Effect of temperature on behavioural isolation : A study with *Drosophila ananassae* populations

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Experiments were conducted to study sexual isolation among two natural populations of *Drosophila ananassae* maintained at 18 °C and 24 °C for 12 generations in the laboratory to see the effect of this environmental variable on behavioural isolation. Multiple choice technique was used and matings were observed directly in Elens Wattiaux mating chamber. Results showed sexual isolation among strains that were maintained at different temperatures, indicating that temperature may have affected the mating behaviour of the flies which resulted in the induction of ethological isolation among the strains.

**Keywords**: Behavioural isolation, *Drosophila ananassae*, Temperature effect

The first steps of animal speciation are thought to be the development of sexual isolating mechanisms that act as barriers to gene flow between incipient species or between populations of the same species. Of great interest is understanding how evolution proceeds at the earliest stages of reproductive divergence. Such mechanisms can occur at three different levels: pre-copulatory or behavioural isolation that hamper interspecific mating such as differences in the recognition system, post-copulatory-pre-zygotic isolation occurring in the female reproductive tract or post-zygotic isolation which results in hybrid sterility or inviability as a consequence of genetic incompatibility.

Among different mechanisms of reproductive isolation, sexual or behavioral isolation is of much importance as the appearance of inferior hybrids is minimized or avoided. Although long recognized as important in intra-population evolution, sexual isolation has more recently been involved as a driving force behind speciation.

In this respect, *Drosophila* is perhaps the most preferred model organism and the most studied genus regarding the development of pre mating isolation through modification of sexual behaviours in natural or genetically manipulated populations. Differences in mating propensity between populations, lines and particular genotypes have been recorded in *Drosophila*.

In many *Drosophila* species, phenotype associated with premating isolation tend to evolve earlier than those associated with postmating isolation in recently diverged sympatric populations. Although recent progress has been made toward understanding the genetic basis of post-zygotic isolation, relatively little is known of the genetic architecture of sexual isolation—arguably the most important form of reproductive isolation in animals.

*Drosophila ananassae* belongs to the *ananassae* species complex of the *ananassae* subgroup of the *melanogaster* species group. It is an especially interesting species as it has a widespread cosmopolitan distribution and is a strictly human commensal. It occupies a unique status among *Drosophila* species as it is endowed with several genetic peculiarities and displays a high population substructure across the whole distribution range. In this species considerable variation in mate discrimination is observed throughout the species range. A number of investigations in sexual behaviour have been reported in *D. ananassae*. Singh and Chatterjee reported sexual isolation based on male choice experiments using isofemale lines and natural populations. The findings provide evidence for positive assortative mating between populations and indicate that laboratory strains of *D. ananassae* have developed behavioural reproductive isolation as a result of genetic divergence. Incipient sexual isolation
was observed between karyotypically different homozygous strains of *D. ananassae*\(^\text{17}\), which shows that chromosome arrangements may affect the mate recognition system in this species. Asymmetrical sexual isolation was observed among drift lines in *D. ananassae* which originated from mass culture stocks collected from three geographically different localities. The drift lines were passed through flush-crash cycle at every generation and maintained for 27 generations before testing the pattern of mating\(^\text{18}\).

Temperature plays an important role in the life history of insects and has a significant influence on fitness. The distribution and abundance of species is often large part determined by environmental variables of which temperature is generally considered to be one of the most important factor\(^\text{19,20}\). Studies showed that species differences in heat or cold resistance commonly match the geographical distribution of species\(^\text{20}\). Studies on the influence of temperature on mating ability in 12 species of *Drosophila* from different ecological and phylogenetic backgrounds showed that temperature dependant mating reflect the distribution of the species’ and the difference among species can be useful in predicting microclimatic differences\(^\text{21}\). Sisodia and Singh\(^\text{22}\) found that females of *D. ananassae* reared to adulthood at 18°C showed a significant increase in body weight compared to females reared at 25 °C. They also found a negative correlation (trade-off) between longevity and productivity in *D. ananassae*.

During the present study, sexual isolation among two populations of *Drosophila ananassae* kept at two different temperatures have been studied to see the effect of this environmental variable on behavioral isolation between the strains.

**Materials and Methods**

*Drosophila* stocks—Two mass culture stocks of *Drosophila ananassae* was established from flies collected from two geographical localities: (a) : A G: This stock was established in 2009 from Agra, UP, India; and (b) : M T: This stock was established in 2009 from Mathura, UP, India.

Both the stocks were established from 50 females each from both the areas. The stocks were maintained on agar corn meal sugar medium under normal laboratory conditions by transferring 50 flies (females and males in equal number) to fresh culture bottles in each generation. The tests for sexual isolation and sexual selection were conducted between the two stocks after a generation of culture. The stocks were then divided into three groups.

Group A : Both the stocks were maintained on standard corn meal medium in BOD incubator at 24 °C temperature under 12:12h L :D condition, stocks named — AGA and MTA.

Group B : Both the stocks were maintained at 18 °C temperature under the above conditions in the BOD incubator, stocks named -- AGB and MTB

The stocks were maintained in the two groups for 12 generations and were then tested for sexual isolation among them, between and within groups.

**CROSSES**—The crosses were as follows:

1. MT × AG : before initiation of the experiment, after one generation of culture
2. MTA × AGB : after 12 generations of culture
3. AGA × MTB : ‘’
4. MTA × AGA : ‘’
5. AGB × MTB : ‘’
6. AGA × AGB : ‘’
7. MTA × MTB : ‘’

**Experiments for sexual isolation**—Virgin females and males were collected and aged for 7 days in food vials. Flies (15 of each sex) were kept in one vial. A day before the behavioural tests, they were transferred to *Drosophila* food medium that had been dyed with green or red food colouring. The flies were housed on colours corresponding to the crosses to be conducted and alternated the colours in replicates. Statistical analysis showed that neither colour had any effect on mating between different stocks. Multiple choice technique was employed to test for assortative mating. Fifteen flies of each sex of the two stocks to be tested were introduced without etherization in an Elens – Wattiaux\(^\text{23}\) mating chamber kept in a room maintained at approximately 24 °C with 60– 80% RH. Mating was observed for 60 min and the mating pair aspirated out and observed under the binocular for identification of the mating types. All the experiments were carried out between 0700 – 1100 hrs. In total 5 trials were run for each combination.

**Measuring sexual isolation**—JMATING software for the analysis of sexual isolation effects from mating frequency data was used\(^\text{24}\). It is a complete and versatile software for mating frequency data analysis and is available at http://www.uvigo.es/web03/XENETICA/XB2/IMsoft.htm. The programme is developed in Java to study sexual selection and sexual isolation effects from laboratory experiments
along with a battery of complementary statistical tests including bootstrapping.

PSI, PSS and PTI coefficients represent the sexual isolation, sexual selection and total deviations of each pair combination calculated for each combination of mating types in a multiple choice design. Heterogeneity G test has been done to assess the statistical significance of sexual selection and sexual isolation effects\(^2\). The coefficients vary from zero to infinity; value of one indicates sexual isolation or no variation in mating propensity. Larger values indicate variation in either mating propensity, mate choice or both. To compare sexual isolation between different crosses, one way ANOVA was used. Bonferroni t test for pairwise analysis was applied where levels of sexual isolation was significant.

### Results

Percentage of homogamic and heterogamic matings between the different pairwise mating combinations in the multiple choice experiments between the two populations of *D. ananassae* kept in different temperature for 12 generations are presented in Table 1. In all the crosses, homogamic matings outnumber the heterogamic matings though in cross MT vs AG the difference was negligible. The heterogeneity G test for sexual selection and sexual isolation showed statistically significant GT values in combinations MTA vs AGB and AGA vs MTB (Table 2) showing evidence of sexual isolation among these combinations. The GS values were not significant in all the pairwise crosses implying no evidence of sexual selection among them. The \(I_{\text{PSI}}\) values were statistically significant in crosses MTA vs AGB and AGA vs MTB (Table 3) providing evidence for deviation from random mating. Significance and \(P\) values were derived by resampling 10,000 times in JMATING software. Table 4 shows the PTI coefficients, their standard deviation and the \(P\) values for all the crosses. Marginally significant \(P\) values were observed in pairwise tests between MTA vs AGB and AGA vs MTB. Table 5 shows the results of one way ANOVA to test the deviation from random mating among the different strains. The \(P\) values were marginally significant between the crosses MTA vs AGB, AGA vs MTB, AGB vs MTB, AGA vs AGB and MTA vs MTB suggesting nonrandom mating in these pairwise combinations. Bonferroni test showed significant differences in pairwise analysis between homogamic and heterogamic matings (\(P < 0.001\), unpublished data).

<table>
<thead>
<tr>
<th>Crosses</th>
<th>GT (GI + GS)</th>
<th>GI</th>
<th>GS</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT × AG</td>
<td>0.17</td>
<td>0.11</td>
<td>0.06</td>
</tr>
<tr>
<td>MTA × AGB</td>
<td>13.28</td>
<td>13.15</td>
<td>0.13</td>
</tr>
<tr>
<td>AGA × MTB</td>
<td>20.86</td>
<td>18.47</td>
<td>2.39</td>
</tr>
<tr>
<td>MTA × AGA</td>
<td>1.14</td>
<td>1.03</td>
<td>0.11</td>
</tr>
<tr>
<td>AGB × MTB</td>
<td>2.08</td>
<td>1.8</td>
<td>0.28</td>
</tr>
<tr>
<td>AGA × AGB</td>
<td>4.6</td>
<td>4.59</td>
<td>0.01</td>
</tr>
<tr>
<td>MTA × MTB</td>
<td>5.48</td>
<td>5.48</td>
<td>0.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Crosses</th>
<th>(I_{\text{PSI}})</th>
<th>SD</th>
<th>(P) - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT × AG</td>
<td>0.0284</td>
<td>0.0843</td>
<td>0.7448</td>
</tr>
<tr>
<td>MTA × AGB</td>
<td>0.3255</td>
<td>0.0843</td>
<td><strong>0.0002</strong></td>
</tr>
<tr>
<td>AGA × MTB</td>
<td>0.3878</td>
<td>0.0848</td>
<td><strong>0.0002</strong></td>
</tr>
<tr>
<td>MTA × AGA</td>
<td>0.0851</td>
<td>0.0853</td>
<td>0.3204</td>
</tr>
<tr>
<td>AGB × MTB</td>
<td>0.1119</td>
<td>0.0830</td>
<td>0.1798</td>
</tr>
<tr>
<td>AGA × AGB</td>
<td>0.1849</td>
<td>0.0852</td>
<td>0.0278</td>
</tr>
<tr>
<td>MTA × MTB</td>
<td>0.1956</td>
<td>0.0825</td>
<td>0.0182</td>
</tr>
</tbody>
</table>

### Table 1—Results of pairwise multiple choice experiments (five replications) between different stocks of *D. ananassae* maintained at 18 and 24°C for 12 generations. Numbers of copulations for each type are shown from left to right locations as \(n_{aa}, n_{ab}, n_{ba}, \text{ and } n_{bb}\), with the left subscript designating females and the right subscript males.

<table>
<thead>
<tr>
<th>Crosses</th>
<th>No. of copulations</th>
<th>(n_{aa})</th>
<th>(n_{ab})</th>
<th>(n_{ba})</th>
<th>(n_{bb})</th>
<th>Homogamic matings (%)</th>
<th>Heterogamic matings (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT × AG</td>
<td>140</td>
<td>37</td>
<td>34</td>
<td>34</td>
<td>35</td>
<td>51.42</td>
<td>48.57</td>
</tr>
<tr>
<td>MTA × AGB</td>
<td>124</td>
<td>40</td>
<td>20</td>
<td>22</td>
<td>42</td>
<td>66.12</td>
<td>33.87</td>
</tr>
<tr>
<td>AGA × MTB</td>
<td>135</td>
<td>49</td>
<td>18</td>
<td>20</td>
<td>48</td>
<td>71.85</td>
<td>28.14</td>
</tr>
<tr>
<td>MTA × AGA</td>
<td>140</td>
<td>39</td>
<td>31</td>
<td>33</td>
<td>37</td>
<td>54.28</td>
<td>45.71</td>
</tr>
<tr>
<td>AGB × MTB</td>
<td>144</td>
<td>41</td>
<td>30</td>
<td>34</td>
<td>39</td>
<td>55.55</td>
<td>44.44</td>
</tr>
<tr>
<td>AGA × AGB</td>
<td>137</td>
<td>40</td>
<td>28</td>
<td>28</td>
<td>41</td>
<td>59.12</td>
<td>40.87</td>
</tr>
<tr>
<td>MTA × MTB</td>
<td>144</td>
<td>43</td>
<td>29</td>
<td>29</td>
<td>43</td>
<td>59.12</td>
<td>40.27</td>
</tr>
</tbody>
</table>
Discussion

It is evident from the present findings that the strains do not mate at random. Initially, the stocks AG and MT did not show sexual isolation. After 10 generations of culture in two different temperature regimes, statistically significant variation between homogamic and heterogamic matings were observed between the stocks raised at different temperatures coming from the two different geographic localities. Interestingly, the stocks coming from different localities and reared under different temperatures showed maximum behavioural isolation. In contrast, flies raised at the same temperature do not develop sexual isolation. This suggests that temperature variation may have acted on the genetic variability of the strains coming from different localities affecting certain aspects of mate recognition system in the populations, indicating the induction of sexual isolation as a result of adaptive divergence.

*D. ananassae* spans great geographical distances and prevail in a wide range of ecological conditions; thus there is a potential to diverge in phenotypic and behavioural traits that may lead to incipient isolation. The factors important in the evolution of widespread species still remains unclear. The question becomes more pertinent as it is expected that migration and gene flow is still going on and is relatively high in the widespread human commensals compared to the species which spread due to natural processes. Partial sexual isolation between US and Caribbean populations was found by Yukilevich and True in *D. melanogaster* revealing that some Caribbean populations exhibit partial sexual isolation with US populations and that there is a substantial geographical variation in the presence and strength of these mating preferences among the islands. They suggested that widespread and human commensal species may harbour and evolve different types of mating preferences across their geographical ranges.
The range of genetic variation within a species limits the degree to which a population can respond to selection. Populations evolve when selection acts on the genetic variations causing a shift in the allele frequency of genes affecting a particular trait. Behavioural responses of adult males and females to temperature and humidity regimes for several years. They performed mating tests to check for reproductive isolation. Positive assortative mating was observed among the lines. They concluded that sexual isolation was produced as a by-product of selection.

Nanda and Singh have reported sexual isolation in *D. ananassae* due to founder effects. According to them, the drift lines may have accumulated considerable amounts of genetic divergences which may have affected the mate recognition system leading to the origin of sexual isolation among the lines. Schug *et al.* found considerable variation in mate discrimination among populations of *D. ananassae* throughout the species range; the discrimination being higher among the populations outside the ancestral Indonesian range and highest in South Pacific. They suggested that colonization and genetic differentiation affect the evolutionary origin of mate discrimination of *D. ananassae*. Further, instability of mate recognition system was reported in *D. ananassae*.

The findings of the present study provide evidence that temperature may have a profound effect on the mate recognition system in *D. ananassae*. This environmental variable may have acted on the genes affecting the mating behavior of the stocks leading to sexual isolation between them.

**Acknowledgement**

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