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REPRODUCTIVE ISOLATION IN AMPHIPOD POPULATIONS

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ABSTRACT

Studies of genetic variation and reproductive isolation among populations are important because they can provide insight into the processes leading to the origin of new species. We hypothesized that freshwater amphipod (*Gammurus minus*) populations in isolated springs should show mating preferences, i.e., males should amplex more frequently with females from their "home" spring than from "foreign" springs. To test this hypothesis we compared the frequency with which males from each of two springs (Ell and Petersburg) in Pennsylvania amplexed with females from each of these springs. Laboratory arenas were set up with a male from one spring and a female from each of the two springs to examine mate selection. Results suggested that both Petersburg and Ell Spring males showed no preference when choosing a female for amplexus, thus providing no evidence for reproduction isolation between the two spring-dwelling populations.

Keywords: Amphipod, Gammarus minus, *reproductive isolation, springs*

INTRODUCTION

Studies of genetic variation and reproductive isolation among populations are important because they can provide insight into the processes leading to the origin of new species. McPeek and Wellborn (1998) studied the degree of reproductive isolation and patterns of genetic structure and diversification within seven populations of freshwater amphipods. Trials demonstrated that there was no interbreeding in crosses involving individuals of dissimilar ecotypes. They proposed that the lack of distinct allele differences and the relative levels of allele frequency differentiation within and between ecotypes suggested recent local speciation.

In this study an analysis of reproductive isolation among populations of the amphipod *Gammarus minus* in both Petersburg and Ell Springs was conducted. Based on the findings of McPeek and Wellborn (1998), we hypothesized that male amphipods from each spring should only amplex with females from their home spring. If this hypothesis proves to be correct, it may indicate that populations of *Gammarus minus* in isolated springs are beginning to become separate species.

The understanding of the possibility of reproductive isolation is vital for species survival. As stated in McPeek and Wellborn (1998), adaptive divergence among populations may restrict gene flow among populations, allowing secondary genetic differentiation and perhaps fostering speciation and extinction. For example, if two populations have become genetically isolated and one is approaching extinction, this population's survival will not be aided by the introduction of individuals from the other population.

FIELD SITE

On March 20th and 27th, 2002, amplexing pairs of *G. minus* were obtained from Ell Spring and Petersburg Spring in Huntingdon County, Pennsylvania. These two springs are similar in most environmental aspects except for predation, as seen in Table 1.

Table 1. Environmental Variables of Ell and Petersburg Springs

Environmental Variable	Ell Spring	Petersburg Spring
Width (m)	2.49	3.0
Depth (cm)	5.47	8.0
Flow Rate (m/sec)	0.482	1.3 (m/sec^{-1})
Discharge (m^3/sec)	0.066	24 (10^{-2} m^3/sec^{-1})
Temperature (C^o)	9.27	10
pH	7.46	7.03
Conductivity(microsiemens)	258.7	138.7
Dissolved O2 (ppm)	11.2	8.8 (mg 1^{-1})
Substrate		
Boulders	3%	<10%
Cobbles	12%	>50%
Gravel	45%	>50%
Sand	30%	10%-50%
Silt	10%	0%
Macrophytic Coverage	40%	>50%

METHODS AND MATERIALS

In the first trial, we collected 20 amplexing amphipod pairs from both Ell and Petersburg springs using small nets, and placed them into plastic buckets for transport back to the laboratory. Detrital leaves were placed into the containers to provide an ample food resource. Additional water was collected at each spring for later use during experiments. Males and females were separated and placed into different containers.

The following day, arenas were constructed by placing one male from one spring and one female from each spring into specimen cups containing water and food from the male's home spring. It was necessary to distinguish between the two females. A failed attempt to use an artificial marking technique using permanent markers led to the differentiation of females by natural color. Arenas were kept in an environmental chamber at 10^{o} C.

After 24 hours, the arenas were checked, and the amplexed females were identified. This process was repeated for a period of three days, after which the remaining amphipods were discarded. Due to the small sample size of this trial, another trial was necessary in order to obtain a total sample size of 76 tests.

The second trial was implemented with two alterations to the method. Amplexing amphipods were immediately separated into two different containers upon their collection at the springs. In addition, arenas were observed for a period of four days.

To analyze the data, we used the Chi Square test for Goodness of Fit and Association to determine whether the males showed a preference for a specific female.

RESULTS

Out of the total 76 arenas, 64 were amplexed after a four-day period, while 12 remained un-amplexed. The totals for each spring are displayed in Table 2.

Table 2. Totals of amplexing and nonamplexing pairs in arenas

	Petersburg male		**Ell male**	
	Ell Female	Petersburg Female	Ell Female	Petersburg Female
Amplexed	15	17*	19	13
Not Amplexed	6^		6	

* = 2 of these had only 1 female present after 4 day period

^ = 1 dead male after 4 day period

Two Chi Square Goodness of Fit tests were implemented for Petersburg Spring. The first, including those arenas with only one female, indicated that Petersburg males showed no preference for either female (χ^2 = 0.063, n = 64, P = 0.802). The second test, excluding the arenas where only one female remained, also showed no preference (χ^2 = 0.0, n = 60, P = 1.0). Similarly, the Chi Square Goodness of Fit test for Ell males showed no preference for either female (χ^2 = 0.567, n = 64, P = 0.451). A Test of Association was used to determine whether there was a difference in preference between Ell and Petersburg Spring males. There was not (χ^2 = 1.004, n = 64, P = 0.316).

DISCUSSION

Results of this experiment suggest that both Petersburg and Ell Spring males show no population preference when choosing a female for amplexus, possibly indicating that mate selection occurs at random. These results suggest that reproductive isolation is not occurring between the Ell and Petersburg Spring amphipod populations. However, further studies are needed due to possible errors. One error may be the lack of an adequate sample size. While there may be a preference for mate selection, our sample size may not have been representative of the total population. A second error may involve the similarities in chemical and physical properties of the springs. Ell and Petersburg Spring were selected based on these similarities to enable us to provide adequate living conditions in the arenas. This may have led to the males' inability to detect a difference between the two females, leading to random selection. A third error deals with the determination of female color when we selected them for the arenas. It is possible that we made mistakes in differentiating between colors when recording which female was amplexed. It is also possible that during collection and separation males and females were placed in the wrong container in addition to un-amplexed individuals accidentally being added to the containers. This error may have led to variations in the arena results.

There are a number of possibilities for further research. These may include a larger sample size, springs with varying characteristics, a greater number of springs being compared and an improved marking technique, to correct the aforementioned errors. In addition, genetic analysis of amphipods from various springs may detect possible reproductive isolation.

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