

File S1

Support for Assumption of 2 to 4 Germ-Line Progenitor Nuclei

There is some contention in the literature regarding the number of progenitor nuclei that found the germline. Estimates range from 2 through more than 8. We have tested these postulates by comparing the clonal composition of germ lines in our observed data to model results while varying several parameters in the model. The term “clone” is used here to denote all the descendants of one replication of an alkylated strand. Clonal composition is defined as the number of different clones represented in the germ line. To make this comparison we first eliminated several experimental lines that had resulted in overlapping clones and were therefore ambiguous. (See Table 6 in the body of the paper – the excluded lines are denoted in bold face.) We summed observed results of data from chromosome 2 and 3 three. We removed 12% from the observed number of monoclonals to account for complete mutations as described in the article. [See discussion of the contribution of completes in the section entitled *Estimating the frequency of complete (whole body) mutations*].

As a result of stochastic events leading up to the selection of germ-line-progenitor nuclei, our model predicts a distribution in the ratio of mono- and polyclonal germ lines. By running the model repeatedly while varying the degree of randomness with which the nuclei are selected from the syncytium, and by varying the number of progenitor nuclei, we are able to confirm that the best fit of the model parameters to our experimental data lies between two and four progenitor nuclei.

We vary the degree of randomness with which nuclei are selected by varying the size of the pool of nuclei from which progenitors are drawn from 20 through 50 (the smaller the pool, the more closely related the nuclei). Varying the pool size from 20 to 50 nuclei had little effect. By varying the number of progenitor nuclei from 1 through 8 we find that the model configured for one progenitor nucleus predicts more monoclonals and fewer polyclonals than observed. Models configured for four through eight progenitor nuclei predict too few monoclonals, and an excess of polyclonals beyond tri-clonal. The model configured for three progenitor nuclei fits the observed data for every clonal count with the exception of five: the model predicts that there will be no nuclei with a clonal count of five while the observed data contain a single instance. This one instance may be an experimental artifact, or the result the inclusion of an alkylated strand that then amplified after germ line selection, the effect of which is not taken into account by the model. Regardless, a complete fit of the model parameters to the observed data is obtained by the reasonable assumption that the observed data result from germ lines descended from a mix of two, three or four progenitor nuclei.

File S1

Table S1 Comparison of model predictions to observed clonal counts assuming one through eight progenitor nuclei.

		Number of Progenitor Nuclei																
			1		2		3		4		5		6		7		8	
Pool	20	Observed	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Clonal Counts	1	71.8%	97.3%	100.0%	59.8%	79.7%	45.7%	72.4%	41.7%	66.9%	38.1%	60.2%	36.9%	60.2%	34.9%	57.1%	32.9%	54.3%
	2	20.9%	0.0%	2.7%	19.0%	39.2%	20.1%	47.2%	24.0%	47.2%	25.0%	44.1%	25.4%	43.7%	26.2%	43.7%	25.4%	43.7%
	3	6.4%			0.0%	4.3%	2.0%	13.0%	2.8%	15.9%	1.6%	15.5%	2.4%	15.4%	2.8%	14.3%	2.8%	12.8%
	4	0.5%					0.0%	3.9%	0.4%	6.6%	1.2%	9.1%	1.2%	10.0%	1.2%	10.4%	1.6%	10.8%
	5	0.5%					0.0%	0.4%	0.0%	3.5%	0.0%	4.8%	0.4%	7.5%	0.4%	7.5%	1.2%	9.4%
	6	0.0%									0.0%	2.4%	0.0%	3.2%	0.0%	4.8%	0.0%	6.0%
	7	0.0%											0.0%	1.6%	0.0%	2.4%	0.0%	2.8%
	8	0.0%											0.0%	0.4%	0.0%	0.8%	0.0%	0.8%
	9	0.0%													0.0%	0.4%	0.0%	0.4%
Pool	50	Observed	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
Clonal Counts	1	71.8%	96.8%	100.0%	59.1%	80.6%	46.8%	72.4%	41.2%	64.8%	37.6%	62.0%	36.8%	57.6%	35.2%	56.8%	34.4%	55.6%
	2	20.9%	0.0%	3.2%	16.7%	39.7%	21.2%	44.8%	24.4%	44.4%	25.2%	44.8%	25.6%	44.8%	26.0%	44.0%	27.6%	43.6%
	3	6.4%			0.0%	4.3%	2.4%	13.2%	2.4%	15.2%	3.1%	15.6%	3.2%	14.8%	3.2%	14.0%	3.2%	14.0%
	4	0.5%					0.0%	4.4%	0.0%	7.6%	0.8%	10.8%	1.2%	10.4%	1.6%	11.2%	2.0%	10.4%
	5	0.5%							0.0%	3.5%	0.0%	5.2%	0.0%	6.8%	0.4%	8.4%	0.4%	10.0%
	6	0.0%									0.0%	2.4%	0.0%	3.6%	0.0%	4.4%	0.0%	5.6%
	7	0.0%											0.0%	1.2%	0.0%	2.4%	0.0%	2.8%
	8	0.0%													0.0%	0.8%	0.0%	0.8%
	9	0.0%																

Table S1 compares the observed ratio of germ lines with those predicted by our model. The upper half of the table shows model results when germ line precursors are drawn at random from 20 adjacent nuclei; the lower half shows results when precursors are drawn from a pool 50. The larger pool size simulates a more random selection of nuclei from the syncytium. Row headings indicate clonal count of one through nine. The observed data appear in the third column. Column headings indicate the number of progenitor nuclei modeled, with the minimum and maximum model results obtained for each out of a thousand independent simulations for each pair of variables. Model predictions with a minimum and maximum of zero are left empty. Cells highlighted in green indicate when the observed values fall between the minimum and maximum model predictions, including those cases when the observed results were zero.

Global Considerations

Extent and Nature of Alkylation: Aaron and Lee (1978) isolated sperm from flies exposed to radio-labeled EMS and measured label incorporation. They estimated that 1.4% of the nucleotides in the sample were alkylated. Since the majority of alkylations involve purines, it follows that nearly 1.4% of the nucleotide pairs were labeled. Table S2 tabulates data from Singer and Grunberger (1983) on the various adducts formed by *in vitro* alkylation of DNA; they noted that similar results are found for DNA alkylated *in vivo*. Sequencing of DNA from flies carrying mutagenized chromosomes (Bentley *et al.* 2000; Cooper *et al.* 2008; Blumenstiel *et al.* 2009) provided estimates of 2–5 sequence changes per million base pairs. Results from Aaron and Lee (1978) imply 14,000 alkylations per million base pairs:

Table S2 Alkylation products of *in vitro* exposure of DNA to EMS (Singer and Grunberger 1983).

Alkylation site	% of alkylations
Adenine	
N-1	1.7
N-3	4.9
N-7	1.1
Σ	7.7
Guanine	
N-3	0.9
O-6	2
N-7	65
Σ	67.9
Cytosine	
N-3	5
Diester	10
Σ	15
$\Sigma\Sigma$	90.6

Transcription data from five-day-old *Drosophila* males contained information regarding more than 15,000 transcripts of which more than 14,000 (14,666) appeared to be protein-encoding genes. The distribution of sizes of genes encoding these transcripts was skewed with half of them under 2kb in length and the remainder distributed in diminishing numbers over 395 kb for a total of approximately 91 million base pairs.

Genomic Effects – Many Alterations, Few Mutations: It has been estimated that there are approximately 3,600 vital genes (among 14,666) in the *Drosophila* genome (Lefevre and Watkins 1986; Brizuela *et al.* 1994 Miklos and Rubin 1996). Rounding these numbers to 600 on the *X* and 1500 on each autosome, we estimate our results concern mutation of 3,000 vital genes. Assuming that the vital genes are representative of the other *Drosophila* genes, they comprise one-fifth of the 91-million base pairs in the total genome. At 2 to 5 EMS-induced changes per million base pairs, we can estimate 36 to 90 changes in vital genes per target genome.

Three thousand vital genes comprising approximately 20% of 91 million base pairs results in 18.2 million base pairs, of which about 1.4% (255 thousand) At $(2 \text{ to } 5) \times 10^{-5}$ altered base pairs among 18.2×10^6 base pairs, there will be 2 to 5 alterations among 14,000 alkylated base pairs. Since there are an estimated 254,800 alkylated base pairs in the target genome, these ratios suggest that 36–120 of them will be abnormal (i.e., mutagenic). This number far exceeds the mutation rates observed. The alkylated base pairs could display eight different adducts of unknown individual effects, some of which may not lead to mutations. Some authors postulate the O-6 alkylation of guanine (2% of the adducts) as the culprit (Vogel *et al.* 1985). Alkylated bases may also fall in nontranslated portions of the

gene, since observed gene lengths used in the calculations are much greater than those required to encode a protein, affect redundant codons, or actually result in altered proteins in ways that do not alter phenotype.

Reexamining Previous Studies in Light of the Model Presented Here

Experiments Involving Specific Locus Mutations

In previous experiments, assessment of the germ line has rarely been followed further than showing whether or not the germ line contains the mutation (Table S3). Lee *et al.* (1970) examined mutations of *yellow*. We discuss this here because *yellow* provides greater anatomical coverage than other loci and can therefore be more accurately scored for somatic mosaics. They found that of 114 *yellow* mutations among 54,243 F₁ females, 16 were complete and presumably premitotic in origin (14% compared with our estimate of 12% for complete lethal mutations). The germ lines of 55 of the 98 mosaic females were examined by progeny testing. Among 55 F₁ females somatically mosaic for *yellow*, the ovaries of 17 were wild type, 33 were *yellow*, and 4 were mosaic. The line separating the two domains of the mosaic bisected the presumptive germline progenitors in fewer than 10% of the mosaic embryos. These numbers suggest that 14% of the *y* mutations were premitotic and that the remaining 86% were mitotic mutations. These results cannot be easily compared to the results predicted by our model; the mutation rate for a single locus is low but the Lee *et al.* (1970) data regard only females in which a mutation has certainly occurred. Their hypothesis, based on the numbers of imaginal discs affected, was that *yellow* mutations occur repeatedly during syncytial mitoses with a probability of 0.54

misreplications per replication of y^+ , compared to our estimate of 0.68 per replication of 3,000 vital genes in males.

Table S3 Specific-locus data.

Reference	Locus	Mutagen	Maternal <i>mus</i> constitution	Complete	Somatic mosaic	Total	% Mutant
Auerbach (1946 a and b)	<i>M</i>	mustard	+	21	17	4,837	0.79
	<i>M</i>	X ray	+	20	1	4,161	0.50
	<i>w sn</i>	mustard	+	12	6	15,300	0.12
	<i>w sn</i>	X ray	+	6	0	3,600	0.17
Carlson and Oster (1962)	<i>dp</i>	ICR100 ^a	+	23	163	21,102	0.88
	<i>dp</i>	X ray	+	137	65	?	
Jenkins (1967a and b)	<i>dp</i>	EMS	+	10	36	7,131	0.65
	<i>dp</i>	EMS	+	34	120	22,388	0.69
Lee <i>et al.</i> (1970)	<i>y</i>	EMS	+	16	98	50,243	0.23
	<i>w</i>	EMS	+	111	106	50,243	0.43
Ondrej (1971)	<i>dp</i>	ENU	+	12	38	14,095	0.35
				34	63	19,345	0.50
				29	52	12,677	0.64
Pastink <i>et al.</i> (1991)	<i>v</i>	EMS	+	21	^b	155,196	0.013531
				16	^c	25,525	0.0509
					^b	94,425	0.0169
			<i>mus201^{D1}</i>		^c	15,624	0.0384

^a 2-methoxy-6-chloro-9-(3[ethyl-2-chloroethyl] aminopropylaminoacridine dihydrochloride

^b mosaics not detectable in F₁ owing to non autonomy of *v*

^c mosaics identified in F₂

Experiments Involving Recessive Lethal Mutations on the X Chromosome

Recessive lethal tests score simultaneously for mutations in large numbers of vital genes (e.g., approximately 600 vital genes on the *X*). Only germ-cell constitutions are testable in these crosses; somatic constitutions cannot be determined because the mutagenized chromosomes are recovered and maintained in balanced heterozygotes. Virtually all recessive-lethal screens have dealt with the *X* chromosome, using the standard *Basc* method. Sex-linked-recessive-lethal data are complex. Mutations designated as complete lethals are recognized by the absence of sons among the offspring of daughters of mutagen-treated males. This class comprises prefertilization mutations and products of monoclonal mutant germ lines, as well as those classes derived from polyclonal gonads of the types scored as double and triple mosaics (i.e., LL and LLL) in our experiment; lethal mutations of the latter source will actually be a mixture of two or three different lethal mutations. The mutagenized *X* chromosome is recovered in F₁ heterozygous females, and whole-gonad mutations are detected by the absence of sons carrying the mutagenized *X* in the F₂ generation. Gonadal mosaics for lethal mutations are hidden among the F₁ females not heterozygous for a complete lethal. They could be gonadal mosaics, whose effect on the F₂ could be a skewed sex ratio in favor of daughters owing to the fact that half of the sons receive a lethal-bearing *X* chromosome; this datum is not ordinarily recorded. Instead F₁ females are again backcrossed to males and the F₂ daughters of gonadal mosaics will comprise a mixture of lethal and nonlethal heterozygotes (Table S4). A number of daughters must be tested individually for the presence of lethality in the F₃; the daughters of gonadal mosaics segregate for lethal mutations. This procedure can be extended for another generation to reveal lethal mutations in the F₄ (Mathew 1964). A shortcoming of

sex-linked-lethal mutation data is that they do not ordinarily admit to complementation analysis for determination of allelism of the mutations recovered. (Note: infrequent lethal mutations located in the region at the base of the X chromosome that is covered by the pseudo-autosomal region *Dp(1;Y)mal⁺* can and have been complementation tested by Lifschytz and Falk (1969).)

In an early study, Auerbach (1946a and b) selected 11 gonadal mosaic daughters of mustard-gas-treated males. From these females she progeny-tested an average of 18 F2 females each. All F1 females tested produced a mixture of **L** and **P** daughters (139:47). This preponderance of lethals probably biased the selection of these F1 females as putative gonadal mosaics. Surprisingly, four of the F1 females also produced one or two gonadal mosaic daughters. Auerbach provided evidence that the mosaic lethal daughters of mosaic lethal mothers were mosaic for the same lethal gene, on the basis of similarity of lethal phenotype and map position in one case and a garnet mutation in another. It appeared that at least six out of 11 gonadal mosaic females tested were carrying replicating instabilities that stabilized as lethal mutations in some of their daughters. The observation that gonadal mosaic females can produce gonadal mosaic daughters suggests that there is no reason that a specific lethal-bearing nuclear type cannot continue to be propagated in future generations.

File S2

Table S4 Sex-linked recessive lethal mutations.

Reference	Mutagen	Maternal <i>mus</i> constitution	F2 Complete mutations			F3 Mosaics		
			Non	Gonadal complete	% Complete	Non	Gonadal mosaic	% Mosaic
Carlson and Oster (1962)	ICR 100	+	295	11	3.6	101	16	13.7
	X ray	+	351	54		285	3	
Carlson and Southin (1963)	ICR 100	+	821	97	9.2	563	91	13.9
	ICR 100	+	688	69	9.1	346	67	16.2
Mathew (1964)	CEMS	+	762	15	1.9	54	2	3.6
Ondrej (1971)	ENU	+	513	29	5.3	550	228	29.3
Sadiq and Mathew (1977)	MNNG	+	950	68	6.7	158	7	4.2
	MNNG	+	890	62	6.5	125	3	2.3
Shvartsman and Romashkina (1995)	Ethylenimine	+	585	6	1			12.4 ^c
		<i>mus201</i> ^{G1}	435	46	9.6			49.2 ^c
	Ethylenimine	+	595	23	3.7			
		<i>mus201</i> ^{G1}	640	178	21.8			
	MMS	+	801	21	2.6			
		<i>mus201</i> ^{G1}	1080	93	7.9			

^a Chloroethylmethane Sulfonate

^b N-Methyl-N'-Nitro-Nitrosoguanidine

^c raw numbers not given (percentage only)

Experiment Involving Autosomal Recessive Lethal Mutations

Autosomal recessive lethals exhibit the same complexity of origin as sex-linked recessive lethals except that double and triple mosaics give rise to normal rather than lethal offspring. They differ from sex-linked recessive lethals in allowing for complementation testing of the mutations. Epler (1966) performed the first screen for autosomal lethal mutations (Table S5). He screened both chromosomes 2 and 3 in the same experiment. He assessed the cellular contents of the germ lines of the sons of EMS-treated males by

establishing 10 lines from each; among these he encountered single mosaics containing some lethal-bearing and some nonlethal lines as well as double mosaics carrying complementing-lethal lines. This first demonstration of multiple mosaicism established the value of complementation testing, and the first suggestion that multiple mosaics likely result from independent misreplications in the generation of multiple clones in biclonal and triclinal germ lines.

The results of the Epler experiment are consistent with our model assuming three nuclei selected as germline progenitors and a mutation rate of approximately 25%.

Chromosome	Non (P)	Gonadal complete (L)	Gonadal mosaic (M)
2	35	9	7
3	21	11	7
Σ	56	20	14
Σ%	62%	22%	16%

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File S2

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Modeling Gonadal Composition

Available for download at www.genetics.org/content/suppl/2015/07/09/genetics.115.178046.DC1