper copy to facilitate accurate formatting. Details are given in the Guide to Authors.

Standing Orders

Several years ago, formal standing orders were discontinued due to the need to obtain prepayment for issues. "Standing Orders" are now handled through a mailing list of active subscribers. All individuals on the active subscriber list will receive notices for forthcoming regular and special issues and a Standing Order Invoice to facilitate prepayment. If you would like to be added to the Drosophila Information Service mailing list, please write to the editor, Jim Thompson, Department of Zoology, University of Oklahoma, Norman, OK 73019.