Annotation for McMillan and McGuire. The homeotic gene spineless-aristapediaaffects geotaxis in *Drosophila melanogaster. Behavior Genetics, 22*;557-573.

TITLE:

The title of an original research paper often seems quite pretentious. Between 1975 and 1989, Senator William Proxmire (Dem. Wis.) used to give out Golden Fleece Awards to government programs and federally funded grants with such pretentious names. However, theses long, convoluted names do have a function. A paper title provides information. They are the first way in which researchers retrieve articles that they are interested in reading. Before the availability of computer searches, it was important to place as much pertinent information as possible into a title.

What information could be obtained from the title of this paper?

The paper is about a homeotic gene named *spineless-aristapedia.* To someone familiar with genetics, this means the paper is about a special type of gene (a homeotic gene) implicated in development. A homeotic gene is responsible for the pattern of a limb or organ. The specific gene is called *aristapedia*. This name implies that the mutation transforms the **arista** (on the antenna) into a foot or leg (***pedia***). **Furthermore, this mutation is an allele of the gene*spineless.*** Since the mutation name does not start with a capital letter, **it is probably recessive**.

The specific behavior being studied in this paper is **geotaxis**. Geotaxis is the movement of an organism toward or away from the pull of gravity. That is, the organisms move up or down. This definition of the behavior geotaxis is in **terms of consequences.** The **physical description of geotaxis,** as measured in this paper, might be described later**.**

Finally, the gene occurs in and the behavior is measured in ***Drosophila melanogaster***. This is the genus and species name for the common fruit fly used in genetics laboratories around the world.

Summarizing from the title, we can infer that fruit flies with the **aristapedia** mutation differ in their movement up or down from flies without the **aristapedia** mutation.

KEY WORDS:

Key words are a second means of indexing. Key words are required by some journals to reduce the size of the titles. That is, not every important word has to be in the title. From the key words given here we can infer that this paper concerns the **genetic analysis** of *Drosophila* for behavior (behavior genetics). These genetic analyses include 1) **biometrical analysis,** and 2)**deletion mapping**. Thus, the approaches in this paper will be mathematical analyses of many hybrid generations (biometrical) and the deletion mapping of the geotaxis effect to a specific site on the chromosome. The term $\mathbf{ss^a}$ is the abbreviation for the *spineless-aristapedia* allele.

ABSTRACT:

The abstract is a mini-paper. It tells the readers all of the pertinent points and lets them decide if the paper is worth further reading. In the abstract we first learn what the allele*spineless-aristapedia* does physically to a fly. This is old information. Next we learn the new information that *spineless-aristapedia* (abbreviated as ss^a) also makes flies move downward (positive geotaxis) in a geotaxis maze of the type invented by Hirsch. This effect is specific toss^a and does not occur with all antennal mutants nor with flies with amputated antennae. Mapping studies using deletions on the third chromosome place the physical effect of ss^a and the behavior effect of positive geotaxis in the same band of chromosome 3. **This implies that the two phenotypes are due to the same gene or to two linked genes**. Finally, the biometrical analyses confirmed the effect of ss^a. The biometrical analyses detected other genes on the X-chromosome that affect geotaxis.

INTRODUCTION:

In the introduction, the authors state the problem that this paper will address and give some background for that problem. The mini-review of previously published papers will help others place this paper into a scientific perspective.

We learn that geotaxis was one of the earliest behaviors used in behavior-genetic experiments. Those earlier analyses have suggested that geotaxis is a polygenic trait. Work by another team (Markow and Merriam, 1977) suggested (but did not prove) that single gene mutations might affect geotaxis.

The authors propose that there are two ways to find single gene traits affecting behavior. The first way is to mutagenize flies and isolate mutations. The second way is to look for preexisting mutations. The authors used the second approach. They started with antennal mutants since the antennae had been implemented in gravity sensing in some insects. In retrospect, this approach might have failed since most antennal mutants, other than ss^a,did not have a big effect on geotaxis.

We then state that the paper concerns itself with an analysis of the effect of ss^a. Studies will include a number of control experiments, testing different ss^a alleles, deletion mapping of the positive geotaxis effect and a biometrical analysis.

METHODS:

In the method section, the authors must give sufficient information so that one could reasonably replicate the experiment.

Subjects: The source of the flies used in the experiments is given. This might allow others to obtain the same strain of flies. Flies were all male and were aged 3-5 days. Although not stated here explicitly, male flies were used since females which are gravid tend to move downward due to their weight.

Mutant Stocks: The stocks used in the experiment are detailed.

Fly Rearing: Each lab has its own rearing techniques. The flies were reared on a cooked medium of cornmeal and sugar. They were maintained at constant temperature on a 16/8 light-dark cycle. The anesthesia was triethylamine (formula given). Other labs might use ether or CO_2. Flies were collected, placed into vials and allowed to recover from the anesthesia for 24 hours.

The Behavior: Although globally the authors said that the behavior was geotaxis, in actuality, the experimenters are only measuring the number of up/down choices that the flies made in a maze. This is geotaxis in terms of *physical description*. Flies are drawn through the maze by a light that equally illuminates the maze. Flies that make 8 up choices are collected in the topmost tube. Flies making 4 up and 4 down choices end up in tube number 4. Only the number of up/down choices is known but not the sequence. For example, there are 70 unique ways of ending in tube 4. Flies making 8 down choices end up in tube 0.The apparatus is well diagramed (Figure 1).

Statistics: A mean and variance could be calculated for each run or mutation. These means are compared using the parametric test - the ANOVA. The *main effect* that is being tested for is**genotype**. Individual mutations were compared to each other using a Scheffe post-hoc test. There are many such tests but the Scheffe is very conservative. This means that any differences that one finds are very significant. One must always evaluate the statistics that one uses. In this case, the experimenter's cite Day and Quinn (1989) to justify their choice of Scheffe test. Actually, we (the experimenters) used another test originally, and the editor of the first manuscript (who was an expert statistician) demanded a better post-hoc test. A final published manuscript is a collaboration between the experimenters, the reviewers and the editor.

Behavioral Controls: It is not enough to say that we are testing the effects of genes. One must also think what other explanations there are for our observed results. In this, case we thought of three possibilities. First, the legs on the head of the flies might be so heavy that the flies were forced to travel downward. If this were the case, then removing the heavy legs should make the flies normal. However, amputation is not a trivial thing to do and hurts the flies. Thus, the correct comparison is amputated ss^a flies to amputated normal flies. A second possibility was that ss^a flies were very slow. Other researchers have suggested that there is a positive correlation between the speed of maze completion and geotaxis. Thus, it was essential to show that wildtype and ss^a flies could complete the maze in the same time. This control was done in a horizontal maze where everything remained the same except the force of gravity. The third possibility was that the ss^a flies could not climb. There is a wonderful story from Rockefeller University where the shop polished a geotaxis maze to such a perfect surface that all flies ended in the bottom tube. They could not climb the walls! If ss^a flies had no trouble climbing, then equal numbers ofss^a and wildtype flies should complete the climbing of a 20-cm plastic tube.

Alleles of spineless: We allowed our fingers to do the walking through the stock books. It was obvious that we should get the allele spineless (ss) which does not transform the antennae. The other alleles were just what were available. Since some alleles were recessive lethals, all alleles were placed over the *ss* allele. This allowed the expression of each allele without lethality. Reciprocal F_1males were tested since at this time we already knew of the X-chromosome effect.

Deletion Mapping: Again, we allowed our fingers to do the walking through the stock books. The deletions all had breakpoints near 89C where the ss^a gene has been mapped. By constructing heterozygotes with a deletion over the ss^a allele we could distinguish the physical phenotypic effect from the behavioral effect.. That is, if the two phenotypes are caused by the same thing, we would only see the positive geotaxis when we also saw the antennal transformation.

Biometrical Analyses: We used an unconventional technique to show that *ss*^{a} affected geotaxis. Rather than back cross the *ss*^{a} gene to a common background (which would take 10 or more generations) we did many different crosses and tested the idea that the behavioral differences between the *ss*^{a} stock and the Canton Special stocks differed only by one gene. This was risky and actually "stacked the deck" against finding any results. We were looking for co-segregation for the geotaxis score and the antennal transformation.

Construction of the models: Understanding these models requires fairly specialized information. The important points to note are that flies were tested from 18 different generational populations. Both males and females (virgin) were tested. The expected model was adjusted for the observed lower viability of the *ss*^{a} allele. The model is given. The models were evaluated using an arc-sign transformation. Since this is so technical, complete details are not included in the paper. Interested scientists would most likely contact the authors.

RESULTS:

All antennal mutants had some effect on geotaxis. The *ss*^{a} gene had the largest and most dramatic effect. Blind flies (ey^D and ey^a) showed normal geotaxis. Because of these results, the*ss*^{a} allele was chosen for further study. The reasons are explained.

Controls: The idea that the heavy legs might actually be weighing down the heads has some basis in fact. Amputated *ss*^{a} flies showed a significant increase in geotaxis scores. This did not make them into wildtype flies, however. Amputation of wildtype flies had no effect.

ss^a flies showed as much activity and were as good at climbing as wildtype flies. The difference in geotaxis scores could be due to either activity differences or differences in climbing.

Alleles: All alleles except *ss* had some effect. *ss* flies were not different from wildtype. There was a trend in that the deficits in geotaxis score were correlated with the degree of transformation but this result is confounded with background genotype. Investigating this would require a completely different study.

Deletion Mapping: In general, wildtype flies showed medium geotaxis (that is, ended up in the center of the maze) Transformed flies showed positive geotaxis. It was quite interesting that the transposition of a wildtype allele of *ss*^{a} on the X-chromosome completely compensated for the *ss*^{a}allele and a deficiency on the third chromosome.

Biometrical Analysis: The biometrical analysis showed that the differences in geotaxis are due to the *ss*^{a} allele and its interactions with genes on the X-chromosome. The X-effect is manifested even if the flies are heterozygous for *ss*^{a}.

DISCUSSION:

In this section we sum up the results, put our own interpretation on the results and suggest additional work.

1. The geotaxis effect and the *ss*^{a} transformation are caused by a single gene or by closely linked genes. The same information comes from the deletion map or and from the biometrical analyses.

2. There are genes on the X-chromosome which also affect geotaxis

3. We suggest that the *aristapedia* effect is due to actual changes in the nervous system of the flies. Deleted at the request of the editor was a short discussion on the neural circuitry of the *ss*^{a} which seems to resemble that of a leg rather than that of an antenna.

4. The final discussion point is how *ss*^{a} flies might be used to generate geotaxis mutants.