The minimal interspecific introgression resulting in male sterility in *Drosophila*

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Summary

Introgression of *Drosophila simulans* genes into the *Drosophila melanogaster* genome provides an ideal system for analysing genetic incompatibility between species. Females and males homozygous for the introgression *Int(2L)S* (cytologically, 30F3-31C5 to 36A2-7) are sterile. Genetic dissection of the proximal part of the introgression (34D1-3 to 36A2-7) has indicated that introgressions of 0.7–1.6 Mb size result in male sterility when homozygous. In the present analysis we examine the distal part of the introgression (30F3-31C to 34D1-3) and reveal that introgressions with similar DNA content (1.8–2.1 Mb) result in male sterility. Compared with introgressions between the more closely related species *Drosophila mauritiana* and *D. simulans*, the minimal introgression resulting in male sterility is smaller by several-fold.

1. Introduction

In order to understand genetic mechanisms of post-zygotic reproductive isolation, interspecific introgressions have been made in *Drosophila*, mice, barley, wheat and rice (e.g. Wu & Palopoli, 1994; Shi & Endo, 1999; Matsubara *et al.*, 2003; Oka *et al.*, 2004). Among them, introgressions of *Drosophila simulans* genes to the *Drosophila melanogaster* genome, which became available recently (Sawamura *et al.*, 2000), offer an especially suitable system for analysis.

It has generally been impossible to make interspecific introgressions between *D. melanogaster* and *D. simulans* because the reciprocal crosses produce sterile unisexual hybrids (Sturtevant, 1920). The discovery of strains which restore hybrid fertility (Davis *et al.*, 1996; Barbash & Ashburner, 2003) brought the hope that introgressions could be introduced by repeated backcrosses. In fact, Sawamura *et al.* (2000) succeeded in introgressing two segments of the left arm of chromosome 2 (2L) from *D. simulans* into *D. melanogaster*; one at the tip of 2L (*Int(2L)D*; cytologically, 21A1 to 22C1-23B1) and the other in the middle of 2L (*Int(2L)S*; 30F3-31C5 to 36A2-7).

One of the introgression segments, *Int(2L)S*, causes female and male sterility when homozygous. The recessive genes responsible for the female sterility have been genetically mapped (Sawamura *et al.*, 2004a). Recombinants between the *Int(2L)S* and *D. melanogaster* chromosomes were made and the boundary of each recombinant introgression was determined by species-specific RFLPs (restriction fragment length polymorphisms). The fertility of females heterozygous for *Int(2L)D+S* and each introgression was examined. Further, the *Int(2L)D+S* chromosome was made heterozygous with a series of deficiency chromosomes of *D. melanogaster*. Because the sterility genes are recessive, females should be sterile if the *D. simulans* genes are made hemizygous by the deficiencies. The female sterility was attributed to a single gene or a cluster of genes in a 170 kb region of the introgression.

The introgression, *Int(2L)S*, also causes inviability in a specific genetic background. Male hybrids from the cross of *D. melanogaster* females and *D. simulans* males are lethal (Sturtevant, 1920), and are rescued by the *Lethal hybrid rescue* mutation of *D. simulans* (Watanabe, 1979), but the male hybrids are not rescued if they retain the introgression (Sawamura,
2. Materials and methods

(i) Chromosomes

We used the same series of recombinant introgressions as did Sawamura et al. (2004a,b). Briefly, the introgression Int(2L)S (cytologically, 30F3-31C5 to 36A2-7) was recombined with the \( P[w^{+}\text{mc} = \text{lacW}]/(2)k4003\) b chromosome (see Fig. 1). In the latter chromosome, a \( P \) element vector carrying the mini-white\(^+\) (\( w^+ \)) gene, which complements the \( w \) mutant phenotype, is inserted at the string-of-pearls locus (\( sop \), 30E1-4). The chromosome also bears another visible marker, \( \text{black} \) (\( b \), 34D1-3). The chromosome is abbreviated as \( P[w^{+}\text{sop} b] \) hereafter. Recombinants were screened for among the offspring of \( +\text{Int}(2L)S+/P[w^{+}\text{sop} b] \) heterozygous mothers, and the recombinant chromosomes maintained by using CyO as a balancer chromosome. In the present analysis, we used only \( sop^+\) recombinants, which presumably retain the distal part of the introgression. This is because the reciprocal \( P[w^{+}\text{sop} b] \) recombinants must always carry male sterility genes on the introgression proximal to \( b \) (Sawamura et al., 2004b).

The following deficiency chromosomes were also utilized (the deleted region is indicated cytologically in parentheses; see Fig. 3): \( \text{Df}(2L)\text{Mdh}(30D-F; 31F) \), \( \text{Df}(2L)\text{J3}(31B1; 32A1-2) \), \( \text{Df}(2L)\text{J3}(31D; 31F2-5) \), \( \text{Df}(2L)\text{J3}(31C; 32D1-E5) \), \( \text{Df}(2L)\text{FCK}(32D1; 32F1-3) \), and \( \text{Df}(2L)\text{Prl}(32F1-3; 33F1-2) \). For detailed descriptions of genes and chromosomes see FlyBase (2003) (http://flybase.org).

(ii) Fertility test

To test the effect of recombinant introgressions on male fertility, males heterozygous for each recombinant and the Int(2L)D\( +S \) chromosome were examined. (Instead of Int(2L)S, Int(2L)D\( +S \) was utilized for the test because sterility could be enhanced with the extra introgression Int(2L)D.) The fertility of viable males homozygous for each recombinant introgression was evaluated. Males heterozygous for Int(2L)D\( +S \) and each deficiency chromosome were also made. Five males to be examined and five Oregon-R females of \( D. \) melanogaster were mated in a single vial with four replicates. Flies were transferred every 3–5 days until all females died, and the offspring were counted. The numbers of offspring per male were truncated at 100, because differences in fertility beyond 100 may not be reliable. Males with >100 offspring were categorized as fertile.

(iii) Molecular analysis

Genomic DNA was extracted, amplified by polymerase chain reaction (PCR), and digested by restriction enzymes to detect RFLPs following the method described previously (Sawamura et al., 2004b). The loci examined (see Fig. 1; for detailed descriptions see Sawamura et al., 2004a) were: basket (\( \text{bsk} \), 31C1-5), daughterless (\( \text{da} \), 31D11-E1), CG4705 (32D2-3), CENP-meta (\( \text{cmet} \), 32E3-4) and paired (\( \text{prd} \), 33B13-14). The big brain locus (\( \text{bib} \), 30F3-5) is outside the introgression.
To check the possibility of double recombination, the *twine* locus (*twe*, 35F6) was analysed (for details see Sawamura et al., 2004b). If *D. simulans*-specific DNA sequence was detected, the chromosome was attributed to a double recombinant carrying the proximal part of the introgression.

3. Results

(i) Recombination mapping

We classified 52 recombinant chromosomes into classes I to VI by molecular markers (Fig. 1). The proximal boundaries of each class of introgression are: between *bib* and *bsk* (class I), *bsk* and *da* (class II), *da* and *CG4705* (class III), *CG4705* and *cmet* (class IV), *cmet* and *prd* (class V) and *prd* and *b* (class VI).

The fertility of males heterozygous for *Int(2L)D+S* and each recombinant introgression is shown in Fig. 2. It should be added here that the order of recombinants within each class is arbitrary with respect to the recombination site. The data are plotted accordingly to the fertility (higher fertility on the left). Males were fertile when introgressions of classes I to

Fig. 1. Recombinant chromosomes used in the present analysis. Recombinants were isolated from offspring of females heterozygous for *Int(2L)S* and *P{w +}sop b*. Fifty-two recombinants were classified into six classes according to the molecular markers indicated below the polytene chromosome map. The chromosome regions from *D. melanogaster* and *D. simulans* are indicated by filled and open bars, respectively. The origin of genes has not been specified in the grey region of the distal end of the introgression. The recombination sites indicated are not known exactly; they are located somewhere in the dotted line regions. The proximal part of the introgression analysed previously (Sawamura et al., 2004b) is indicated as a reference.

Fig. 2. Fertility of males heterozygous for *Int(2L)D+S* and each recombinant introgression. The mean number of offspring per male is shown. Note here that the order of recombinants within each class is arbitrary with respect to the recombination site. The data are plotted according to fertility (higher fertility on the left). An asterisk (*) indicates a double recombinant chromosome; this chromosome presumably retains male sterility genes in the introgression proximal to the *b* gene.
IV were utilized (more than 100 offspring per male produced), except for one case (no offspring, indicated by asterisk). But the molecular analysis of the twe locus indicated that this exceptional chromosome also contained another introgression. We assume that this chromosome retained male sterility genes on the proximal introgression by double recombination (Fig. 1).

Among nine recombinants of class V, seven were fertile (>100) but two showed reduced fertility (59.9 and 37.9 offspring). Among 21 recombinants of class VI, one showed reduced fertility (59.9 offspring) but the others were almost completely sterile (2.3±2.26 (SD)). We assume that the two exceptional recombinants in class V carry larger introgressions than the others and that the one exceptional recombinant in class VI carries a smaller introgression than the others, although we do not have molecular evidence for these assumptions. There seems a transition zone around theprdlocus. Generally, large introgressions covering far beyond prd lead to male sterility. The transition zone suggests the presence of at least two genes which synergistically cause male sterility. This is consistent with the view that hybrid male sterility is multi-genic and epistatic (Wu & Palopoli, 1994; Wu et al., 1996; Naveira & Maside, 1998). The proximal part of the introgression was also found to be such a case (Sawamura et al., 2004b).

The fertility of males homozygous for an introgression overInt(2L)D+S exhibited semi-sterility (seven introgressions of class VI), homozygous introgressions also resulted in male semi-sterility. On the other hand, if males with an introgression over Int(2L)D+S exhibited intermediate fertility (one each of classes V and VI), homozygous males were fertile (>100). This confirms that dominant enhancers of male sterility exist somewhere in the introgression, which has been suggested previously (Sawamura et al., 2004b).

(ii) Deficiency mapping

Males heterozygous for Int(2L)D+S and a series of deficiency chromosomes (Fig. 3) were examined for their fertility. Males were fertile when heterozygous with Df(2L)Mdh, Df(2L)J2, Df(2L)J3 or Df(2L)FCK-20, but were sterile over Df(2L)J39 or Df(2L)Prl (data for Df(2L)Mdh, Df(2L)J39 and Df(2L)Prl are from Sawamura et al., 2000). (Only seven males were tested in Df(2L)J39 because the deficiency causes semi-lethality. As this region might contain loci of haplo-insufficiency (even D. melanogaster flies with this deficiency are semi-lethal), the

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Table 1. Male fertility of heterozygous introgressions over Int(2L)D+S and of homozygous introgressions. Mean number of offspring per male is shown

<table>
<thead>
<tr>
<th>Heterozygotes over Int(2L)D+S</th>
<th>Homozygotes</th>
</tr>
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<tbody>
<tr>
<td>(i) 1 recombinant of class V&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.9 (IF) 134.6</td>
</tr>
<tr>
<td>(ii) 8 recombinants of class VI&lt;sup&gt;b&lt;/sup&gt;</td>
<td>59.9 (IF) 187.6</td>
</tr>
<tr>
<td>5.5 (SS)</td>
<td>10.4</td>
</tr>
<tr>
<td>2.6 (SS)</td>
<td>0.2</td>
</tr>
<tr>
<td>2.5 (SS)</td>
<td>0</td>
</tr>
<tr>
<td>1.7 (SS)</td>
<td>0</td>
</tr>
<tr>
<td>1.5 (SS)</td>
<td>18.4</td>
</tr>
<tr>
<td>0.8 (SS)</td>
<td>5.4</td>
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<tr>
<td>0.4 (SS)</td>
<td>0.2</td>
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<sup>a</sup> Data plotted in Fig. 2. IF, intermediate fertility; SS, semi-sterility.

<sup>b</sup> Classes as depicted in Fig. 1.
male sterility might not be caused by the introgression but by a side-effect of the deficiency.) We can, at least, conclude that the introgression regions 30F3-31C5 to 32A1-2 and 32D1 to 32F1-3 do not contain recessive genes solely responsible for male sterility.

4. Discussion

The smallest introgression resulting in male sterility when homozygous is in class VI. According to FlyBase (2003) the physical size of the introgression (bsk/bib-prd) is 1.8–2.1 Mb and covers 86–93 polytene chromosome bands. Previously, Sawamura et al. (2003a) indicated that homozygous introgressions covering the region of Adh/Su(H)–CycE/twe (0.7–1.6 Mb; 12–27 bands) cause male sterility. The minimal introgression resulting in male sterility is similar at the physical level, although very different at the cytological level (the density of bands varies depending on the chromosome region).

Here we compare the D. melanogaster/D. simulans data with the previous analyses of introgression from D. mauritiana to D. simulans. Precise mapping of hybrid male sterility genes at a comparable level has been done for chromosomes X and 3 (Cabot et al., 1994; Perez & Wu, 1995; Davis & Wu, 1996; Tao et al., 2003a,b), but the resolution of the previous mapping on the chromosome 2 does not reach the same level (Hollocher & Wu, 1996; True et al., 1996). Therefore, we use data from different chromosomes to compare the physical length of introgressions which result in male sterility. Because there still remains the controversy of whether the density of hybrid male sterility genes is similar between the X chromosome and the autosomes (Hollocher & Wu, 1996; True et al., 1996), comparison among and within autosomes seems most reasonable. Data for chromosome 3 (Tao et al., 2003a) are used for the comparison.

In the D. simulans/D. mauritiana pair the minimal introgression resulting in male sterility includes 138 polytene chromosome bands (Tao et al., 2003a). This is 5-1 times larger than that in the D. melanogaster/D. simulans pair, where at most 27 polytene chromosome bands are enough to cause male sterility (the present calculation). Because only two regions of D. melanogaster/D. simulans introgression have been tested, the present calculation might underestimate the value. We therefore introduce another method of calculation. According to Tao et al. (2003a), the genome has 15 times the minimum number of incompatibility factors necessary to cause complete hybrid male sterility (HMS “equivalents”) in the D. simulans/D. mauritiana pair. Because the euchromatin size of the species seems similar to D. melanogaster (i.e. 120 Mb; Adams et al., 2000), every 8 Mb must have 1 HMS “equivalent” on average. This is 4-7 times larger than that in the D. melanogaster/D. simulans pair, where a 1.7 Mb size of introgression corresponds to 1 HMS “equivalent” (the present calculation). There is much molecular evidence suggesting that D. melanogaster and D. simulans diverged 2–3 MYA (million years ago), and that D. simulans and D. mauritiana diverged 0.3–0.4 MYA (Lachaise & Silvain, 2004). The minimal introgression resulting in male sterility is several times smaller in the species pair that has been isolated from each other for several-fold longer time period.

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