Increased production of genetic mosaics in *Habrobracon juglandis* by cold shock of newly oviposited eggs

By R. M. PETTERS\(^1\) AND D. S. GROSCH\(^1\)

*From the Department of Genetics, North Carolina State University*

**SUMMARY**

Eggs from an *ebony* stock exposed to 5.5 °C prior to syngamy exhibited increased production of genetic mosaics in comparison with untreated eggs from the same females. No increase in mosaic production occurred for cold-shocked cleavage-stage embryos from the *ebony* stock or from pre-cleavage cold-shocked eggs from a wild-type stock. Heat shock of pre-syngamy eggs also failed to increase the production of genetic mosaics. These findings are consistent with predictions based on the post-cleavage fertilization theory of mosaic origin in *Habrobracon* or with a hypothesis of differential mortality.

**INTRODUCTION**

Theories of mosaic origin have been extensively discussed by Stern (1968). In the honey bee, *Apis mellifera*, Boveri proposed that gynandromorphs arise through a delayed fertilization mechanism which allows cleavage of the egg nucleus prior to syngamy. With the finding that polyspermy normally occurs in the honey bee, the theory of mosaic origin in the bee was modified to feature the division of supernumerary sperm. Genetic evidence indicates that fertilization of binucleate eggs and rare development of accessory sperm are the major mechanisms of gynandromorph origin in *Habrobracon* (Whiting, 1943). Genetic mosaics derived from crosses involving the *ebony* mutation are believed to result from post-cleavage fertilization (Clark, Gould & Potts, 1968). Evidence supporting this hypothesis has been obtained using a test-cross method (Clark & Gould, 1972). Cold shock of newly laid eggs from *ebony* braconid females should further delay syngamy and allow an increased frequency of precocious cleavage. Temperature effects on gynandromorph production have been documented for *Habrobracon* and *Apis* (Greb, 1933; Drescher & Rothenbuhler, 1963).

**MATERIALS AND METHODS**

*Habrobracon juglandis* Ashmead (= *Bracon hebetor* Say), a braconid wasp, is ectoparasitic on the larvae of the Mediterranean flour moth, *Ephestia kuehniella*.

\(^1\) *Authors' address*: Department of Genetics, North Carolina State University, Raleigh, North Carolina 27607, U.S.A.
Zeller (= Anagasta kuehniella Zeller). *Habrobracon* females normally produce haploid male progeny from unfertilized eggs, while diploid female and male offspring develop from fertilized eggs. Diploid males arise through homozygosity at the sex-determining locus and are present only in crosses involving parents of common ancestry.

The mutations *ebony* body color, *cantaloupe* eye color and *honey* body color were used as genetic markers in this investigation. All three are recessive and non-allelic. By crossing homozygous *ebony* females to *cantaloupe honey* males (*e/e??? × c; ho???*), the cuticular structures of the offspring can be classified according to sex and origin. Haploid male tissue will either be *ebony* (gynogenetic in origin) or *cantaloupe honey* (androgenetic in origin) in phenotype. Diploid tissue (zygogenetic in origin) will appear wild type (brown) due to heterozygosity at the mutant loci. Cellular autonomy of these mutations in gynandromorphs has been demonstrated. Mosaic individuals were identified through the use of (a) these incorporated eye and body color mutants, (b) external genitalia and (c) the secondary sex characteristics of antennal length and abdominal sternite sclerotization (Clark & Egen, 1975).

The time course of early *Habrobracon* development is well documented (Amy, 1961; Speicher, 1936). Prior to oviposition the mature eggs are arrested in the first meiotic metaphase. Syngamy occurs between 30 and 45 minutes after oviposition at 29–30 °C. Samples of eggs no older than 30 min were obtained by transferring mated *ebony* females every 30 min. The eggs from every other transfer were treated with a cold shock of 1 h in a refrigerator at 5–5 °C. This temperature was chosen to slow cellular processes and still permit survival of the eggs. Upon removal from the refrigerator the eggs were counted, placed on caterpillars and stored in the rearing incubator (29 °C). The alternate samples of eggs were used as controls. The foregoing experiments were designed for treatment of pre-syngamy eggs (Replicate Expts. I and II). Modification of this procedure to treat eggs in cleavage required a 30-min waiting period before the application of the cold treatment (Expt. III). A heat-shock experiment (1 h at 35 °C) was conducted for pre-cleavage eggs (Expt. IV). The use of Whiting no. 33 wild-type females instead of *ebony* females was another experimental modification.

The percentage of mosaics is used as a measure of mosaic production. It is calculated by dividing the number of mosaics by the number of fertilized progeny (females, diploid males and mosaics). Standard errors on percentage of mosaics, percentage survival and percentage of progeny fertilized were calculated according to the formula,

\[
\text{s.e. (s.d.)} = \sqrt{\frac{p(100-p)}{n}}
\]

described in Snedecor & Cochran (1967, p. 207). When \( p \) represents % mosaics, \( 100-p \) is that % not mosaics.

Where a comparison between two treatment groups was made, a \( 2 \times 2 \)}
Increased production of genetic mosaics

Table 1. *Progeny from e/e♀♂ x c; ho♂♂ crosses of Habrobracon*

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Percent survival</th>
<th>Types of survivors</th>
<th>% of progeny fertilized</th>
<th>% mosaics</th>
</tr>
</thead>
<tbody>
<tr>
<td>(<strong>Eggs treated</strong></td>
<td></td>
<td><strong>Geno-</strong></td>
<td><strong>Zygo-</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>genetic</td>
<td>genetic</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>♀♀</td>
<td>♂♂</td>
<td>Mosaics</td>
</tr>
<tr>
<td>Cold shock</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-cleavage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>1111</td>
<td>39.4±1.5</td>
<td>242</td>
<td>34</td>
</tr>
<tr>
<td>Treated</td>
<td>1182</td>
<td>32.7±1.4***</td>
<td>243</td>
<td>45</td>
</tr>
<tr>
<td>Cold shock</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-cleavage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>1056</td>
<td>27.8±1.4</td>
<td>125</td>
<td>24</td>
</tr>
<tr>
<td>Treated</td>
<td>1175</td>
<td>21.0±1.2**</td>
<td>125</td>
<td>47</td>
</tr>
<tr>
<td>Cold shock</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>During cleavage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>1107</td>
<td>23.3±1.3</td>
<td>117</td>
<td>17</td>
</tr>
<tr>
<td>Treated</td>
<td>1275</td>
<td>23.3±1.2</td>
<td>135</td>
<td>16</td>
</tr>
<tr>
<td>Heat shock</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-cleavage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>1205</td>
<td>16.1±1.1</td>
<td>72</td>
<td>14</td>
</tr>
<tr>
<td>Treated</td>
<td>1111</td>
<td>13.8±1.0</td>
<td>56</td>
<td>10</td>
</tr>
</tbody>
</table>

* denotes significance at 5 % level.
** denotes significance at 1 % level.
*** denotes significance at 0.1 % level.

Only one diploid male was found during these experiments.

contingency $\chi^2$ test was used (Snedecor & Cochran, 1967, p. 217). Those comparisons that are significant are indicated in Table 1.

**Observations**

Cold shock (Table 1, Replicate Expts. I and II) of pre-cleavage eggs significantly increased the proportion of mosaics among the fertilized offspring. However, the survival rate and the percentage of progeny fertilized in the treated group were lower in both experiments. In Expt. I, 39.4 % of the control eggs survived, while only 32.7 % of the treated eggs produced adults. The decrease in the proportion of progeny fertilized parallels the decrease in survival rate (Expt. I: controls 44.8 %, treated 37.0 %). Cold shock (Expt. III) of eggs during cleavage did not change any of the parameters measured. Heat shock (Expt. IV) of pre-cleavage eggs also failed to alter the proportion of genetic mosaics. Variation in the control survival rate between experiments can be
attributed to differences in the quality of the host caterpillars. No gynandro- morphs resulted from cold shock prior to cleavage of 1267 wild-type eggs (not shown in Table 1).

DISCUSSION

A central theme in Clark's hypothesis of post-cleavage fertilization is the delayed migration of the pronuclei. 'This delay may allow for precocious cleavage of the egg or sperm pronucleus or cleavage before the union of egg and sperm nuclei' (Clark et al. 1968). At low temperature, the pronuclei in pre-syngamy eggs should experience even more delay.

Indeed, cold-shocked pre-syngamy eggs from ebony females showed an increase in mosaic production, but identical treatment 30 min later did not alter the proportion of mosaics obtained. These results are consistent with the premise that mosaicism of an embryo is determined at syngamy in Habrobracon. Furthermore, since no mosaics could be found from cold-shocked wild-type eggs, it appears that some factor in the ebony stock is necessary for mosaic production to reach a recognizable level.

The heat shock experiment was performed mainly because of Greb's (1933) success when used to increase mosaic male production. Our failure to increase mosaic production with heat shock, coupled with the data collected by Clark et al. (1968), suggests the mechanism in crosses involving the ebony stock to be different from that involved in the production of mosaic males from unmated heterozygous females. Whiting and his students gave particular attention to the latter type of experiment (Whiting & Whiting, 1927).

It is evident from the survival rate that pre-syngamy cold shock kills some embryos. Surprisingly, the percentage of progeny fertilized also drops in the treated group, indicating that more females and mosaics are killed than haploid males. We do not know why individuals with diploid tissue should be more sensitive to low temperature. Perhaps the time required to complete mitosis or the cytoplasmic: nuclear volume ratio are of vital importance when the embryo is cold shocked prior to cleavage. At least in plants there is evidence that DNA content is related to the minimum time required to complete mitosis (Van't Hof & Sparrow, 1963).

A portion of the increase in mosaic production with pre-syngamy cold shock may be due to the differential mortality of diploid individuals (females). To examine this possibility, the progeny expected in the treated group was calculated using the control values of survival and percentage of progeny fertilized. In the extreme case that all fertilized progeny dying are females, the treated group's rate of mosaic production would decrease from 31·5 % to 21·6 % in Expt. I and from 38·5 % to 25·0 % in Expt. II. The treated group's mosaic production still exceeds that of untreated eggs in both cases. In the second experiment the difference is still statistically significant (0·05 > P > 0·01), suggesting
that the increased incidence of mosaics among cold-shock-treated eggs cannot be entirely attributed to increased female mortality.

In honey bees, cold shock of newly oviposited eggs increased mosaic production from a control value of zero (Drescher & Rothenbuhler, 1963), but data on differential mortality were not presented. Since gynandromorph production in Apis involves polyspermy and subsequent androgenesis (Rothenbuhler, Kulincevic & Kerr, 1968), the cold shock may affect mechanisms that would ordinarily cause the degeneration of accessory sperm.

In the experiments presented above, the differential mortality of diploids obscures the conclusion that the production of genetic mosaics was increased via pre-syngamy cold shock. Both differential mortality and a cold-shock influence on a post-cleavage fertilization mechanism remain as possible explanations.

The authors are grateful for support in part by a United States Public Health Service Traineeship (5 TO1 GM00296) to Robert M. Petters from the National Institute of Health.

The authors wish to thank Dr A. M. Clark for reading the manuscript and suggesting revisions.

Paper no. 4885 of the journal series of the North Carolina Agricultural Experiment Station, Raleigh, N.C.

REFERENCES


(Received 19 January 1976; revised 2 April 1976)