Epistatic interactions among the P element-induced high interspecific crossability strains in 
*Drosophila melanogaster*

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ABSTRACT

Five *Drosophila melanogaster* strains showing high interspecific crossability with *D. simulans* males, derived from the previous screening of a set of autosomal *plwB* transposants, were selected and the effect of the *plwB* insertion under the same genetic background (*white* strain) on the interspecific crossability was examined. This phenotype was recessive in the two strains but semidominant in the other three strains. Trans-heterozygotes, however, showed high interspecific crossability compared with the parental homozygotes, suggesting some epistatic interactions between them. In three strains, the effect of the *plwB* insertion region in different backgrounds (*w¹¹⁸* strain) on the crossability was also tested. Homozygotes of a strain (#687) showed high interspecific crossability comparable to the *w* background, while homozygotes of both #68 and #783 strains showed lower crossability than *w¹¹⁸*. Although #783 heterozygotes showed intermediate values between #783 homozygotes and *w¹¹⁸*, #68 heterozygotes showed a significantly higher insemination rate than *w¹¹⁸* and the #68 homozygotes. These results suggest that the region around the *plwB* insertion sites of #68 and #783 affects the interspecific crossability either positively or negatively depending on the genetic background. In all the stocks, positive correlation between interspecific crossability and the intraspecific mating speed was detected.

1. INTRODUCTION

The process where one species is divided into two species, speciation, requires both origination and development of reproductive isolation mechanisms. To clarify genetic mechanisms operating on reproductive isolation between related species is a very important task.

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Since there is sexual isolation between *Drosophila melanogaster* and its sibling species, *D. simulans* with sympatric distribution, low hybridization frequency is observed in nature, and even if the hybridization occurs, this cross produces unisexual and sterile hybrid progeny (Sturtevant, 1920; Inoue et al., 1990). In general, hybridization between *D. melanogaster* females and *D. simulans* males occurs more easily than the reciprocal cross (Sturtevant, 1920). It is known that many factors, such as genotype, age, and density, affect the degree of sexual isolation in the laboratory (Manning, 1959; Barker, 1967; Parsons, 1972; Watanabe et al., 1977).

Among the factors affecting sexual isolation, strain differences have a large effect on the success of hybridization particularly for females (Parsons, 1972; Watanabe et al., 1977). Recently, a positive correlation between hybridization ability of *D. melanogaster* females with *D. simulans* males and her receptivity to her own males has been shown (Carracedo et al., 1991). *D. melanogaster* females showing high interspecific crossability with *D. simulans* males exhibit high receptivity to *D. melanogaster* males.

In order to clarify the nature of sexual isolation between them, we have begun to isolate the mutations in *D. melanogaster* affecting sexual isolation by using single marked *P* element mutagenesis (Oguma et al., 1995). From the screening of about six hundred lines having a single *plwB* insertion on the autosome, 36 lines showing high interspecific crossability with *D. simulans* males were recovered. To determine the association of the *plwB* insertion with the high crossability phenotype, 36 lines were backcrossed to the *white* (*w*) strain for 10 to 16 generations to substitute the background except for the *plwB* insertion, and the crossability of these backcrossed strains was examined. These studies revealed that several backcrossed strains consistently showed higher crossability than the *w* control, suggesting the *plwB* insertion is responsible for the high crossability phenotype.

In the present study, we further analyzed these backcrossed lines with high crossability for clarifying genetic interactions between these lines and the effect of different genetic background on the mutant expression. This report also includes the examination of the correlation between interspecific crossability and intraspecific mating speed.

2. MATERIALS AND METHODS

*Fly stocks*

*Drosophila melanogaster* original stocks having a *plwB* insertion on the autosome and backcrossed stocks are described elsewhere (Oguma et al., 1995). Designation of these stocks is as follows: for example #687 means original stock and also used to indicate a mutant gene(s) residing at the *plwB* insertion site or nearby region responsible for the high crossability. X10(w)687 is the #687
stock, which was backcrossed to the \( w \) stock for 10 generations and made homozygous for the \( plwB \) insertion. Since then, these stocks were maintained by mass mating. Oregon-R (OR) and \( w \) strains used as controls have been maintained in our laboratory for many years. The \( w^{1118} \) stock was provided by Prof. K. Kaiser of Glasgow University. As the standard \( D. \ simulans \) strain, Otsuki isofemale line established 15 years ago from a population in Otsuki, Yamanashi, was used. All flies were reared in a shell vial (3 cm diameter \( \times \) 10 cm height) with agar-yeast-glucose-cornmeal medium at 25\( \pm \)1°C with a 14 h light and 10 h dark cycle. In the course of all experiments, except for distinguishing heterozygotes and homozygotes for \( plwB \) derived from the backcross experiment (see below), no anesthesia was used.

**Measurement of interspecific crossability**

Interspecific crossability was measured as follows unless otherwise indicated. Twenty \( D. \ melanogaster \) females aged 5-6 days were mixed with twenty \( D. \ simulans \) males aged 5-6 days in a vial with food. After 3 days, all females were dissected and the presence of sperm in seminal receptacle was examined. In each line, about 10 replications were made, and frequency (%) of inseminated females per total dissected females is presented. For detecting correlation between mating ability and mating speed, mating frequency was transformed to Arcsin \( \sqrt{\%} \) scale.

In situ hybridization to polytene chromosomes

In situ hybridization to salivary polytene chromosome was performed according to the method of Engels et al. (1986). pGEM DNA (Promega) was used as a probe for detecting \( plwB \).

**Backcrosses**

To substitute a background except for the \( plwB \) insertion, four original strains \#68, \#687, \#788, and \#812 were backcrossed to the \( w^{1118} \) strain as shown in Fig. 1. Backcrossing scheme is the same as described earlier for substituting the background to the \( w \) strain (Oguma et al., 1995). During the first three generations, the X chromosome and cytoplasm are replaced by the \( w^{1118} \) strain. In the following generations, red eyed females were backcrossed to the \( w^{1118} \) males. Because \( plwB \) possesses the \( w^+ \) gene, this backcross procedure gives the substitution of background to the \( w^{1118} \) strain except for the \( plwB \) insertion and nearby region. Theoretically, after \( \tau \) generations of backcross, approximately 100/t cM on each side of a \( plwB \) insertion is expected to be conserved (Crow and Kimura, 1970). After 8 or 12 generations, red eyed females and males were crossed with each other, and resultant three genotypes, homozygous and heterozygous for \( plwB \), which were distinguished from each other by their eye color, and white eyed females were used for behavioral tests.
Fig. 1. Mating scheme for backcrossing to the \( w^{118} \) strain.

**Measurement of mating speed**

One female from a desired stock was introduced into a mating chamber (13 mm diameter \( \times \) 6 mm height) with one male from the OR stock. Both the female and male were aged for 4–5 days before the test. The time until copulation was measured during a 20 min observation period. Female receptivity was estimated as mating speed defined as follows (Carracedo et al., 1991): 75 pairs from each strain were observed and the time of the 37 fastest mating pairs (this number of pairs is 50% pairs of total observed pairs) was transformed to a decimal log and averaged.

3. RESULTS

**Interspecific crossability of the selected plwB insertion strains under the white background**

The first task of this study is to know whether the mutant phenotype is retained
in the backcrossed stocks and to select strains for further analysis. Table 1 shows interspecific insemination rates of selected backcrossed stocks. All strains except X15(w)585, X(18)812, and X16(w)957, showed much higher insemination rate than the controls (w and OR). High crossability was maintained during several generations after the stock establishment. These strains have the same genetic background except for the region of the plwB insertion, suggesting that these insertions are responsible for the high crossability. Five stocks, X10(w)68, X15(w)140, X16(w)430, X10(w)687 and X18(w)783 were chosen for further analyses because of their constant high crossability.

Table 1. Interspecific insemination rate (%) of plwB insertion strains under the w background

<table>
<thead>
<tr>
<th>Stock</th>
<th>Insemination rate (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X10(w)68</td>
<td>16 (220)</td>
</tr>
<tr>
<td>X18(w)140</td>
<td>18 (240)</td>
</tr>
<tr>
<td>X7(w)412</td>
<td>12 (199)</td>
</tr>
<tr>
<td>X16(w)430</td>
<td>22 (219)</td>
</tr>
<tr>
<td>X15(w)585</td>
<td>1 (219)</td>
</tr>
<tr>
<td>X10(w)687</td>
<td>19 (219)</td>
</tr>
<tr>
<td>X18(w)783</td>
<td>16 (219)</td>
</tr>
<tr>
<td>X18(w)812</td>
<td>1 (90)*</td>
</tr>
<tr>
<td>X18(w)825</td>
<td>8 (199)</td>
</tr>
<tr>
<td>X18(w)833</td>
<td>5 (220)</td>
</tr>
<tr>
<td>X16(w)957</td>
<td>3 (215)</td>
</tr>
<tr>
<td>w</td>
<td>2 (220)</td>
</tr>
<tr>
<td>OR</td>
<td>2 (260)</td>
</tr>
</tbody>
</table>

* Insemination rate was determined using 10 females and 15 males in a vial.

**Determination of the cytological sites of the plwB insertion in the mutant strains**

Although there is no direct evidence that the plwB insertion is solely responsible for high crossability, it is clear that the region near the plwB insertion site affects the mutant phenotype at least under the w background. We determined the plwB insertion site of the four mutant strains on the salivary polytene chromosome by in situ hybridization (Table 2). The insertion site of the #687 strain was previously defined at 89A on the right arm of chromosome 3 (Oguma et al., 1995). Thus all five insertions are on the third chromosome, but in different sites. At present, no behavioral mutants which were mapped to the same positions are known (Lindsay and Zimm, 1992).

**Genetic interactions between five mutant strains**

Interspecific insemination rates of homozygotes and heterozygotes for the five
Table 2. Cytological sites of the *plwB* insertions in the four high crossability strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Cytological site</th>
</tr>
</thead>
<tbody>
<tr>
<td>#68</td>
<td>82C</td>
</tr>
<tr>
<td>#140</td>
<td>61A</td>
</tr>
<tr>
<td>#430</td>
<td>83F</td>
</tr>
<tr>
<td>#783</td>
<td>62A</td>
</tr>
</tbody>
</table>

Table 3. Interspecific insemination rate (%) of homo, hetero, and trans-heterozygotes for the five *plwB* insertion strains

<table>
<thead>
<tr>
<th>Genotype* (Maternal/Paternal)</th>
<th>Insemination rate (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>68/ 68</td>
<td>17 (218)</td>
</tr>
<tr>
<td>+ / 68</td>
<td>6 (198)</td>
</tr>
<tr>
<td>140/140</td>
<td>7 (240)</td>
</tr>
<tr>
<td>+ /140</td>
<td>2 (200)</td>
</tr>
<tr>
<td>430/430</td>
<td>23 (220)</td>
</tr>
<tr>
<td>+ /430</td>
<td>5 (220)</td>
</tr>
<tr>
<td>687/687</td>
<td>10 (240)</td>
</tr>
<tr>
<td>+ /687</td>
<td>2 (239)</td>
</tr>
<tr>
<td>783/783</td>
<td>17 (235)</td>
</tr>
<tr>
<td>+ /783</td>
<td>8 (239)</td>
</tr>
<tr>
<td>+ / +</td>
<td>2 (240)</td>
</tr>
<tr>
<td>783/ 68</td>
<td>22 (240)</td>
</tr>
<tr>
<td>687/783</td>
<td>13 (240)</td>
</tr>
<tr>
<td>68/687</td>
<td>19 (220)</td>
</tr>
<tr>
<td>430/ 68</td>
<td>27 (259)</td>
</tr>
<tr>
<td>140/430</td>
<td>15 (219)</td>
</tr>
<tr>
<td>140/783</td>
<td>9 (120)</td>
</tr>
<tr>
<td>783/430</td>
<td>35 (220)</td>
</tr>
<tr>
<td>68/140</td>
<td>20 (240)</td>
</tr>
<tr>
<td>430/687</td>
<td>20 (240)</td>
</tr>
<tr>
<td>687/140</td>
<td>17 (218)</td>
</tr>
</tbody>
</table>

* Genotype is represented by only stock No. and + means *w* strain.

Backcrossed strains (heterozygotes were F₁ progeny from the crosses between the *w* females and each of backcrossed strains males) and all possible trans-heterozygous combinations using the five backcrossed strains were examined (Table 3). Several homozygotes showed some differences in crossabilities between Table 1 and Table 3, but either test consistently showed that the mutant strains exhibited higher insemination rates than the control value. Heterozygotes showed lower insemination rates compared to homozygotes. The rates of both #140 and #687 heterozygotes with the *w* strain were not significantly
different from the control (χ²-test, \( P > 0.05 \)), indicating that the phenotypes were recessive characters; while the rates of the heterozygotes of \#68, \#430 and \#783 with the \( w \) strain were significantly higher than the control, indicating semidominance. Surprisingly, in all trans-heterozygous combinations, high insemination rates were observed compared to the parental homozygotes. Since the \( plwB \) insertion sites of five strains are different (Table 2; Oshima et al., 1995), these results imply complex epistatic interactions between five mutant genes.

**Effects of different genetic background**

The experiments described above were carried out using the stocks with the same \( w \) background. To know the effect of different background on the expression of the five mutant genes, we backcrossed original stocks of three high crossability strains, \#68, \#687 and \#783, and one low crossability strain, \#812, to the \( w^{118} \) stock as shown in Fig. 1. After 8 or 12 generations, females of three genotypes, heterozygous and homozygous for \( plwB \) and \( w^{118} \) females, derived from the same parents (Fig. 1), were subjected to the mating test with \( D. simulans \) males respectively (Table 4). Only the \#687 homozygotes gave significantly high insemination rate among the three mutant homozygotes. On the contrary, the \#68 and \#783 homozygotes exhibited lower insemination rates than the control. Furthermore, although the \#783 heterozygotes showed an intermediate value between homozygote and \(+\( w^{118} \)), the \#68 heterozygotes gave a significantly higher insemination rate than both homozygous for \#68 and for +.

<table>
<thead>
<tr>
<th>Genotype*</th>
<th>Insemination rate (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>68/68</td>
<td>1 (257)</td>
</tr>
<tr>
<td>68/+</td>
<td>17 (216)</td>
</tr>
<tr>
<td>687/687</td>
<td>24 (217)</td>
</tr>
<tr>
<td>687/+</td>
<td>18 (239)</td>
</tr>
<tr>
<td>783/783</td>
<td>0 (197)</td>
</tr>
<tr>
<td>783/+</td>
<td>5 (198)</td>
</tr>
<tr>
<td>812/812</td>
<td>4 (197)</td>
</tr>
<tr>
<td>812/+</td>
<td>7 (197)</td>
</tr>
<tr>
<td>+/+</td>
<td>9 (238)</td>
</tr>
</tbody>
</table>

* Genotype is represented by only stock No. and + means \( w^{118} \) strain.

**Correlation between interspecific crossability and intraspecific receptivity**

Female receptivity can be estimated as mating speed. Mating speed of females using females of thirteen genotypes with the conspecific OR males was measured
Table 5. Interspecific insemination rate and intraspecific mating speed of females of thirteen genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Insemination rate* (Aresin √%)</th>
<th>Mating speed (log)</th>
<th>Mating speed (sec)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>X10(w)68</td>
<td>24.33</td>
<td>2.09</td>
<td>124</td>
</tr>
<tr>
<td>X10(w)687</td>
<td>18.83</td>
<td>2.13</td>
<td>135</td>
</tr>
<tr>
<td>X18(w)783</td>
<td>24.69</td>
<td>2.03</td>
<td>107</td>
</tr>
<tr>
<td>X18(w)812</td>
<td>6.05</td>
<td>2.24</td>
<td>174</td>
</tr>
<tr>
<td>X12(w&lt;sup&gt;1118&lt;/sup&gt;)68</td>
<td>5.06</td>
<td>2.48</td>
<td>302</td>
</tr>
<tr>
<td>+/X12(w&lt;sup&gt;1118&lt;/sup&gt;)68</td>
<td>24.45</td>
<td>2.27</td>
<td>187</td>
</tr>
<tr>
<td>X12(w&lt;sup&gt;1118&lt;/sup&gt;)687</td>
<td>29.00</td>
<td>2.09</td>
<td>122</td>
</tr>
<tr>
<td>+/X12(w&lt;sup&gt;1118&lt;/sup&gt;)687</td>
<td>25.41</td>
<td>2.16</td>
<td>143</td>
</tr>
<tr>
<td>X12(w&lt;sup&gt;1118&lt;/sup&gt;)783</td>
<td>0</td>
<td>2.28</td>
<td>193</td>
</tr>
<tr>
<td>+/X12(w&lt;sup&gt;1118&lt;/sup&gt;)783</td>
<td>12.31</td>
<td>2.33</td>
<td>214</td>
</tr>
<tr>
<td>X8(w&lt;sup&gt;1118&lt;/sup&gt;)812</td>
<td>10.87</td>
<td>2.26</td>
<td>181</td>
</tr>
<tr>
<td>+/X8(w&lt;sup&gt;1118&lt;/sup&gt;)812</td>
<td>14.89</td>
<td>2.16</td>
<td>145</td>
</tr>
<tr>
<td>w&lt;sup&gt;1118&lt;/sup&gt;</td>
<td>17.28</td>
<td>2.22</td>
<td>165</td>
</tr>
</tbody>
</table>

* This value is calculated from the data in Tables 1, 3, and 4.
** This value is back-transformed value of the mean mating speed in the log scale.

![Fig. 2. A correlation between the interspecific crossability (in Aresin √%) and the intraspecific mating speed (in log of sec.).](image)

and plotted against the interspecific insemination rate (Aresin √%) (Table 5, Fig. 2). Coefficient of correlation was −0.71907 (P<0.01), indicating significant negative correlation. Thus, *D. melanogaster* females with the higher interspecific crossability showed the faster mating speed to her own males.
4. DISCUSSION

We selected and examined five \( P \) element induced high interspecific crossability strains under the same genetic background of the \( w \) strain. Although there is no direct evidence that the high crossability is brought by the \( plwB \) insertion except for \#687, the backcross analysis suggests that the gene(s) responsible for the high crossability must reside within ca. 20 map unit around the \( plwB \) insertion site (Crow and Kimura, 1970). In \#687, the analysis of the excision lines of the \( plwB \) links the association of the \( plwB \) insertion with the high crossability (Oguma et al., 1995).

In the trans-heterozygotes between five mutants, insemination rates are the same as the parental homozygotes or rather high. This result cannot be explained by the additive effect of each strains, because heterozygotes of each strain showed much lower crossability (Table 3). Thus explanation of this result requires epistatic interaction between gene(s) on the each strains.

Both \#68 and \#783 showed lower insemination rates under the \( w^{1118} \) background than controls (Table 4). Of course, this result would be explicable if the gene(s) responsible for the high crossability were not linked to the \( plwB \) insertion, and were lost by recombination during the backcross to \( w^{1118} \). Inference is, however, possible that the high insemination rates observed under the \( w \) background are brought about by epistatic interactions between \#68 and \#783, and the \( w \) background. Furthermore, under the \( w^{1118} \) background, \#68 and \#783 homozygotes showed lower insemination rates than the \( w^{1118} \), and \#68 heterozygotes showed higher insemination rate than both \( w^{1118} \) and the \#68 homozygotes, although the \#783 heterozygotes showed intermediate rates between \( w^{1118} \) and the \#783 homozygotes (Table 4). These results suggest that the region around the \( plwB \) insertion site of \#68 or \#783 actually possesses some effects on the interspecific crossability, however, these effects are exerted either positively or negatively depending on the epistatic interactions in the different genetic background.

Sexual isolation between \( D. \ melanogaster \) and \( D. \ simulans \) is thought to be caused mainly by male discrimination, because no \( D. \ simulans \) males court \( D. \ melanogaster \) females well (Manning, 1959; Wood and Ringo, 1980). Preliminary observation on matings between the females of the high crossability strains and \( D. \ simulans \) males suggested that the male discrimination is also effective in the high crossability strains. Possibly, this is the reason why the expression of the high crossability phenotype is incomplete (maximum insemination rate over three days is 24% in the \#687 strain in Table 4). The mutants therefore may exert their effects through reduced female discrimination.

Recently, it has been shown that \( D. \ melanogaster \) females showing high interspecific crossability with \( D. \ simulans \) males exhibit high receptivity to conspecific males (Carracedo et al., 1991; Izquierdo et al., 1992; Pineiro et al.,
Our results also confirm this relationship (Fig. 2). Their results were obtained from the analysis of genetic variations in the natural population (Carracedo et al., 1991) or artificially selected strains (Izquierdo et al., 1992; Pineiro et al., 1993), whereas our results are derived from the analysis of a possible $P$ element induced single mutation, thus suggesting that some part of two traits is regulated by the same gene.

Taking into account the fact that our strains are probably induced by the $P$ element insertions, the mutant phenotype might be caused by the loss of function of the genes. In this context, two types of the mutant characters can be explained as loss or lowering of female discrimination ability against both heterospecific and conspecific males. In this explanation, it is postulated that a part of female receptivity to own males is accounted for by female discrimination ability towards her own males. That is, females with low receptivity may result from her high discrimination ability, and this situation may lead to sexual selection from a female standpoint.

Characters, such as interspecific crossability and mating speed, are quantitative traits. The nature of quantitative trait loci (QTLs) operating on the quantitative traits has not been clarified. Recently, attempts have been made to identify each QTLs by P-M hybrid dysgenesis, with some success (Mackay, 1984, 1985; Lai and Mackay, 1993). In the present study we employed a more sophisticated approach, that is, combination of single marked $P$ element mutagenesis and backcrosses (Boyton and Tully, 1992). Consequently, we can identify some candidate QTLs and find the complex epistatic interactions between them. This approach will be powerful to shed light on the nature of the QTLs and complex epistatic interactions at the molecular level.

We express our thanks to Dr. D. Macer for his critical reading of the manuscript.

REFERENCES


Mutations affecting sexual isolation in Drosophila


