Undergraduate research in epigenetics using *Drosophila melanogaster*

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Abstract

Position Effect Variegation – the silencing of a gene in some of the cells in which it is normally expressed – occurs when a gene normally found in euchromatin is juxtaposed with heterochromatin through rearrangement or transposition. This silencing is due to stochastic assembly of heterochromatin over the reporter gene, an epigenetic phenomenon. Because modifications of PEV phenotypes of reporter genes such as *white* are easily scored, undergraduates can use PEV to study epigenetic mechanisms. We have available three sets of lines for such studies. 1) Lines carrying a *P* element reporter with *hsp70-white* in different heterochromatic domains (pericentric heterochromatin, telomeres, 4th or Y chromosome) can be used to examine the impact of genetic modifiers, environmental conditions (diet, temperature), etc. on the different types of heterochromatin. Selection for high and low levels of expression can be used to identify background modifiers and reveal distinctive patterns of variegation, potentially providing new mechanistic insights. 2) Lines with that reporter inserted at sites along the 4th chromosome allow investigation of genes present, and active, in a heterochromatic domain, both those dominated by H3K9me2/3-HP1a and those dominated by H3K27me3-Pc. 3) Lines exhibiting ectopic silencing induced by a *1360* element or by a tandem array of repeats, either $[GAA]_{310}$ or $[lacO]_{256}$, display different sensitivities to modifiers, implying different mechanisms for heterochromatin targeting and assembly; this suggests multiple investigations. Important studies can be done using genetic crosses, with the eye phenotype scored by a pigment assay (quantitative results; requires a spectrophotometer) and pattern characteristics (requires an iphone with jpeg software). Many modifiers of PEV [Su(vars)s and E(var)s] as well as the group 1 lines are available from the Bloomington Stock Center. Group 2 and 3 lines are available through August 30 from S Elgin lab, and thereafter from A Arsham or C Reinke. The excellent annotation of *D. melanogaster*, including ChIP results for many chromosomal proteins and histone modifications, plus extensive annotation of transcripts, from modENCODE and others, enables students to frame sophisticated questions (browsers at FlyBase and GEP http://gander.wustl.edu). Examples of student work using these resources are shown. Come chat with us or email (selgin@wustl.edu, aarsham@bemidjistate.edu, Catherine.reinke672@gmail.com) if you think your students might enjoy a project in epigenetics! The Elgin lab has been funded by NIH and NSF.

Investigating epigenetics with fruit flies

Inexpensive and easy to culture,
- short life cycle (2 wks);
- easily visible phenotypes;
- good genetic approaches

Biochemical approaches

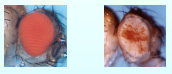
Polytene chromosomes: excellent cytology

Simple genome, good reference sequence, extensive annotation

Position Effect Variegation (PEV) reporter for gene silencing by heterochromatin formation

Metazoan useful for behavioral, developmental. and human disease research

PEV: a reporter of gene silencing



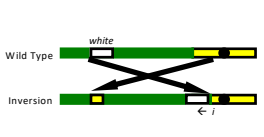
In its normal euchromatic environment the *white* gene is fully expressed, leading to a red eye; if juxtaposed with heterochromatin, it is silenced in some of the cells where it should be active due to stochastic packaging as heterochromatin.

Scalable, scientifically interesting, unique, inquiry-driven projects for undergraduates
Requires doing
- genetic crosses;
- pictures of fly eyes (iPhone)
- image analysis (Image J from NIH)

Does NOT require
- molecular biology
- expensive microscopes

although these can be incorporated

Placement of a reporter next to or within a heterochromatic domain results in a PEV phenotype



An inversion on the X chromosome places *white* next to a breakpoint in heterochromatin; one sees similar stochastic silencing if the gene is inserted into a hetero-chromatic domain by P element transposition, apparently due to spreading of heterochromatin.

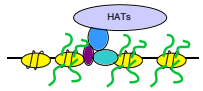
The PEV phenotype allows investigations of trans-acting modifiers

Crossing in loss-of-function mutations in genes required for heterochromatin formation result in loss of silencing, *Suppression of variegation*, while such mutations in genes required for euchromatin formation or transcription result in a gain in silencing, *Enhancement of variegation.*

Su(var)
(mutations in HP1, H3K9 methyl transferases, histone deacetylases)

E(var)
(mutations in RNApol II, histone acetyl transferases)

DNA packaging plays a major role in regulating gene expression

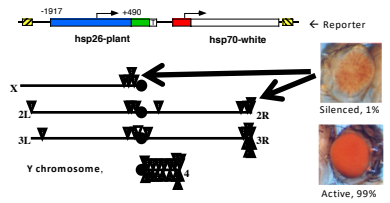


- Euchromatin
  - Less condensed
  - Chromosome arms
  - Unique sequences; gene rich
  - Replicated throughout S
  - Recombination during meiosis
- Heterochromatin
  - Highly condensed
  - Centromeres and telomeres
  - Repetitious sequences; gene poor
  - Replicated in late S
  - No meiotic recombination

Transcriptional activators
Hyper-acetylated histone tail
Heterochromatin Protein 1 complex
Hypo-acetylated histone tail; methylated H3K9

While these characteristics are broadly applicable, specific domains (e.g., pericentric hetero-chromatin vs sub-telomeric heterochromatin) will differ in their response to various *Su(var)* and *E(var)* mutations (available from Bloomington), revealing characteristics of the underlying structure.

Transposition of a P element reporter has provided the starting lines for exploration of different heterochromatin domains



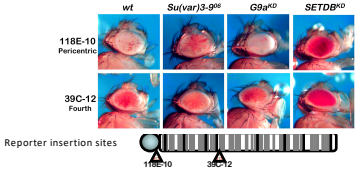
Wallrath & Elgin,1995, Genes Dev 9: 1263-77

A screen of 3000 P element mobilizations recovered 30 lines exhibiting PEV. Lines carrying the *P* element reporter with *hsp26-plant; hsp70-white* in different heterochromatic domains (peri-centric heterochromatin, telomeres, 4th or Y chromosome) are available and can be used to examine the impact of genetic modifiers [*Su(var)*s and *E(var)*s], environmental conditions (diet, temperature), etc. on the different types of heterochromatin.

Example #1: Do all heterochromatic domains use the same H3K9 histone methyltransferase?

Drosophila has three H3K9 methyltransferases:

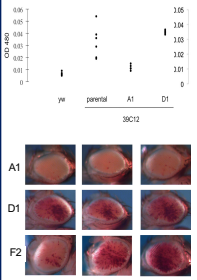
Su(var)3-9 mutations → loss of silencing in pericentric heterochromatin, gain of silencing on 4th;
G9a knock-down → no impact on either domain;
SETDB knock-down → some loss of silencing in pericentric het, complete loss of silencing on 4th.



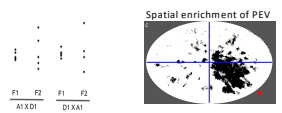
The results indicate that 4th chromosome heterochromatin maintenance requires SETDB activity, but not G9a or SU(VAR)3-9 activity, while pericentric heterochromatin requires SU(VAR)3-9 as well as SETDB. Loss of heterochromatic proteins from the pericentric heterochromatin on loss of SU(VAR) 3-9, and their redistribution to the 4th chromosome probably accounts for the gain of silencing on the 4th.

B Brower-Toland et al., 2008, Genetics 181: 1303-19.

Example #2: To what extent do background genetic variants contribute to the variation in PEV phenotype? Does the pattern of variegation depend on the heterochromatic domain?



Wang & Elgin, 2019, Epigenetics Chromatin 12: 70 doi: 10.1186/s13072-019-0314-5.

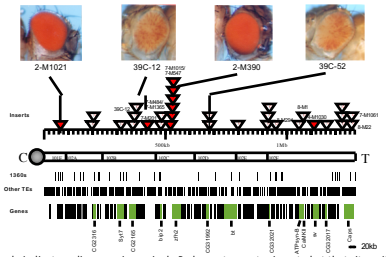


Flies from variegating line 39C-12 (4th chromosome) were inbred for six generations, selecting those with the least pigmentation (A1) and most pigmentation (D1). Inbred reporter lines show a very consistent degree and pattern of PEV; whether this is true for different heterochromatin domains, and how similar the pattern would be, is unknown. Crossing A1 X D1 or D1 X A1 gives a tight median pigment level in F1, while crossing F1 results in variation in F2 similar to the parental lines, as would be expected from random segregation of independent modifier loci. (Eye pigment extracted and measured at OD_{480}.) (Environmental impacts?)

Selection for high and low levels of expression could be used to identify QTLs via bulk segregant analysis if NextGen sequencing is accessible, potentially providing new insights.

Example #3: Mapping the fourth chromosome subdomains

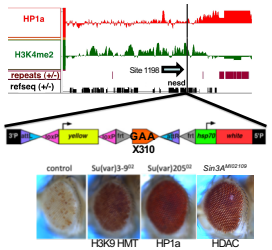
Lines with the *hsp70-white* reporter inserted at sites along the 4th chromosome allow investigation of genes present, and active, in a heterochromatic domain, both those dominated by H3K9me2/3-HP1a and those dominated by H3K27me3-Pc

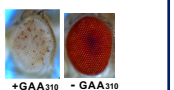


Each triangle indicates a line carrying a single P element reporter inserted at that site, with the eye pheno-type shown, red or variegating, indicating a permissive (Pc: H3K27me3) or silencing (heterochromatic: H3K9me2/3) environment; green genes have one or more P elements inserted.

Sun et al 2004 Mol Cell Bio 24: 8210; Riddle et al 2008 Genetics 178: 1177.

Example #4: A Friedrich's Ataxia model in Drosophila



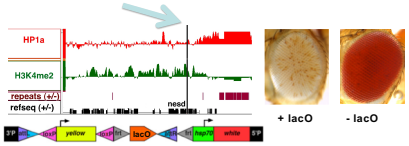


Triplet repeats induce human mutations by causing local hetero-chromatin formation, silencing the gene. Here we put a human DNA fragment of GAA310 into a site in the fly genome where we observe repeat-induced silencing, first shown using a transposable element, *1360*. The presence of the repeat leads to 8X silencing. This is due to heterochromatin formation, as shown by loss of silencing when *Su(var)* mutations are crossed in.

Gracheva & Elgin, unpublished; Sentmanat & Elgin, 2012, PNAS 109:14104

Example #5: A tandem repeat of a bacterial DNA sequence, *lacO*, induces a different kind of heterochromatin formation

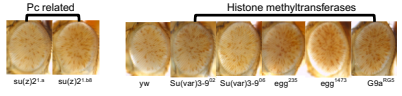
T Gu & SCR Elgin, unpublished

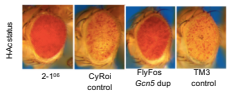


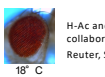
Li et al, Wallrath (2003) *Development* 130: 1817

An tandem array, 256 copies of the 36 bp bacterial DNA fragment *lacO*, also induces 8-fold silencing when inserted at the 1198 site. This foreign repeat results in different sensitivities to known *Su(var)*s, and to temperature, implying different mechanisms for heterochromatin targeting and assembly; see the following panel for examples. This suggests several lines of investigation.

Variegation of 1198-*lacO*256 is sensitive to
- mutations in the HP1a complex, and H-Ac status;
- not to individual histone H3K9 HMTs;
- but to temperature!







(others shown above at 25° C)

H-Ac and TS sensitivity- collaboration with Reuter, Sanger, & Walther

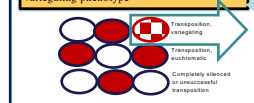
Example #6: What euchromatic locations in the genome are prone to repeat-induced heterochromatin formation and silencing?

P transposon reporter with *lacO*256 x transposase

F1 expressing transposon reporter and transposase → germ line transposition

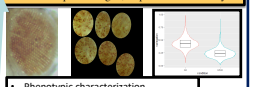
F2 removal of transposase, individual flies screened for stable transposon insertions with variegating reporter expression

Students set up crosses and screen for rare variegating phenotype



- Genetics lab skills
  - Maintaining stocks
  - Assessing sex and age of adult flies
  - Timing and experimental setup
  - Microscopy → screening phenotypes

Students acquire images, import to R for analysis



- Phenotypic characterization
  - Variegation
  - Temperature sensitivity
  - Repeat-dependence

Students extract/digest DNA, inverse PCR to map

Line	Phenotype	Position	Chromatin State	Yeap
1	Variegating	2L: 2163001	Active	Yes
2	Variegating	2L:202103	Polycomb	No
3	We	3R:2790309	Heterochromatin	Yes
4	Variegating	2: TBD	?	?
5	Variegating	3: TBD	?	Yes
6	Variegating	3R:7955524	Intergenic Euchromatin	No
7	Variegating	2:TBD	?	?

- Bioinformatics
  - Inverse PCR
  - BLAST mapping → Position
  - Chromatin state

Arsham et al, unpublished results