The Zuker Collection: A Resource for the Analysis of Autosomal Gene Function in *Drosophila melanogaster*

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ABSTRACT

The majority of genes of multicellular organisms encode proteins with functions that are not required for viability but contribute to important physiological functions such as behavior and reproduction. It is estimated that 75% of the genes of *Drosophila melanogaster* are nonessential. Here we report on a strategy used to establish a large collection of stocks that is suitable for the recovery of mutations in such genes. From ~72,000 F_3 cultures segregating for autosomes heavily treated with ethyl methanesulfonate (EMS), ~12,000 lines in which the treated second or third chromosome survived in homozygous condition were selected. The dose of EMS induced an estimated rate of $1.2–1.5 \times 10^{-3}$ mutations/gene and predicts five to six nonessential gene mutations per chromosome and seven to nine alleles per locus in the samples of 6000 second chromosomes and 6000 third chromosomes. Due to mosaic mutations induced in the initial exposure to the mutagen, many of the lines are segregating or are now fixed for lethal mutations on the mutagenized chromosome. The features of this collection, known as the Zuker collection, make it a valuable resource for forward and reverse genetic screens for mutations affecting a wide array of biological functions.

THE search for mutations in genes whose protein products are known is complicated by the uncertainty regarding the phenotypes expected for such mutations. To search for mutations in a gene encoding a protein of interest, an F_2 screen can be used if a mutant or chromosome deficiency is available for the locus. In the absence of a chromosome deficiency, it is necessary to carry out an F_3 screen to produce flies homozygous for a mutagenized chromosome. The interest in this laboratory has been in genes encoding eye-specific proteins, the mutations of which are expected to result in viable adults. In the past this approach has been effective in recovering mutations of a number of genes; however, each recovery required a large F_3 screen involving thousands of individual lines (Table 1).

On the basis of our knowledge of the Drosophila phototransduction cascade, we knew that mutations for every gene had not yet been generated and that a definitive saturation study was warranted. Our goal was to conduct a screen in such a way that chromosomes were efficiently mutagenized with ethyl methanesulfonate (EMS) and that statistically each nonessential autosomal locus would have at least one new mutant allele. In addition, the collection should be of such quality that it would be worth maintaining and making available to other Drosophila investigators. Accordingly, we have generated and now maintain a collection of 12,336 lines with EMS-treated chromosomes, with 6079 lines with mutagenized second chromosomes and 6257 lines with mutagenized third chromosomes. Here we describe the strategy used to generate this collection and report on the features that make it a valuable resource for genetic analyses of a wide variety of physiological processes in Drosophila.

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MATERIALS AND METHODS

Deciding on the optimal level of EMS required us to strive for a dose high enough to engender a high mutation rate, but not so high as to produce too many homozygous lethal chromosomes. We arbitrarily settled on the criterion that at least 75% of the treated chromosomes in a run must be homozygous lethal. The reliable achievement of 75% lethality required experimentation with mutagenic protocols, in the process of which many F_3 runs were discarded. A modification of the procedure of Lewis and Bacher (1968) was finally adopted. We fed sexually isolated, 5-day-old adult males for 6 hr at room temperature a solution containing 25 mm EMS in 3% sucrose and 2.5% green food coloring. The flies were then scored on the basis of the quantity of EMS ingested as indicated by the intensity of the green in their abdomens. Flies with dark abdomens were returned to the EMS for an additional 18 hr. We again selected the flies for green abdominal color and placed those selected in new food vials for 24 hr prior to mating to maximize the sperm exposure to the ingested EMS; during this time the green coloring was completely excreted.

The genetic scheme we used followed the standard protocol for Drosophila F_3 screens (*e.g.*, see Greenspan 1997). For the second-chromosome mutagenesis, groups of 10 EMS-treated isogenized *cn bw* males were mated *en masse* to 25–30 *CyO/l(2)DTS91 b pr cn sca* virgin females in quarter-pint bottles (see

Lindsley and Zimm 1992 and http://flybase.bio.indiana.edu/genes/ for descriptions of mutations used in this study). After 3 days at room temperature, the males were cleared and the females returned to the bottles for 4 more days. F_1 males, either *cn bw*/l(2)DTS91 b pr cn sca* or *cn bw*/CyO*, were crossed individually to two *CyO/l(2)DTS91 b pr cn sca* virgins. The vials were incubated at 29° for 4 days to kill all embryos or larvae carrying the *l(2)DTS91*. We then cleared the adults and returned the vials to 29° for 4 more days before moving to room temperature. Newly eclosed *cn bw*/CyO* F_2 flies were transferred to new medium. The transferred vials were examined for the presence of non-Curly flies indicating the presence of dominant temperature-sensitive lethal (DTS) escapers; such vials were discarded. Transferred vials were kept at 25° for 12 days. F_3 progeny were scored in vials for the presence of white-eyed flies (*cn bw* homozygotes). The presence of at least one moving white-eyed fly indicated homozygous viability, and the culture was retained. If no white-eyed adults, but white-eyed pupae were observed, the adults were cleared and the vial was kept at 25° for 4 more days and scored again; the vial was then recorded as homozygous viable or as a pharate-adult lethal to be further screened for putative mechanosensory mutants (PMM). Any vials with previously undetected DTS escapers were discarded.

For the third-chromosome crossing scheme, EMS-treated isogenized *bw; st* males were mated to *bw; TM6B-Tb/ l(3)DTS4 D* virgins. F_1 males, either *bw*; *st*/TM6B-Tb* or *bw*; *st*/l(3)DTS4 D*, were crossed individually to two *TM6B-Tb/l(3)DTS4 D* virgins. As *l(3)DTS4*-bearing females lay no eggs at 29°, these individual crosses were held at 19° for 4 days to allow oviposition before clearing parents and transferring the cultures to 29° for 2 days and then to room temperature to complete development. F_2 *bw; st*/TM6B-Tb* progeny were then inbred, after removing any cultures segregating *Dichaete* (wings held out), indicative of DTS escapers. The F_3 vials were examined for non-Tubby pupae, the presence of which was indicative of *bw; st** homozygotes. The progeny produced were examined for the absence of *Dichaete* and for survival of white-eyed (*bw; st**) adults or for the presence of pharate-adult lethals.

The entire crossing scheme required 8 weeks. A new run was set up weekly such that at steady state there were eight runs in the pipeline; the project was completed in ~3 years. The lines that were retained for the collection carry the balanced mutagenized autosome, a mutagenized Y chromosome, and, since the treated males were exposed to two generations of outcrossing, one-fourth of the alleles on the unbalanced autosomes (*3* and *4* or *2* and *4*) are EMS derived; these alleles are segregating in each line. The second-chromosome collection is composed of 6079 lines (Z2-0001–Z2-6079) from 37,944 F_3 lines (or 16.0% homozygous viable). The third-chromosome collection is composed of 6257 lines (Z3-0001–Z3-6257) from 34,586 F_3 lines (or 18.1% homozygous viable).

TABLE 1

Results of F_3 screens for eye-specific mutations

Locus	No. of tests	No. of alleles recovered	References
arr1	20,800[a]	3	Dolph *et al.* (1993)
arr2	15,481[a]	2	Dolph *et al.* (1993)
trpl	1,093	1	Niemeyer *et al.* (1996)
Gcα1	20,137[a]	5	Gibbs *et al.* (2001)
Gα	4,403	3	Scott *et al.* (1995)
Gβ	?	2	Dolph *et al.* (1993)
inaD	6,190[b]	5	Tsunoda *et al.* (1997)
Ipp	4,377	3	Acharya *et al.* (1998)

[a] A mixture of F_3 and F_2 screens performed after the first allele was found.

[b] From the current screen.

RESULTS AND DISCUSSION

Simple Poisson assumptions can be employed to provide an estimate of the number of mutations per chromosome in this collection. For the second-chromosome screen, 16% lethal-free lines imply 1.83 lethal mutations/chromosome. The number of essential genes on the second chromosome is 1247, among 5290 (S. Celnicker, personal communication) second-chromosome genes, on the basis of extrapolation from counts of 267 lethal complementation groups in 415 of 1939 (Bridges and Bridges 1939; Bridges 1942) polytene bands subjected to saturation mutagenesis (Brizuela *et al.* 1994; Miklos and Rubin 1996). These numbers lead to an estimate of the average mutation rate of essential genes of $\sim 1.5 \times 10^{-3}$. Assuming that this mutation rate applies to all genes, simply multiplying the mutation rate by the number of chromosomes tested provides an estimate of the number of alleles per gene in the sample. This number is 9 for the surviving sample of 6079 chromosomes and 57 for the total sample of 37,944 mutagenized chromosomes.

Following the same procedure, 18% lethal-free third chromosomes imply 1.7 lethal mutations/chromosome. Extrapolating from 235 lethal complementation groups recorded in 343 of a total of 2062 bands (Bridges 1941a,b; Miklos and Rubin 1996) leads to an estimate of 1412 essential genes among 6178 (S. Celnicker, personal communication) genes on chromosome 3 and a lethal mutation rate of 1.2×10^{-3}. This mutation rate leads to an estimate of 7.5 alleles/gene in the surviving sample of 6257 chromosomes and 41 for the total sample of 34,586 mutagenized chromosomes.

Our observations indicate that the above estimates have some validity. In the process of balancing the mutagenized chromosomes and in screening the collection for visible phenotypes, the homozygotes were incidentally recorded. The collection contains at least 15 black and 15 ebony mutations, testifying to the efficacy of the mutagenesis and showing that large numbers of alleles were induced for certain genes. We note that the mutation rates indicated in Table 1 are lower than the above two estimates. This may indicate that our Poisson-based estimates are inflated or that the mutation rates of the specific genes tested depart from the mean and that the doses employed in those cases were lower than those used in the present study.

The rough estimates of the effectiveness of the mutagenesis presented earlier suggest that, despite some suggestions to the contrary (Peters *et al.* 2002), the second and third chromosomes have been truly saturated with

TABLE 2

Representative results of screens of the Zuker collection

Basis for screen	No. of lines screened[a]	Representative results	References
Electroretinogram recordings	7,504	253 lines with abnormal electroretinograms	E. J. Koundakjian (unpublished results)
Male sterility/male chromosome loss	11,502	2,216 male-sterile lines; 62 chromosome 4 loss lines	Wakimoto *et al.* (2004, accompanying article)
Male sterility due to cytokinesis defects		34 lines defective in cytokinesis; 19 complementation groups	M. Giansanti and R. Farkas (personal communication)
Mutagen sensitivity	6,275	78 lines mutagen sensitive; 33 complementation groups	Laurencon *et al.* (2004, accompanying article)
Meiotic chromosome nondisjunction in females	3,733 Z2	22 lines with increased levels of X chromosome NDJ; ≥6 complementation groups	Giunta *et al.* (2002); Manheim and McKim (2003)
	1,908 Z3	105 lines with increased levels of X chromosome NDJ	S. McMahan and J. Sekelsky (personal communication)
Abnormal wing veins	11,706	20 lines with wing-vein defects; 11 complementation groups	K. Lunde and E. Bier (personal communication)
Mechanosensory deficient	569	17 lines with mechanosensory defects	T. Avidor-Reiss (personal communication)

[a] The lines screened are Z2 and Z3 lines as noted except for those of T. Avidor-Reiss who screened the PMM, pharate adult lethal collection.

mutations. That is to say, every gene has probably been mutated several times, but not every possible allele of every gene has been generated. Some mutations that appear to be unique may simply represent rare alleles of genes, most of whose mutations are not recognized by the screens employed. For example, it is possible that rare viable alleles of essential genes might be found in appreciable numbers, given the enormous numbers of essential-gene hits estimated for the total sample.

A feature of the collection, as it is currently constituted, is that in approximately one-third of the lines, the mutagenized chromosomes have become homozygous lethal or nearly so, despite having been originally isolated as homozygous viable. We attribute this to the fixation of lethal mutations that were mosaic in the original F_1 of the treated male. If, for example, an F_1 male produced sperm carrying two types of mutagenized chromosomes, one bearing a lethal mutation and one a wild type or a nonallelic lethal, then inbreeding the F_2 will generate three types of offspring homozygous for the mutagenized autosome, *lethal/lethal*, *lethal/+* (or *lethal #1/lethal #2*), and *+/+* (or *lethal #2/lethal #2*), and they would have been saved as homozygous viable. Using a dose of EMS that produced 22% autosomal recessive lethals, Epler (1966) found that 20% of the lines producing viable homozygotes segregated for lethal mutations. It follows that in this study the dose of mutagen generating >80% recessive lethals would produce significant numbers of apparently homozygous viable lines derived from animals multiply mosaic for recessive lethal mutations and thus segregating for several lethals. Multiple mosaicism would also have contributed to the reduced survival of homozygotes observed when the lines were originally isolated. Had we carried the crossing scheme through a fourth generation to resolve the mosaics, the lethal frequency would have been greater, and the dose of EMS would have had to be adjusted accordingly. In spite of this frequency of now homozygous lethal lines, all of the lines in the collection remain useful and are currently being maintained. Mutations in these lines may be identified genetically by deletion mapping or by complementation analyses with existing alleles. In addition, the EMS-induced lesion can be molecularly identified in heterozygous flies via heteroduplex formation and detection by targeting-induced local lesions in genomics (McCallum *et al.* 2000) or denaturing high-preformance liquid chromatography methods (Bentley *et al.* 2000).

The Zuker collection has been used for a variety of screens in our laboratory. To identify new mutations in the *inaD* locus, Tsunoda *et al.* (1997) assayed homozygotes from the second-chromosome collection for the absence of the InaD protein on Western blots of proteins extracted from heads. The recovery of five protein null alleles testifies to the utility of their approach. This type of protein screen can be extended to other genes since we have stored Western blots of head proteins for the second- and third-chromosome lines (423 nitrocellulose blots of 15 lanes each for the second chromosome and 412 blots for the third chromosome). The second-chromosome lines have also been screened for defective deep pseudopupils (Zelhof *et al.* 2003). Approximately 7500 second- and third-chromosome lines have been screened for abnormal electroretinograms (E. J. Koun-

DAKJIAN, unpublished results). In the process of establishing the Zuker collection, we also established a collection of ~600 strains of pharate-adult lethals from the original EMS mutagenesis. This collection, known as the Zuker PMM collection, has been screened for mechanosensory defects (T. AVIDOR-REISS and C. ZUKER, personal communication).

The Zuker collection has also proven to be a valuable resource for other Drosophila geneticists. Over 100 laboratories worldwide have screened the entire collection or subsets of it for phenotypes of interest. Representative results from several of the largest screens are shown in Table 2. We and others (WAKIMOTO *et al.* 2004, accompanying article in this issue) are maintaining databases to catalog the phenotypes reported for each line. This information, along with continued use of this community resource, will facilitate our understanding of the genetic networks that regulate physiological functions in Drosophila. The collections are currently available from Charles Zuker (czuker@ucsd.edu).

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LITERATURE CITED

ACHARYA, J. K., P. LABARCA, R. DELGADO, K. JALINK and C. S. ZUKER, 1998 Synaptic defects and compensatory regulation of inositol metabolism in the inositol polyphosphate 1-phosphatase mutants. Neuron **20:** 1219–1229.

BENTLEY, A., B. MACLENNAN, J. CALVO and C. R. DEAROLF, 2000 Targeted recovery of mutations in Drosophila. Genetics **156:** 1169–1173.

BRIDGES, C. B., and P. N. BRIDGES, 1939 A new map of the second chromosome: a revised map of the right limb of the second chromosome of *Drosophila melanogaster*. J. Hered. **30:** 475–476.

BRIDGES, P. N., 1941a A revised map of the left limb of the third chromosome of *Drosophila melanogaster*. J. Hered. **32:** 64–65.

BRIDGES, P. N., 1941b A revision of the salivary gland 3R-chromosome map of *Drosophila melanogaster*. J. Hered. **32:** 299–300.

BRIDGES, P. N., 1942 A new map of the salivary gland 2L-chromosome of *Drosophila melanogaster*. J. Hered. **33:** 403–408.

BRIZUELA, B. J., L. ELFRING, J. BALLARD, J. W. TAMKUN and J. A. KENNISON, 1994 Genetic analysis of the *brahma* gene of *Drosophila melanogaster* and polytene chromosome subdivisions 72AB. Genetics **137:** 803–813.

DOLPH, P. J., R. RANGANATHAN, N. J. COLLEY, R. W. HARDY, M. SOCOLICH *et al.*, 1993 Arrestin function in inactivation of G protein-coupled receptor rhodopsin *in vivo*. Science **260:** 1910–1916.

EPLER, J. L., 1966 Ethyl methanesulfonate-induced lethals in Drosophila: frequency-dose relations and multiple mosaicism. Genetics **54:** 31–36.

GIBBS, S. M., A. BECKER, R. W. HARDY and J. W. TRUMAN, 2001 Soluble guanylate cyclase is required during development for visual system function in Drosophila. J. Neurosci. **21:** 7705–7714.

GIUNTA, K. L., J. K. JANG, E. A. MANNHEIM, G. SUBRAMANIAN and K. S. MCKIM, 2002 *subito* encodes a kinesin-like protein required for meiotic spindle pole formation in *Drosophila melanogaster*. Genetics **160:** 1489–1501.

GREENSPAN, R., 1997 *Fly Pushing*, pp. 23–46. Cold Spring Harbor Laboratory Press, Plainview, NY.

LAURENCON, A., C. M. ORME, H. K. PETERS, C. L. BOULTON, E. K. VLADAR *et al.*, 2004 A large-scale screen for mutagen-sensitive loci in Drosophila. Genetics **167:** 217–231.

LEWIS, E. B., and F. BACHER, 1968 Methods of feeding ethylmethane sulfonate (EMS) to Drosophila males. Dros. Inf. Serv. **43:** 193.

LINDSLEY, D. L., and G. G. ZIMM, 1992 *The Genome of Drosophila melanogaster*. Academic Press, San Diego.

MANHEIM, E. A., and K. S. MCKIM, 2003 Synaptonemal complex component *C(2)M* regulates meiotic crossing over in Drosophila. Curr. Biol. **13** (4): 276–285.

MCCALLUM, C. M., L. COMAI, E. A. GREEN and S. HENIKOFF, 2000 Targeted screening for induced mutations. Nat. Biotechnol. **18:** 455–457.

MIKLOS, G. L. G., and G. M. RUBIN, 1996 The role of the genome project in determining gene function: insights from model organisms. Cell **86:** 521–529.

NIEMEYER, B. A., E. SUZUKI, K. SCOTT, K. JALINK and C. S. ZUKER, 1996 The Drosophila light-activated conductance is composed of two channels, TRP and TRPL. Cell **85:** 651–659.

PETERS, H. K., C. M. ORME, D. T. HARRIS, A. LAURENCON, R. S. HAWLEY *et al.*, 2002 A large scale screen for repair deficient mutants in Drosophila. 43rd Annual Drosophila Conference (Abstr. 204C), April 10–14, 2002, San Diego.

SCOTT, K., A. BECKER, Y. SUN, R. HARDY and C. S. ZUKER, 1995 Gq alpha protein function *in vivo*. Genetic dissection of its role in photoreceptor cell physiology. Neuron **15:** 919–927.

TSUNODA, S., J. SIERRALTA, Y. SUN, R. BODNER, E. SUZUKI *et al.*, 1997 A multivalent PDZ-domain protein assembles signaling complexes in a G-protein-coupled cascade. Nature **388:** 243–249.

WAKIMOTO, B. T., D. L. LINDSLEY and C. HERRERA, 2004 Toward a comprehensive genetic analysis of male fertility in *Drosophila melanogaster*. Genetics **167:** 207–216.

ZELHOF, A. C., E. KOUNDAKJIAN, A. L. SCULLY, R. W. HARDY and L. POUNDS, 2003 Mutation of the photoreceptor specific homeodomain protein Pph13 results in defects in phototransduction and rhabdomere morphogenesis. Development **130:** 4383–4392.

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