Cytogenetics of New Zealand blackflies of the genus *Austrosimulium* (Diptera: Simuliidae) II. Heterozygote deficiency and non-random association of inversion heterozygotes*

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**Abstract**

We present population cytogenetic data from an analysis of polytene chromosomes of individuals of the common New Zealand blackfly *Austrosimulium australense* (Schiner). These data show that, in an area of the central North Island of New Zealand (Tarawera), there are, firstly, a number of abrupt changes in inversion polymorphisms between this area and adjacent collection sites. Secondly, there is non-random association of heterozygous inversion pairs in samples from the Tarawera area. Thirdly, we present evidence of a deficiency of heterozygotes for a number of inversions found in this area. We conclude that this may provide evidence for the existence of cryptic species within our samples. We discuss this possibility in terms of the morphological and genetical changes which are likely to occur at speciation, and briefly discuss the mounting evidence for the ubiquity of cryptic species in Diptera.

**Introduction**

*Austrosimulium australense* (Schiner) is the most common species of blackfly in the North Island of New Zealand. A cytogenetic analysis of this species, using polytene chromosomes taken from salivary glands, has revealed the presence of ten paracentric inversions plus a supernumerary band. Some of these chromosomal rearrangements were polymorphic over large regions of the North Island (McLea & Lambert, 1983). A region of particular interest was the Tarawera area in the central North Island where there were abrupt changes in inversion polymorphisms between this area and adjacent collection sites. Some details of these changes were outlined in a previous contribution (McLea & Lambert, 1983). Three inversions and a supernumerary band which occurred in the Tarawera area, were absent from, or were only recorded at very low frequencies at adjacent more northern collection sites. These changes in inversion polymorphisms between the Tarawera area and adjacent sites suggested the necessity for an analysis of inversion data which tested for random association of heterozygote pairs and/or possible deviations from Hardy-Weinberg equilibrium.

**Material and methods**

All larvae were collected and prepared by the methods outlined in a previous paper (McLea & Lambert, 1983). In this paper we presented a chromosomal map of *A. australense* and details of inversion breakpoints. In that study, larvae were sampled from 49 collection sites over the North Island of New Zealand. Only the Tarawera area and some selected sites are considered here (Fig. 1).

As discussed by McLea and Lambert (1983), with one exception, inversions were very small and regular scoring of homozygote configurations was con-

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sequently difficult. For this reason it was necessary to use a modified Hardy-Weinberg test, in which not all homozygote configurations were scored. The modified test assumes that homozygotes are equally easy to score.

O (observed) - observed data, all heterozygote inversions are scored but not all homozygote standards (ss) and homozygote inversions (ii) can be scored.

Thus

\[
\begin{array}{c|c|c|c|c}
\text{Std (ss)} & \text{Hetz. (si)} & \text{Inv. (ii)} & \text{Total Sample} \\
\hline
x & y & z & w \\
\end{array}
\]

where \( x + y + z < w \)

O’ - homozygote numbers are raised to fit total number in sample. They are raised in proportion to their observed numbers.

\[
x' = x + \frac{w - (x + y + z)}{x + z} \cdot z \\
y' = y \\
z' = z + \frac{w - (x + y + z)}{x + z} \cdot z
\]

The frequency at which the standard chromosome configuration occurs is given by

\[ p = \frac{x' + y'}{2w} \]

The frequency at which the inverted chromosome configuration occurs is given by

\[ q = \frac{z'}{2w} \]

or \( q = 1 - p \)

\( E' \) - expected values for the three chromosomal configurations can now be calculated.

\[
\begin{align*}
E'x' &= p^2w \\
E'y' &= 2pqw \\
E'z' &= q^2w
\end{align*}
\]

A chi squared value can now be calculated and deviation from Hardy Weinberg equilibrium tested using \( x', y', z' \) as observed values and \( E'x', E'y', E'z' \) as expected values.

Genotypes of inversion polymorphisms IS-2, III-1, and IIIIL-3 were tested in this way. Since this is a modified test we considered a 1\% level of significance was more appropriate than the conventional 5\% level.

In addition to a modified Hardy-Weinberg test, larvae from the Tarawera populations were analysed for random association of inversion heterozygotes. Considering two different heterozygotes, X and Y, with frequencies in a sample of \( r \) and \( s \) respectively, the expected frequency of both heterozygotes occurring in the same animal, is given by the product \( r \times s \). This expected value based on the assumption of random association, was then tested against an observed value. All heterozygote pair combinations for inversions in the Tarawera region were tested in this way. Thus the term 'heterozygote pair' in the following discussion refers to the presence of two heterozygous inversion configurations occurring together in the same individual. The terms 'attraction' and 'repulsion' refer to a significant excess of observed heterozygous pairs and to a deficiency of observed heterozygous pairs respectively.

**Results**

Expected values and observed values of heterozygous pair combinations for the three Tarawera localities (sites 28, 29, 30) are shown in Table 1. These samples were pooled in subsequent analyses since they are within 3 km of each other. Requirements for Chi squared testing are that no expected

<table>
<thead>
<tr>
<th>IS-2/Std</th>
<th>IIS-1/Std</th>
<th>III-1/Std</th>
<th>IIIIL-1/Std</th>
<th>IIIIL-2/Std</th>
<th>IIIIL-3/Std</th>
<th>96A/Std</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS-2/Std</td>
<td>5.49* (3)</td>
<td>2.48 (4)</td>
<td>5.22* (2)</td>
<td>0.62 (0)</td>
<td>8.33* (5)</td>
<td>1.68 (0)</td>
</tr>
<tr>
<td>IIS-1/Std</td>
<td>7.32* (3)</td>
<td>15.43* (22)</td>
<td>1.83 (2)</td>
<td>24.59* (32)</td>
<td>4.97* (6)</td>
<td></td>
</tr>
<tr>
<td>III-1/Std</td>
<td>6.97* (3)</td>
<td>0.83 (0)</td>
<td>11.11* (4)</td>
<td>2.24 (0)</td>
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<tr>
<td>IIIIL-1/Std</td>
<td>1.74 (0)</td>
<td>23.40* (34)</td>
<td>4.73 (1)</td>
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<tr>
<td>IIIIL-2/Std</td>
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<td>2.78 (4)</td>
<td>0.56 (1)</td>
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<tr>
<td>IIIIL-3/Std</td>
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<tr>
<td>96A/Std</td>
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</table>

* Only expected values greater than five can be further used in a \( \chi^2 \) test against observed values.