

# The Effects of Rapid Cold Hardening on Courtship in *Drosophila melanogaster*

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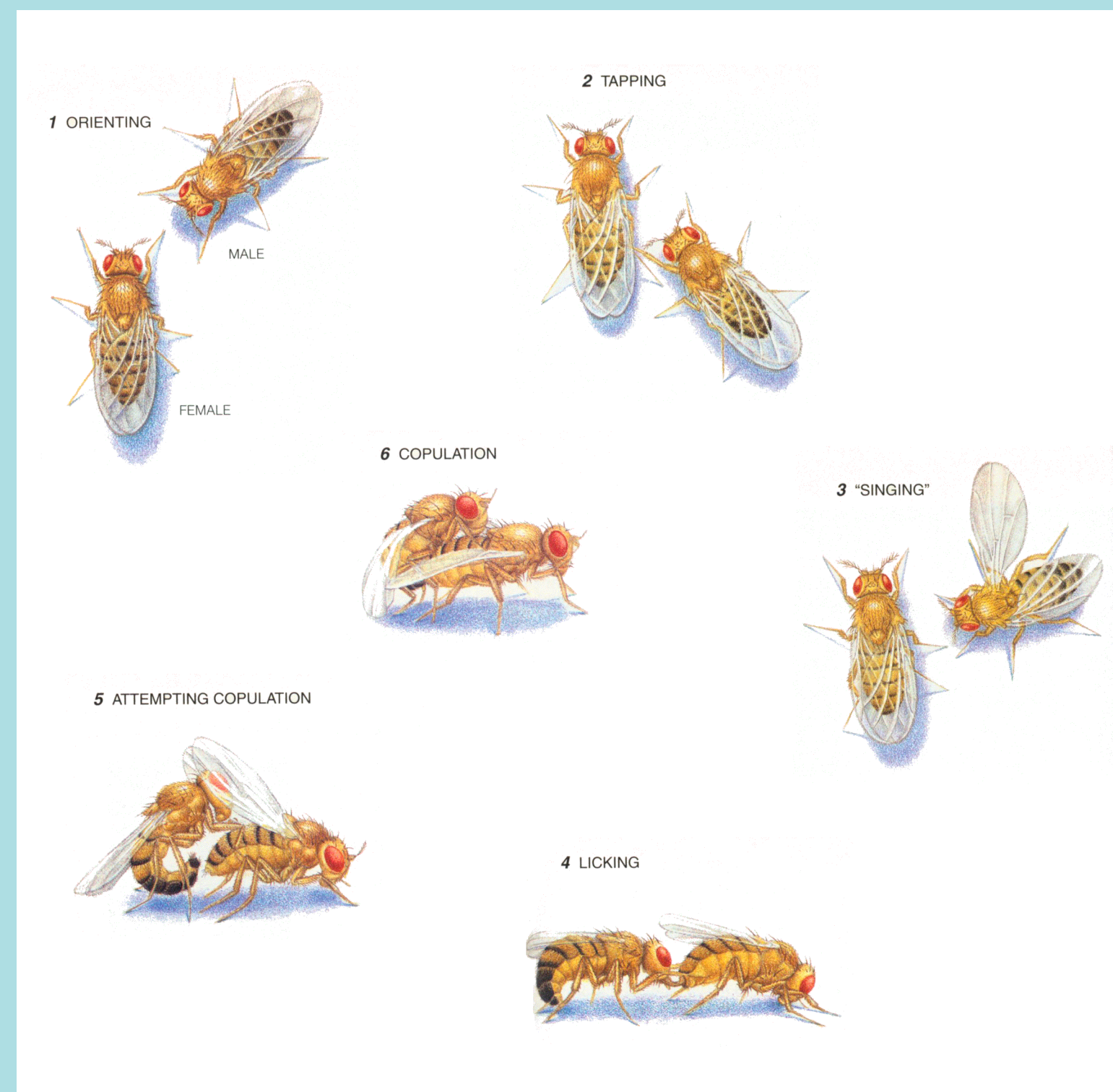
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## Abstract

The ability to survive through harsh winter conditions is a problem faced by many small animals, specifically exotherms, each year. Rapid Cold Hardening (RCH) is a phenomenon observed in many species of insects, including *Drosophila melanogaster*, and results in an enhanced tolerance to extreme cold conditions. With the ability to rapidly cold harden, the fly is at an ecological advantage because it will have greater reproductive success than those flies that do not have these abilities. This project will observe courtship bouts between Oregon-R wild type *Drosophila melanogaster*, after being placed in extreme cold conditions. Upon acquiring sufficient data, the procedure will be repeated using a bang-sensitive mutant strain, *easily shocked* (*eas*). The *eas* strain has a mutation that affects the nervous system, which can be hypothesized to affect the fly's ability to rapid cold harden. The goal of the project is to see if the flies from the mutant strain, *eas*, are able to preserve their reproductive behaviors relative to their control counterparts, the Oregon-R strain.

## Introduction

*Drosophila melanogaster*, has been recognized as a model organism since the early 1900s, since its genome has been completely sequenced and because its behaviors can be easily studied (Beckingham *et al.*, 2003). One behavior of interest is courtship, which involves a series of complex events that if successfully completed leads to copulation.



Initially, the male will orient toward his potential partner and tap her with his front leg. This is then followed by his first wing vibration which aids in the female's recognition of the male. Subsequently, the male will begin to chase the female while continuing his wing vibrations. If the female is receptive to the male she will slow her locomotion, which allows the male to curl his abdomen in an attempt to copulate. During most courtship bouts the male will attempt to copulate multiple times before being successful (Greenspan & Ferveur, 2000).

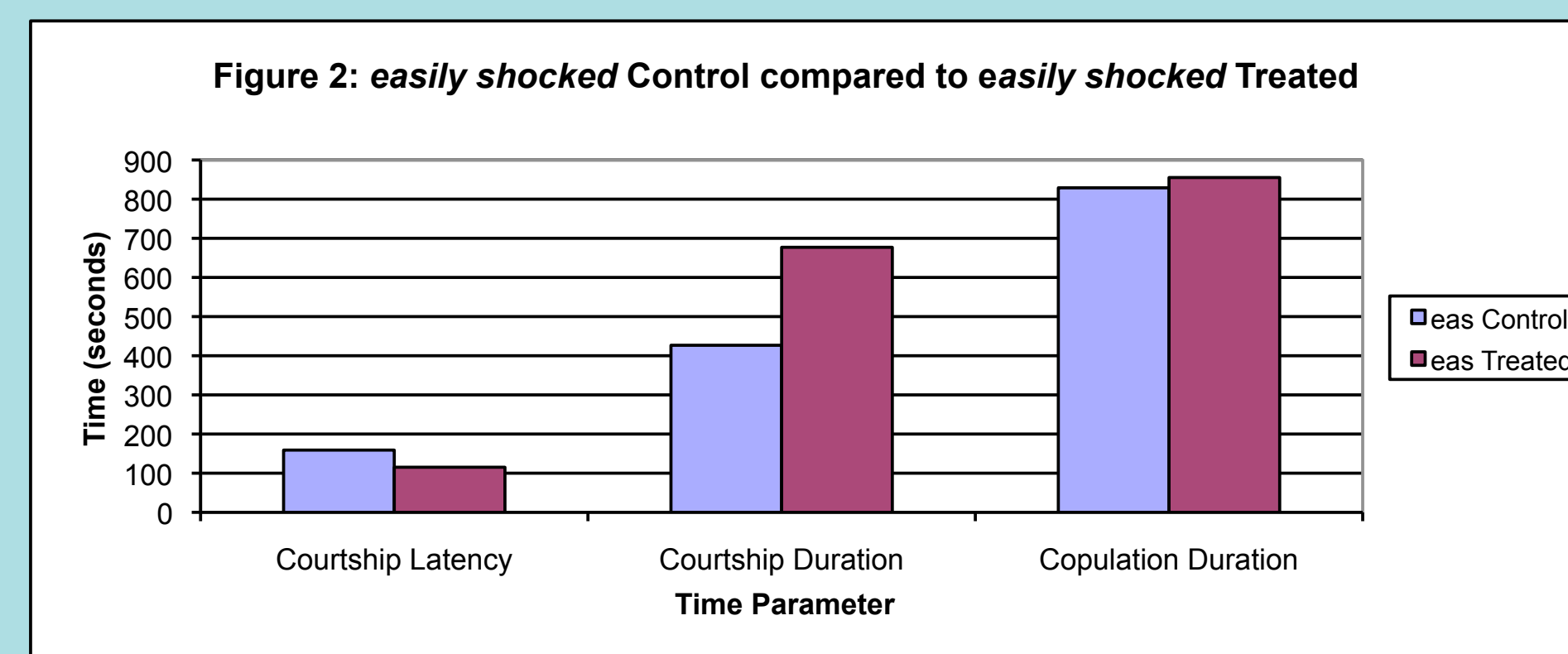
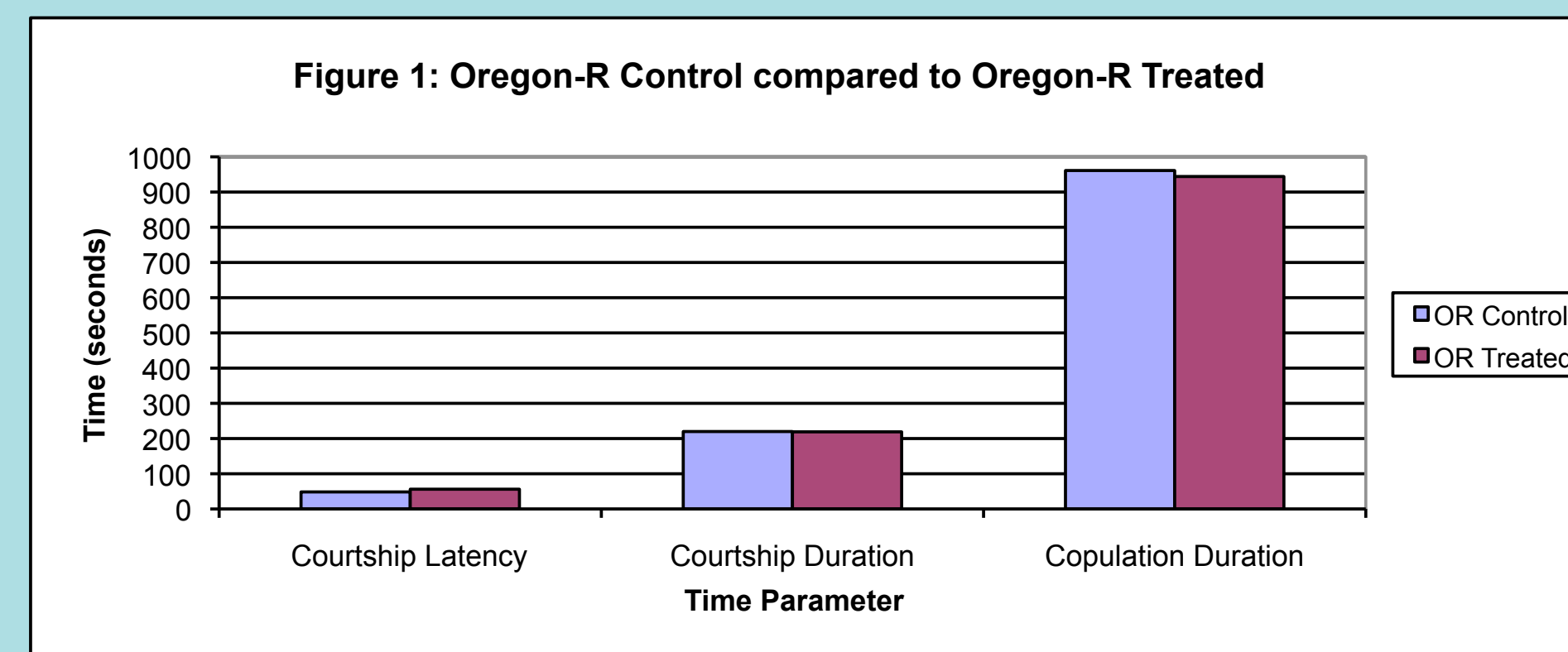
Rapid Cold Hardening (RCH) is defined as the "acquisition of enhanced tolerance to low temperatures over a short period of time" (Shreve *et al.*, 2004) and is of interest to scientists so they can understand how insects tolerate extreme cold conditions. Shreve and his colleagues concluded that *D. melanogaster* were able to preserve their reproductive behaviors when given an acclimation period in the cold, with the use of RCH. They

concluded that flies subjected to the RCH conditions engaged in courtship for longer periods of time when compared to the control group. Such RCH capabilities can alter the flies' evolutionary fitness giving them a reproductive advantage.

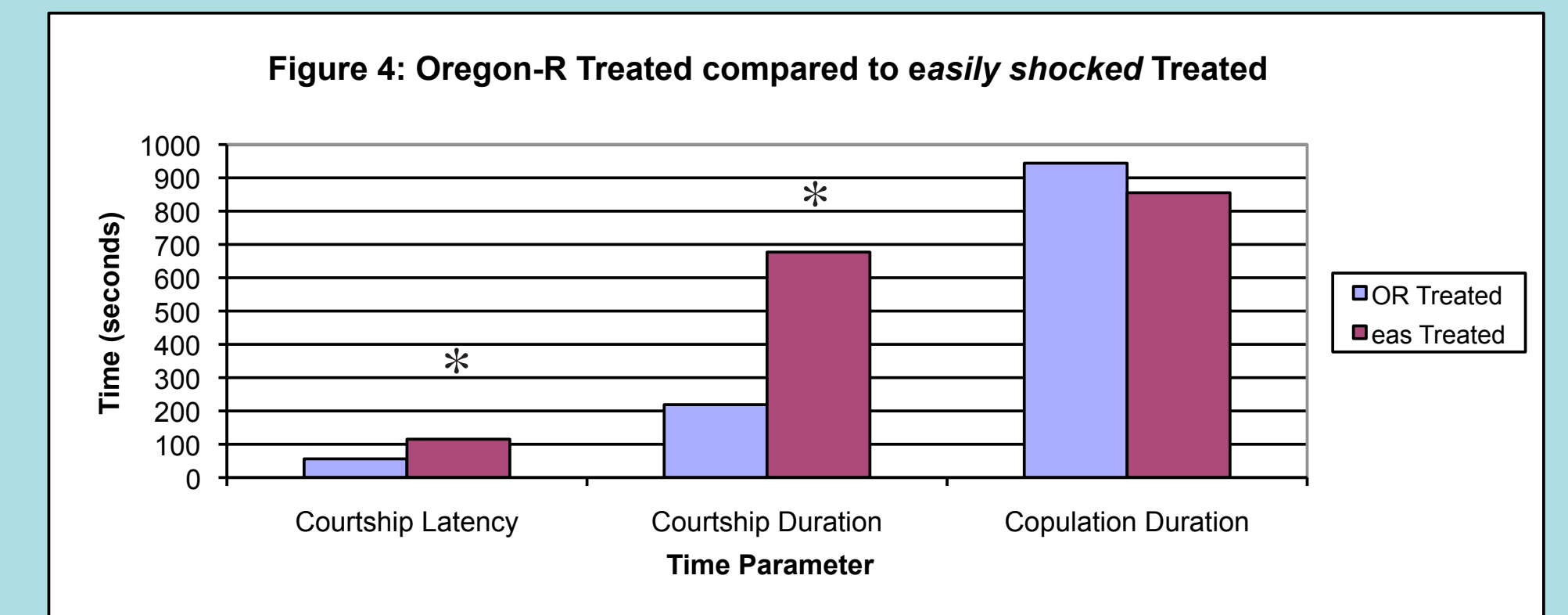
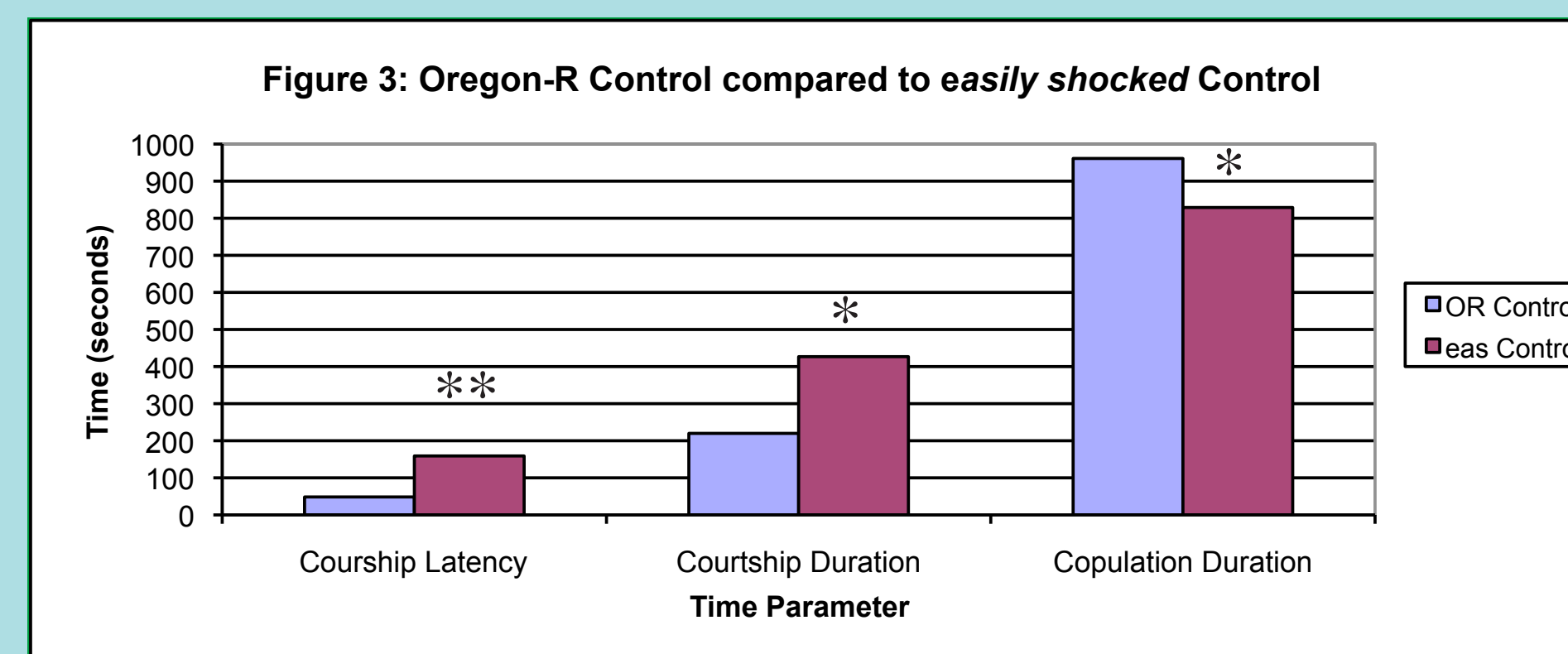
The *easily shocked* mutation falls in the category of bang-sensitive mutations which produce hyperexcitability followed by a period of paralysis upon mechanical stimulation. Specifically, the *eas* mutation has a defect in the structural gene for ethanoamine kinase which is required for the synthesis of phosphatidylethanolamine (PE), a lipid found in the cell membrane. This defect causes altered levels of PE which is known to cause the neuronal excitability exhibited by the flies (Pavlidis *et al.*, 1994).

## Results and Discussion

The data collected suggests that Rapid Cold Hardening does not have any negative effect on the reproductive ability of the Oregon-R and *easily shocked* strains of *Drosophila*, represented in Figures 1 and 2.



The experiment also concluded that there is a difference in the ability to court and mate between the genotypes, represented in Figures 3 and 4.



These differences could be an effect of the mutation on the nervous system within the *easily shocked* strain. Many aspects of courting rely heavily on the nervous system, such as physical movements and recognition of visual and chemical stimuli. If these are affected from the mutation, then courting may also be affected. Another explanation is that the *easily shocked* mutation may produce non-neural effects on the reproductive system which could cause the shortened copulation duration seen in the mutant strain. Further experimentation will need to be conducted to determine the relationship between the effects seen on the nervous and reproductive systems.

## Experimental Methods

This experiment involved the use of two strains of *Drosophila melanogaster*. The wild-type strain, Oregon-R, was obtained from the Bloomington Drosophila Stock Center at Indiana University. The mutant strain *easily shocked* (*eas*) was provided by Professor Elaine Reynolds of Lafayette College. All flies used for testing were collected zero to eight hours after eclosion to insure they were virgins.

In order to complete the Rapid Cold Hardening portion of the experiment, insulated black ice buckets were used. Large vials were taped to the perimeter of the buckets, which allowed for smaller vials to be inserted into them. The smaller vials contained the individual flies for testing. Water was added to a level which met the bottom of the foam stoppers in the small vials. With the addition of ice, the temperature was held constant (10-15°C). Each male-female pair was exposed to the cold conditions for a two-hour acclimation period and then removed for a one-hour recovery period before courtship testing.

Courtship assays were performed in an apparatus formed by a 0.25 cm thick Plexiglas plate with holes of 1.27 cm in diameter that lay on top of a glass plate with microscope slides covering each individual hole. Courtship was then observed through the top of the apparatus. For all baseline experiments, the male and female flies came from the Oregon-R strain. For all experimental trials, the males were obtained from the *eas* strain while females were collected from the Oregon-R strain.

All data collection was based on time measurements:

- Courtship Latency (CL) - the time that passes between the introduction of the male to the female until the first observed wing vibration by the male.
- Courtship Duration (CD) - the time that passes between the male's first wing vibration and his successful attachment to the female.
- Copulation Duration (CopD) - the time that passes from the point of attachment until the male separates from the female.

## Works Cited

Beckingham, K.M., Armstrong, J.D., Texada, M.J., Munjal, R., & Baker, D.A. (2005). *Drosophila melanogaster* - The Model Organism of Choice for the Complex Biology of Multi-Cellular Organisms. *Gravitational and Space Biology*, **18**(2), 17-30.

Greenspan, R.J., & Ferveur, J. (2000). Courtship in *Drosophila*. *Annual Review of Genetics*, **34**, 205-232.

Pavlidis, P., Ramaswami, M., & Tanouye, M.A. (1994). The *Drosophila easily shocked* Gene: A Mutation in a Phospholipid Synthetic Pathway Causes Seizure, Neuronal Failure, and Paralysis. *Cell*, **79**, 23-33.

Shreve, S.M., Kely, J.D., & Lee, R.E. (2004). Preservation of reproductive behaviors during modest cooling: rapid cold-hardening fine-tunes organismal response. *The Journal of Experimental Biology*, **207**, 1797-1802.