DO AMPHIPODS (GAMMARUS MINUS) FROM TWO ISOLATED POPULATIONS INTERBREED?

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ABSTRACT

We tested the hypothesis that speciation is occurring between two populations of the freshwater amphipod Gammarus minus that inhabit isolated alkaline, thermally stable springs (Petersburg and Ell) in central Pennsylvania. Our test focused on the degree to which males and females from the two springs would engage in amplexus in the laboratory. Males from each spring were placed with females from their own spring and from the other spring to see if amplexing would occur at differing frequencies. A Chi-square test for independence showed that amplexus occurs with equal frequencies between males and females regardless of which spring they originated from ($\chi^2 = 0.174, P > 0.05$). Therefore we found no evidence that speciation is occurring between the study populations.

Key words: alkaline springs, amphipods, Gammarus minus, speciation

INTRODUCTION

Amphipods are small crustaceans that are widely dispersed throughout Pennsylvania freshwater systems. Amphipods are sometimes referred to as “scuds” or “side swimmers” due to their tendency to turn on their side or back while moving. They are on average 5 to 20 mm in length. The species Gammarus minus is abundant in cool, alkaline springs. Amphipods do not appear in acidic springs due to a lack of calcium for carapace development. The abundance and behavior of amphipods in areas where predators exist are limited by and due to the stable environmental conditions of their spring water habitat. Amphipods can be used for year round ecological and evolutionary studies (Glazier, 1998).

Aquatic amphipods engage in amplexus or precopulatory mate guarding. Male amphipods will attach to the female’s back, and carry her around for a few days until they are ready to mate. Due to sexual selection, large males are favored because they are more likely to amplex with a female than a smaller male. The importance of amplexus with regards to the Gammarus minus is vital for the reproduction process within the species (Fong, 1989).

In the amphipod Hyallela azteca, there is evidence that recent speciation has occurred between populations. Populations in ponds with fish predators have become phenotypically different from those in ponds without fish. Furthermore, amphipods from these populations are genetically distinct and do not interbreed (McPeek and Wellborn, 1998).

We tested whether G. minus from a spring with fish (Ell) are able to interbreed with those from a spring without fish (Petersburg). These springs were chosen because they have similarly constant temperatures and water chemistry (e.g., pH). We carried out our test by comparing the frequency of amplexus between males and females from their home spring with those from the other spring. We
hypothesized that amplexus should be more frequent between males and females from their home population than from the “foreign” population. This hypothesis assumes that these isolated populations are undergoing genetic differentiation and geographic speciation.

METHODS AND MATERIALS

Ell Spring in Birmingham, Pennsylvania and Petersburg Spring in Petersburg, Pennsylvania, both alkaline springs, are known to contain large numbers of *Gammarus minus*. At each spring, small nets were held in the water by one researcher while another kicked up the sediment about a foot upstream of the net. All amphipods were placed in small buckets with water from their own springs. This was repeated in various parts of the springs until a sufficient number of amphipods were collected. The buckets were taken back to the lab. Four new containers were obtained and labeled so the males from Ell, the females from Ell, the males from Petersburg, and the females from Petersburg could be kept separate. All amplexing pairs were removed from the buckets of samples. The pairs were separated and the males and females were placed in their appropriate storage containers. The number of amplexing pairs from Ell Springs became the limiting factor, as in every trial, there were fewer amplexing pairs found in Ell than in Petersburg. Four shallow trays were obtained, and labeled as follows; 1. “Male Ell, Female Ell”, 2. “Male Ell, Female Petersburg”, 3. “Male Petersburg, Female Petersburg”, and 4. “Male Petersburg, Female Ell”. Trays 1 and 2 were filled with water and decaying leaves from Ell Springs and trays 3 and 4 were filled with water and decaying leaves from Petersburg Springs. Half of the males from Ell were placed in tray 1 and half in tray 2. A similar process was repeated, placing identical numbers of each gender in each tray. The trays were placed in a walk-in refrigerator with aerators to keep the amphipods alive. Twenty-four hours later, the number of amplexing pairs in each tray was recorded. A similar process was repeated two more times, and the frequency of amplexus in the above groups were compared using a Chi-square contingency test for independence.

RESULTS

We collected data for a total of 256 amphipods, 32 males and 32 females in each grouping, as shown in Table 1. Of the 32 pairs in each grouping, 24 pairs amplexed in the grouping of Petersburg males with Petersburg females, 24 pairs amplexed in the grouping of Petersburg males with Ell Females, 25 pairs amplexed in the grouping of Ell Males with Ell Females, and 25 pairs amplexed in the grouping of Ell males with Petersburg females.

Table 1. Shows total number of pairs and number of amplexing pairs after 24 hours for each combination of male (M) and female (F) amphipods from Ell and Petersburg Springs.

<table>
<thead>
<tr>
<th>Test #</th>
<th># Pairs per grouping</th>
<th>Petersburg M + Petersburg F amplexing</th>
<th>Petersburg M + Ell F amplexing</th>
<th>Ell M + Ell F amplexing</th>
<th>Ell M + Petersburg F amplexing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>9</td>
<td>6</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>7</td>
<td>8</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Totals</td>
<td>32</td>
<td>24</td>
<td>24</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

% of total pairs amplexing: 75, 75, 78, 78
A Chi-square contingency test for independence showed the frequency of amplexus did not differ significantly among the four test groups ($\chi^2 = 0.174, P > 0.05$; Table 2).

**Table 2.** Shows each setup of amphipod pairings along with column and row totals for use in a Chi-Square Contingency Test for Independence.

<table>
<thead>
<tr>
<th>Setup</th>
<th>Amplexed</th>
<th>Did Not Amplex</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>P m + P f</td>
<td>24</td>
<td>8</td>
<td>32</td>
</tr>
<tr>
<td>P m + E f</td>
<td>24</td>
<td>8</td>
<td>32</td>
</tr>
<tr>
<td>E m + E f</td>
<td>25</td>
<td>7</td>
<td>32</td>
</tr>
<tr>
<td>E m + P f</td>
<td>25</td>
<td>7</td>
<td>32</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>98</strong></td>
<td><strong>30</strong></td>
<td><strong>128</strong></td>
</tr>
</tbody>
</table>

Determining Expected Counts:

$$\frac{(R \times C)}{T}$$

$$\frac{(98 \times 32)}{128} = 24.5 = \text{expected count for number of amphipod pairs amplexed in each setup}$$

$$\frac{(30 \times 32)}{128} = 7.5 = \text{expected count for number of amphipod pairs not amplexed in each setup}$$

$$\text{Sum of (obs-exp)}^2/\text{exp} = \frac{(24-24.5)^2}{24.5} + \frac{(25-24.5)^2}{24.5} + \frac{(8-7.5)^2}{7.5} + \frac{(7-7.5)^2}{7.5} = 0.174 – \text{Chi Square Test Statistic}$$

$$X^2_{(0.05, 3)} = 7.81$$

**DISCUSSION**

We found no evidence of reproductive isolation between the two populations of *Gammarus minus* from Petersburg and Ell Springs. We can draw this conclusion because when males from St. Petersburg were mixed with females from Ell and when females from St. Petersburg were mixed with males from Ell, they amplexed just as frequently as when males and females from the same spring were mixed. These results contradict our hypothesis, which stated that amphipods from the same spring should amplex more frequently than amphipods that were combined from different springs. Perhaps *G. minus* has not speciated like *H. azteca* because it lives in springs that are interconnected underground (thus facilitating gene flow) rather than in isolated ponds. It is also possible that results could have been different, if there had been a larger sample size. If a test with a larger sample size does not yield a different result, we suggest conducting a study involving the geology of the area, and the level to which the springs in the area are interconnected. Assessments of gene flow among spring-dwelling populations of *G. minus* would also be useful.
REFERENCES

