Ecologically driven genetic variation in *Daphnia magna* swimming behavior

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Introduction

In recent years, interest has grown in the neurobiology of organisms and the implications for their behavioral patterns. Though the nervous system of *Daphnia magna* is less complex than vertebrate nervous systems, studies have revealed *D. magna* share several neurotransmitters, and related gene pathways, with vertebrates.[1] Anti-predator behavioral responses in daphniids is well recorded throughout the literature,[1] and thereby confirms possible variation in *D. magna* swimming behavior due to ecologically driven genetic differences.[2]

The purpose of this study is to examine the individual swimming behavior in a horizontal plane of four unique *D. magna* genotypes from three ecologically distinct environments (Finland, Germany, and Israel). Each locale presents unique ecologies that may drive the evolution of disparate behaviors. Specifically, Finland ponds are characterized by arctic brackish water with invertebrate predators, German ponds are temperate freshwater ponds with vertebrate predators, and Israel ponds are tropical freshwater ponds with invertebrate predators. This study provides evidence for variation in behavior across genotypes, likely due to predator-driven genetic differences.

Materials and Methods

Daphnia magna genotypes used in this experiment were clonal descendants of lineages originally collected from three ecologically unique environments: Finland (F), Germany (G), and Israel (I). The four different genotypes used were FC, GA, GC, and IC. The goal in using unique ancestral populations was to uncover innate ecological and genetic differences.

Prior to conduction of the experiment, the water fleas were reared in 3 L jars containing Aachener Daphnien Medium (ADaM)[3] under a 16L:8D photoperiod at a constant temperature of 18 °C. Biweekly, the *Daphnia* were fed the unicellular green algae, *Scenedesmus obliquus*, cultivated at room temperature in autoclaved Bold's Basal Medium (BBM).[4]

During the experiment, individual adult females were transferred from the jars into 150 mL beakers containing 100 mL of ADaM and *S. obliquus* at a concentration of 600,000 cells/mL. Beakers were maintained at 18 °C under a 16L:8D photoperiod for 48 hours prior to behavior assessment.

After the acclimation period, the female was then transferred into a petri dish with 40 mL of the solution from the beaker it had been habituating and allowed to acclimate for 30 seconds. Twenty-five second videos of each individual were then recorded and analyzed to quantify behavior. Thirty individuals from each genotype were recorded, totaling 120 videos overall.

Tracker video analysis and modeling tool software was used to analyze the individual swimming behavioral videos of the *Daphnia*. Six aspects of behavior were quantified using the tracking software: mean velocity, maximum velocity, standard deviation of velocity (to estimate the erratic nature of behavior), mean acceleration, maximum acceleration, and standard deviation of acceleration.

Data for each trait was analyzed using one-way ANOVA followed by post-hoc tests in Program R.[5]

Results and Discussion

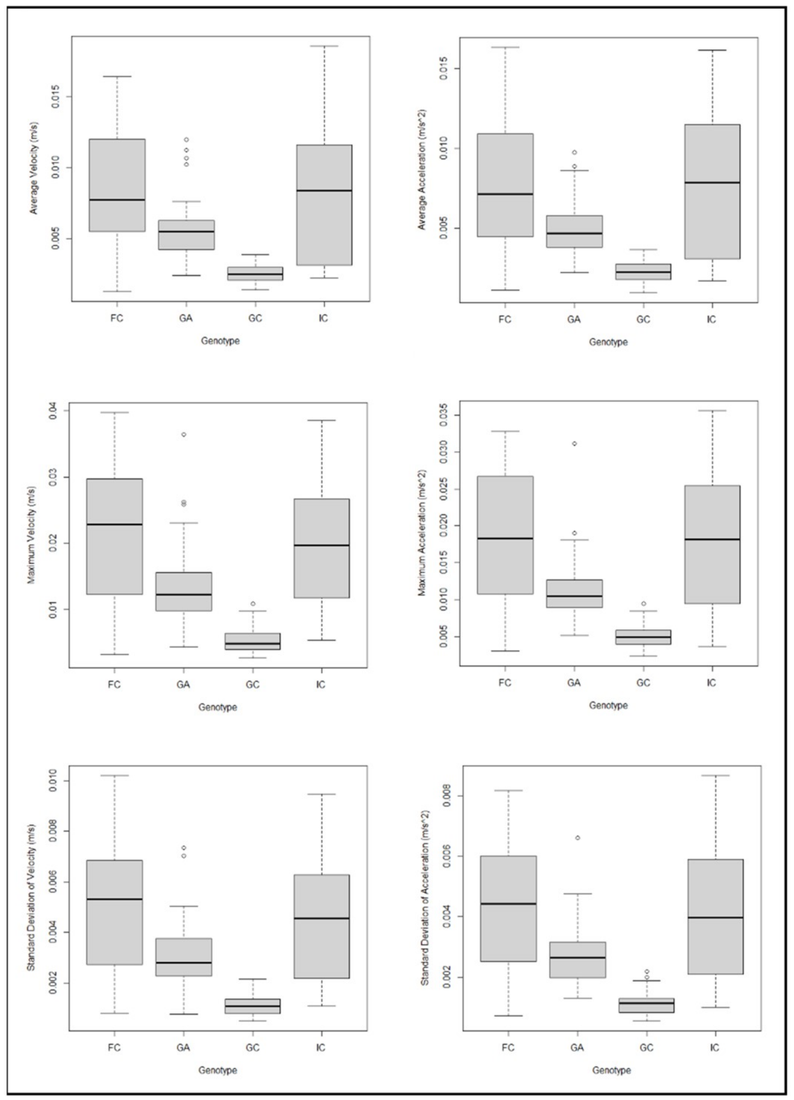

Figure 1. Summary plots of behavioral traits in various *Daphnia magna* genotypes

Throughout the literature, morphological evolution in daphniids induced by predators is well established.[1] Additionally, it has been found that the types of predators present, i.e., vertebrate or invertebrate, can also influence *D. magna* responses.[6, 7] These anti-predator behavioral differences have been shown to have a genetic basis.[2]

In general, behavioral analysis concluded FC and IC clones were quicker while GA and GC clones were consistently slower. In a previous study on similar genotypes, corresponding findings were recorded.[8] These results could reflect genetically predisposed anti-predator behaviors. IC clones may exhibit faster swimming behavior due to a lack of vertebrate, visual predators which would allow the water fleas to move freely without worry. Ancestral Israeli environments also tend to be warmer, and therefore, the rapid movement the animals displayed could have been compensating for the lack of warmth the creatures were accustomed to.[8]

FC clones also had quicker swimming behavior. Finland *D. magna* environments are known to contain high salt concentrations as they are near the ocean. Higher salinity habitats are typically characterized by invertebrate predators, in which case, the F clones were positioned to moving quicker and reacting faster to flee from such predators. Because slow behavior is a customary avoidance behavior observed in *Daphnia,*[6] it would appear the German clones (GA and GC) were exposed to numerous vertebrate predators.

Table 1. ANOVA summary statistics between *Daphnia magna* genotypes and behavioral traits

Trait	df	F value	p value
Average Velocity	3/116	19.1	<0.001
Maximum Velocity	3/116	24.3	<0.001
Standard Deviation of Velocity	3/116	24.1	<0.001
Average Acceleration	3/116	19.5	<0.001
Maximum Acceleration	3/116	24.0	<0.001
Standard Deviation of Acceleration	3/116	24.5	<0.001

Conclusions

The ecological and environmental impacts on organisms can lead to behavioral differences across genotypes.[2, 6, 7] This experiment established pre-existing genetic differences in behavior across four unique *D. magna* genotypes from three ecologically distinct environments.

Future Work

Genetic variation in behavior could also be driven by a myriad of other factors.[9] Of interest, putative behavioral differences may also reflect a correlation between gut microbiome composition and neural function.[10] Such a relationship could open the door to connecting higher level organisms, their gut microbiomes, and their behaviors.[9, 10]

Acknowledgements

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