A Three-Part Laboratory Exercise Using Flightless Fruit Flies (*Drosophila melanogaster*) to Study Modes of Inheritance

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Abstract: This is an inquiry-based investigation of genetic modes of inheritance using flightless *Drosophila* as the experimental organism. We present the three-part laboratory writeup, suitable for use as a student handout. We describe the flightless mutant strains and where they may be obtained, how to use carbon dioxide as fly anesthesia, various molecular websites for students to research the mutant genes, and helpful hints for setting up the laboratory. Practical benefits of using flightless flies include convenience in handling the organisms by inexperienced students, and reduced likelihood of flies escaping to invade other areas of the school building.

Introduction

This objective of this exercise is for students to perform an inquiry-based investigation of genetic modes of inheritance using flightless fruit flies as the experimental organism (see Chinnici *et al.*, 2005). We have used this exercise in introductory general education science college courses for non-science majors, and many high school advanced biology courses also use this exercise. Students learn how to anesthetize the flies, distinguish male and female fruit flies, identify unknown (to them) mutant traits by comparing mutant and wild-type flies, and determine genetic modes of inheritance for their mutant type by setting up parental, F1, and F2 generation crosses and observing the offspring of these crosses. They analyze their F2 generation data using chi-square analyses. The exercise consists of three 90-120 minute sessions each separated by two weeks (performed on days 1 [week 1], 15 [week 3], and 29 [week 5]) to allow the offspring of each generation to develop into adults. Other lab exercises may be performed on days 8 [week 2] and 22 [week 4].

Materials

Here, we describe the flightless flies and where they may be purchased, how to use carbon dioxide as an alternative to "Fly Nap" for fly anesthesia, various molecular websites for students to research the different mutants, and helpful hints for setting up the laboratory.

Flightless Fruit Flies

Ten years ago, one of the authors (JPC) constructed the various mutant strains of flightless flies used in this exercise. The available flightless strains of *Drosophila* are *white eyes, white-apricot eyes, yellow body, singed wings, and cut wings* (all recessive X-linked traits); *Bar eyes* (a dominant X-linked trait); *dumpy wings, vestigial wings, scarlet eyes, sepia eyes, ebony body, apterous wings,* and *eyeless eyes* (all autosomal recessive traits). Each of these mutant strains has the X-linked recessive trait *miniature wings* fixed in its genetic background. The "wildtype" or normal strain used in exercises with these mutants is the *miniature wing* strain. Thus, *miniature wing* is the genetic "standard" for all these strains. The practical benefit of using flightless flies is convenience in handling flies by typically inexperienced students, and no likelihood of flies escaping the laboratory to invade other areas of the school building.

Carolina Biological Supply Company carries the flightless fruit fly kits and individual strains. Go to <carolina.com>, type in "flightless fruit flies" and select "teacher resources" for more information about use of the flies. At the end of the article *Using Flightless Fruit Flies in the Genetics Teaching Lab*, click on "Flightless Fruit Fly Kits" and, then, "Flightless Fruit Fly Mutants" for more information.

Using CO₂ As An Anesthetic

As an alternative to using "Fly-Nap" for fly anesthesia, one of us (RK) uses carbon dioxide. Once a CO_2 delivery system is constructed in a lab room, it provides a convenient, odor-free means for students to anesthetize flies. Flies stay "out" as long as CO_2 is supplied, but they recover within minutes when removed from the CO_2 . Different CO_2 delivery systems can vary a lot in details of construction. Here, we describe the system we built into RK's teaching labs at the University of Delaware and add some comments on where the system at the University of Kentucky differs from ours.

Tanks, regulators, control valves. We installed two CO_2 tanks in each of our lab rooms. Each tank has its own two-stage regulator (Fisher 10-572E) and the piping from the two tanks come together in a **T**, where a 3-way selector valve (McMaster Carr 4373K51) allows the operator to choose which tank is in use. This makes it possible for a lab instructor to restore the CO_2 supply simply by throwing the selector valve to the second tank if one tank is emptied during a class activity. Each tank has a lever-style shut off valve (McMaster Carr 4726K72) installed between its regulator and the selector valve.

Piping from tank to work stations. The University of Kentucky has an ideal set up for distributing CO_2 around the room. Their labs were built with gas cocks at each student seat and they converted that system to CO_2 . At Delaware, we had to install our piping from scratch. An early version consisting of 1/2" Tygon tubing running down each bench was workable but cumbersome. We upgraded by installing 3/4" c-PVC tubing underneath each bench, with a branch point at each student station. Each branch consists of a length of amber gas-line tubing (Fisher 14-178 2B), which steps down to aquarium airline tubing.

Student stations. Our student stations use flexible silicone aquarium airline tubing (Penn Plax STD25) and a two-valve aquarium airline gang valve (Penn Plax VN2). From the gang valve, one line is attached to a 16G 3" hypodermic needle (Fischer 14-817-103) with the tip cut off; students use this by inserting the needle into a vial or bottle to anesthetize the flies before dumping them onto a working platform. The second line from the gang valve goes to the working platform, which students use to inspect and sort flies under the dissecting microscope. Connections in the airline tubing are made using Luer fittings (Value Plastics FTLL230-1 and MTLL230-1).

My choice of material for building student work platforms is floral foam. It has high resistance to gas flow but disperses the gas very uniformly. I buy bricks (9" x 3" x 41/4") at a local florist, and cut them to 1" x 3" x 23/4" blocks on a band saw (wearing a respirator is important - the dust is irritating). A channel for the gas to enter the block is created by pushing the handle of a flysorting paint brush (Carolina 17-3094) down the center of the block, starting in the center of the smallest face and extending about 7/8 of the length of the block. Each block is covered on the bottom and four sides with card stock, as a gas barrier. A hole punched into the card stock at one end accommodates a Luer fitting (FTLL230-1) that fits into the opening of the gas channel down the center of the block. The top and four sides of the block are covered with Whatman #1 filter paper, cut, folded, and taped to the bottom of the block to form a flat, smooth, gas-permeable working surface for sorting flies.

Several other materials may make suitable substitutes for floral foam. I have used cellulose sponges, Styrofoam (though most Styrofoam packaging is impervious to gas), and upholsterer's foam. The University of Kentucky built working platforms using the bottom half of pipette-tip boxes covered with Mylar fabric. The CO_2 in their system is dispersed by an aquarium air stone in the hollow base of the pipette-tip box.

Student Outline

We present the three-part laboratory exercise write-up, suitable for use as a student handout, in APPENDIX A. MS-Word formatted files of these three exercises are available by emailing JPC (joechin@vcu.edu).

Materials and Equipment

Initially, (week 1) students work in groups of two or three. Each group receives three *miniature wing* cultures; a "wild-type" culture containing male and female flightless flies with the *miniature wing* trait only; an "unknown" mutant culture with males and females possessing one mutant trait; and, a culture containing only virgin wild-type females. Each group also receives an empty culture vial (in which to place sleeping flies) and an anesthetizing chamber (an empty vial with a foam stopped through which a wand from the "Fly-Nap" kit is placed) if "Fly-Nap" is used. Each group also uses a dissecting microscope, index cards (on which sleeping flies are placed for viewing and sorting), and either toothpicks or a small artists watercolor brush (for pushing the flies around on the index cards). For the second part of the exercise (week 3), each student in the groups receives a food vial without flies, in which to place some F1 flies to generate the F2 generation. For the third part of the exercise (week 5), each student in the class will need a dissecting microscope in order to collect data from his/her vial of flies set up in week 3, as well as an individual anesthetizing chamber.

Notes for the Instructor

Culture Medium

We use Instant *Drosophila* food as the culture medium for maintaining our stocks and for the experimental procedures: go to <www.carolina.com > and type in "17 3200" (for white medium) or "17 3210" (for blue medium). To prepare student fly cultures, first, we add the dry Instant *Drosophila* medium to a vial. Then, we sprinkle in some dry yeast (obtained from the supermarket). When adding water, we use distilled water if available, or jugs of drinking water from the supermarket. We avoid using tap water due to the risk of introducing mold into the cultures.

In addition, we add a small rectangle of white paper toweling impregnated with "Tegosept" mold inhibitor to each vial. Tegosept may be ordered fro Carolina Biological Supply Co.: <<u>https://www2.carolina.com/webapp/wcs/stores/servlet/ProductDisplay?jdeAddressId=&catalogId=10101&storeId=10151&productId=23738&langId=-1&parent_category_rn=&crumbs=n></u>. We mix 10 grams of Tegosept powder into 100 ml of 100% ethyl alcohol and soak full sheets of white paper toweling, squeezing the excess fluid out, and then hanging the wet towels up on a clothesline until the alcohol evaporates. Then, we cut the paper towels into small rectangles (1 x 4 inches = 2.5 x 10 cm). We then push the "tego-strip" into the surface of the fly-food in the vial with the handle of a small artist's watercolor brush. These "tego-strips" accomplish two purposes: protect against mold infestation, and give the larvae more surface area for pupation.

Collecting "Virgin" Female Drosophila

In the parental generation, "virgin" (previously unmated) wild-type females are crossed with mutant males. We collect the virgin females for the students to use, since the entire experiment will be ruined if the parental generation females are not virgins, a trivial reason for students to have their experiments fail. Virgin females are easy to collect, since female fruit flies cannot accept sperm from males until they are at least four hours post-emergence from their pupal cases (it takes that time for them to expel larval wastes from their seminal receptacles). So, a few days before the beginning of the exercise, we clear all the wild-type culture vials of adults at 10:00AM, then return three hours later (1:00PM) and collect any "new" females which have emerged, knowing that they are virgins. We repeat this again three hours later (4:00PM) and collect more virgin females. If needed, we do this again the following day. Then, the day before the exercise begins, we place 6-8 virgin females each in fresh food vials and have students add mutant males to these vials to begin the parental generation crosses.

Alternatively, one may ask the students to collect their own virgin females, but this would be burdensome to the instructor who would have to clear the wild-type vials for each group of students three hours before they collect the virgins. In addition, in very young flies, it is more likely for inexperienced students to mis-sex the flies since the pigmentation is quite pale in newly emerged flies, increasing the chance for error. Adding virgin males to the parental cross would ruin the experiment.

On days 8 (week 2) and 22 (week 4), either the instructor or the students must remove the adult flies from their vials, so that when the next generation of adults emerges several days later, they will not intermingle and mate with their parents (thus ruining the experiment).

Molecular Websites for Researching the Mutant Genes

Since we live in a molecular age, students should become exposed to some of the molecular aspects of fruit fly genetics. One way to incorporate some molecular biology into this exercise is to have students submit a report on some molecular aspects of the particular mutant gene they are following in their crosses. A good place for them to begin is at the "WWW Virtual Library: Drosophila" website: <<u>http://www.ceolas.org/fly/</u>>. Then, the student might go to the "National Biotechnology Information" website for Drosophila Center for melanogaster <http://www.ncbi.nlm.nih.gov/genome/guide/fly/> and finally to <http://www.ncbi.nlm.nih.gov/sites/entrez> where one selects "Gene" for Search and then types in the mutant name (for instance, "white"). APPENDIX B lists some results for various mutant genes. An alternative molecular approach is to go to the FlyBase website, "A Database of Drosophila Genes & Genomes" < http://www.flybase.org/>. Here, one can simply select "genes" for Data Class (or "alleles" for "cut-6") and then type in the mutant name (for instance, "miniature"). APPENDIX C lists some results for various mutant genes or alleles.

Literature Cited

Chinnici, J. P., A. M. Farland, and J. W. Kent. 2005. An Inquiry-Based Investigation of Modes of Inheritance Using "Flightless" Fruit Flies. The American Biology Teacher 67:38-44.

About the Authors

Dr. Joseph P. Chinnici received his A.B. in Biology from LaSalle University in 1965, and his Ph.D. in Biology from the University of Virginia in 1970. He has been a faculty member in the Biology Department at Virginia Commonwealth University in Richmond, VA since 1970. Currently, he is an Emeritus Associate Professor of Biology and Life Sciences at VCU. In 2001, he was awarded the Distinguished Teaching Award from the College of Humanities and Sciences at VCU. Dr. Chinnici has published over 35 papers in a variety of research and teaching journals and has been a PI or co-PI on several federal and state research grants totaling over five million dollars.

Dr. Robert B. Ketcham earned his undergraduate degree at Wesleyan University and his Ph.D. degree at the University of Delaware. He currently works as Laboratory Coordinator in the Department of Biology at the University of Delaware, managing the laboratory component of the non-science majors' biology class.

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APPENDIX B. Molecular Websites of Interest for Drosophila Mutants

<u>http://www.ceolas.org/fly/</u> [The WWW Virtual Library: *Drosophila*. This directory points to internet resources for research on the fruit fly *Drosophila melanogaster*]

http://www.ncbi.nlm.nih.gov/ [National Center for Biotechnology Information]

<u>http://www.ncbi.nlm.nih.gov/sites/entrez</u> [type in Drosophila and mutant name (e.g., Drosophila white)]. Some results are listed below. If one clicks on the gene symbol, a complete description appears (the longer website at the end of each summary).

white [Drosophila melanogaster] Other Aliases: Dmel_CG2759, BACN33B1.1, CG2759, DMWHITE, EG:BACN33B1.1, unnamed, w(AT)[[13]] Other Designations: white CG2759-PA Chromosome: X; Location: 3B6-3B6 Annotation: Chromosome X, NC_004354.3 (2684632..2690499, complement) GeneID: 31271 http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=31271&ordinalpos=9&ito ol=EntrezSystem2.PEntrez.Gene.Gene ResultsPanel.Gene RVDocSum

ebony [Drosophila melanogaster] Other Aliases: Dmel_CG3331, CG3331 Other Designations: ebony CG3331-PA Chromosome: 3R; Location: 93C7-93D1 GeneID: 42521 http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=42521&ordinalpos=1&ito ol=EntrezSystem2.PEntrez.Gene_Gene_ResultsPanel.Gene_RVDocSum

yellow [Drosophila melanogaster] Other Aliases: Dmel_CG3757, CG3757, EG:125H10.2, T6 Other Designations: yellow CG3757-PA Chromosome: X; Location: 1A5-1A5 Annotation: Chromosome X, NC_004354.3 (250542..255278) GeneID: 30980 http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=30980&ordinalpos=17&it ool=EntrezSystem2.PEntrez.Gene.Gene_ResultsPanel.Gene_RVDocSum

vestigial [Drosophila melanogaster] Other Aliases: Dmel_CG3830, CG3830, VG, vg21 Other Designations: vestigial CG3830-PA Chromosome: 2R; Location: 49E1-49E1 GeneID: 36421 http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=36421&ordinalpos=15&it ool=EntrezSystem2.PEntrez.Gene.Gene_ResultsPanel.Gene_RVDocSum

cut [Drosophila melanogaster] Other Aliases: Dmel_CG11387, CG11387, Ct, Cut, kf Other Designations: cut CG11387-PA, isoform A; cut CG11387-PB, isoform B Chromosome: X; Location: 7B4-7B6 Annotation: Chromosome X, NC_004354.3 (7503181..7570056) GeneID: 44540 http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=44540&ordinalpos=2&ito ol=EntrezSystem2.PEntrez.Gene.Gene_ResultsPanel.Gene_RVDocSum

apterous [Drosophila melanogaster] Other Aliases: Dmel_CG8376, CG8376, LIM, S-2a, Xa, blt Other Designations: apterous CG8376-PA, isoform A; apterous CG8376-PB, isoform B Chromosome: 2R; Location: 41F8-41F8 GeneID: 35509 http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=35509&ordinalpos=27&it

ool=EntrezSystem2.PEntrez.Gene.Gene_ResultsPanel.Gene_RVDocSum

Bar [Drosophila melanogaster] Other Aliases: FBgn0000154, BB, Bar eye, BarH1, InfraBar, Ultrabar, bar Chromosome: 1; Location: 1-57.0 GeneID: 44798 This record was discontinued. http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=44798&ordinalpos=1&ito ol=EntrezSystem2.PEntrez.Gene.Gene_ResultsPanel.Gene_RVDocSum

BarH1 [Drosophila melanogaster] Other Aliases: Dmel_CG5529, BH1, Bar, Bar H1, Bar-H1, BarHI, CG5529, barH1 Other Designations: BarH1 CG5529-PA Chromosome: X; Location: 16A4-16A5 Annotation: Chromosome X, NC_004354.3 (17291534..17297312) GeneID: 32724 http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=32724&ordinalpos=4&ito ol=EntrezSystem2.PEntrez.Gene.Gene_ResultsPanel.Gene_RVDocSum

BarH2 [Drosophila melanogaster] Other Aliases: Dmel_CG5488, B, BH2, Bar, Bar-H2, CG5488 Other Designations: BarH2 CG5488-PA Chromosome: X; Location: 16A1-16A1 Annotation: Chromosome X, NC_004354.3 (17208614..17218195) GeneID: 32723 http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=32723&ordinalpos=5&ito ol=EntrezSystem2.PEntrez.Gene.Gene ResultsPanel.Gene RVDocSum

dumpy [Drosophila melanogaster] Other Aliases: Dmel_CG33196, CG15637, CG33196, CT35799, DP, SP460 Other Designations: dumpy CG33196-PB Chromosome: 2L; Location: 24F4-25A1 GeneID: 318824 http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=318824&ordinalpos=1&it ool=EntrezSystem2.PEntrez.Gene.Gene ResultsPanel.Gene RVDocSum

singed [Drosophila melanogaster] Other Aliases: Dmel_CG32858, CG1536, CG32858, Sn, fs(1)K418, fs(1)M45 Other Designations: singed CG32858-PA, isoform A; singed CG32858-PB, isoform B; singed CG32858-PC, isoform C Chromosome: X; Location: 7D1-7D2 Annotation: Chromosome X, NC_004354.3 (7858057..7880134) GeneID: 31717 http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=31717&ordinalpos=1&ito

ol=EntrezSystem2.PEntrez.Gene.Gene ResultsPanel.Gene RVDocSum

scarlet [Drosophila melanogaster] Other Aliases: Dmel_CG4314, CG4314 Other Designations: scarlet CG4314-PA Chromosome: 3L; Location: 73A3-73A3 GeneID: 39836 http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=39836&ordinalpos=1&ito ol=EntrezSystem2.PEntrez.Gene.Gene ResultsPanel.Gene RVDocSum

[sepia] CG6781 [Drosophila melanogaster] Other Aliases: Dmel_CG6781 Other Designations: CG6781-PA Chromosome: 3L; Location: 66D5-66D5 GeneID: 38973 http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=38973&ordinalpos=1&ito ol=EntrezSystem2.PEntrez.Gene.Gene_ResultsPanel.Gene_RVDocSum

brown [Drosophila melanogaster] Other Aliases: Dmel_CG17632, CG17632, Pm, Su(w[coJ]), unnamed Other Designations: brown CG17632-PA Chromosome: 2R; Location: 59E2-59E3 GeneID: 37724 http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=37724&ordinalpos=60&it ool=EntrezSystem2.PEntrez.Gene.Gene_ResultsPanel.Gene_RVDocSum

eyeless [*Drosophila melanogaster*] Other Aliases: Dmel_CG1464, CG1464, DPax-6, EYEL, Ey, Ey/Pax6, Pax-6, Pax6, eye, l(4)33 Other Designations: eyeless CG1464-PA, isoform A; eyeless CG1464-PB, isoform B; eyeless CG1464-PC, isoform C; eyeless CG1464-PD, isoform D Chromosome: 4; Location: 102C2-102C2 Annotation: Chromosome 4, NC_004353.3 (718315..741787) GeneID: 43812 http://www.ncbi.nlm.nih.gov/sites/entrez?Db=gene&Cmd=ShowDetailView&TermToSearch=43812&ordinalpos=1&ito ol=EntrezSystem2.PEntrez.Gene.Gene ResultsPanel.Gene RVDocSum

APPENDIX C. More Molecular Websites of Interest for Drosophila Mutants

http://www.flybase.org/ [FlyBase: A Database of Drosophila Genes & Genomes]

choose "genes", type in "miniature": <u>http://www.flybase.org/cgi-</u> <u>bin/uniq.html?species=Dmel&field=SYN&db=fbgn&context=miniature&authors=&year=&alltext=&caller=quicksearch</u> select "m": <u>http://www.flybase.org/reports/FBgn0002577.html</u>

choose "genes", type in "Bar": <u>http://www.flybase.org/cgi-</u> <u>bin/uniq.html?species=Dmel&field=SYN&db=fbgn&context=Bar&authors=&year=&alltext=&caller=quicksearch</u> select "B-H1": <u>http://www.flybase.org/reports/FBgn0011758.html</u> select "B-H2": <u>http://www.flybase.org/reports/FBgn0004854.html</u>

choose "genes", type in "white": <u>http://www.flybase.org/cgi-</u> <u>bin/uniq.html?species=Dmel&field=SYN&db=fbgn&context=white&authors=&year=&alltext=&caller=quicksearch</u> select "w": <u>http://www.flybase.org/reports/FBgn0003996.html</u>

choose "genes", type in "white apricot": <u>http://www.flybase.org/cgi-</u> <u>bin/uniq.html?species=Dmel&field=SYN&db=fbal&context=white&authors=&year=&alltext=&caller=quicksearch</u> select "w^a": <u>http://www.flybase.org/reports/FBal0018195.html</u>

choose "genes", type in "yellow": <u>http://www.flybase.org/cgi-</u> <u>bin/uniq.html?species=Dmel&field=SYN&db=fbgn&context=yellow&authors=&year=&alltext=&caller=quicksearch</u> select "y": http://www.flybase.org/reports/FBgn0004034.html

choose "genes", type in "cut": <u>http://www.flybase.org/cgi-</u> <u>bin/uniq.html?species=Dmel&field=SYN&db=fbgn&context=cut&authors=&year=&alltext=&caller=quicksearch</u> select "ct": http://www.flybase.org/reports/FBgn0004198.html

choose "alleles", type in "cut": <u>http://www.flybase.org/cgi-</u> <u>bin/uniq.html?species=Dmel&field=SYN&db=fbal&context=cut&authors=&year=&alltext=&caller=quicksearch</u> select "ct^6": <u>http://www.flybase.org/reports/FBal0001934.html</u>

choose "genes", type in "dumpy": <u>http://www.flybase.org/cgi-</u> <u>bin/uniq.html?species=Dmel&field=SYN&db=fbgn&context=dumpy&authors=&year=&alltext=&caller=quicksearch</u> select "dp": http://www.flybase.org/reports/FBgn0053196.html choose "genes", type in "vestigial": <u>http://www.flybase.org/cgi-</u> <u>bin/uniq.html?species=Dmel&field=SYN&db=fbgn&context=vestigial&authors=&year=&alltext=&caller=quicksearch</u> select "vg": http://www.flybase.org/reports/FBgn0003975.html

choose "genes", type in "scarlet": http://www.flybase.org/reports/FBgn0003515.html

choose "genes", type in "sepia": http://www.flybase.org/reports/FBgn0086348.html

choose "genes", type in "brown": <u>http://www.flybase.org/cgi-</u> <u>bin/uniq.html?species=Dmel&field=SYN&db=fbgn&context=brown&authors=&year=&alltext=&caller=quicksearch</u> select "bw": <u>http://www.flybase.org/reports/FBgn0000241.html</u>

choose "genes", type in "ebony": <u>http://www.flybase.org/cgi-</u> <u>bin/uniq.html?species=Dmel&field=SYN&db=fbgn&context=ebony&authors=&year=&alltext=&caller=quicksearch</u> select "e": <u>http://www.flybase.org/reports/FBgn0000527.html</u>

choose "genes", type in "eyeless": <u>http://www.flybase.org/cgi-</u> <u>bin/uniq.html?species=Dmel&field=SYN&db=fbgn&context=eyeless&authors=&year=&alltext=&caller=quicksearch</u> select :ey": <u>http://www.flybase.org/reports/FBgn0005558.html</u>