Chromosomal rearrangement *In(2)TY* and linkage maps of the second chromosome of *Drosophila virilis*

Kendo TSUNO¹⁾ and Osamu YAMAGUCHI²⁾

¹⁾Biological Laboratory, Meikai University, 8 Akemi, Urayasu, Chiba 279

²⁾Department of Biology, Hyogo University of Teacher Education,
Yashiro, Kato-gun, Hyogo 673-14

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ABSTRACT

A chromosomal rearrangement *In(2)TY*, found in a natural population of *Drosophila virilis*, was examined cytologically, and its genetical characterization was carried out by the use of some marker isozymes and visible marker loci. From the interspecific hybridization between *D. virilis* and *D. americana* or *D. texana*, we deduced that the origin of *In(2)TY* was from a member of a closely related species of *virilis*.

1. INTRODUCTION

Drosophila virilis is characterized as a species having the primitive or telocentric type of chromosomes and no polymorphic inversions. Many visible mutant markers in this species have been reported by several authors (reviewed by Alexander, 1976), but most of these markers have unfortunately been lost. The reconstruction of the genetic map is important to study genetic relationships among the members of the *Drosophila virilis* group.

Since a new chromosomal rearrangement, named *In(2)TY*, was found in 32 chromosomes obtained from a Toyama (Japan) population of *D. virilis* in 1988, we have examined various cytological and genetical aspects of this rearrangement based on the mapping of some biochemical and visible mutations on the second chromosome.

2. MATERIALS AND METHODS

In(2)TY inversion

This rearrangement, *In(2)TY*, was originally obtained as a chromosome displaying no recombination between the *Est-α* and *Est-β* loci on the 2nd chromosome. The strain was inbred to establish a line homozygous for *In(2)TY*, but, we failed in making it because of a lethal gene(s) on the chromosome. So, some other lethal chromosomes were extracted from a natural population by the use of *In(2)TY*; and, thus, the line was established as a lethal-balanced line heterozygous for a non-homologous lethal(s).

For a cytological study of *In(2)TY*, lethal-balanced heterozygotes for the inversion were used, and their third-instar larvae were examined by an aceto-orcein staining method. In the course of making our observations, we suspected that this inversion might have come from one of some closely related species. So, referring to the Table 75 and Fig. 49 of Patterson and Stone (1952), the inversion line was crossed with *D. americana* and *D. texana* flies in the hope of observing the complete pairing of the hybrid 2nd chromosome in the salivary gland chromosomes. Moreover, in order to confirm these observations, we carried out several crosses among *americana*, *texana*, and *virilis* having a standard gene arrangement (ST) with one another.

Mapping of selected genetic markers

Of visible mutants or isozyme variants collected so far, candidates for a linkage group on the 2nd chromosome were mapped by nine cross experiments. The loci for esterase (*Est- α* , *Est- β*), octanol dehydrogenase (*Odh*), and acid phosphatase (*Acph*) enzymes, and those for the visible mutants taxi (*tx*) and curled (*cul*) were employed. The visible mutant *cul* probably corresponds to the *cu* (2-50.0) of *Drosophila melanogaster*. However, as the symbol *cu* is reserved for the curved wing locus in *D. virilis* (Chino, 1936; a review by Alexander, 1976), the curled wing locus of this species is indicated as *cul*.

Electrophoresis

Esterase isozymes were examined according to Tsuno (1981). ACPH and ODH isozymes were electrophoresed under the following conditions: Potassium phosphate buffer (pH 6.8) in the electrode vessels, and 1 g agarose, 2 g polyvinylpyrrolidone K-90 (Wako Pure Chemical Industries, Ltd.), 50 ml 0.05 M Tris-HCl buffer (pH 7.9) and 50 ml H₂O in the gel. Thin-layer agar gel electrophoresis was carried out for 90 min at a constant 16.5 V/cm. For ACPH staining, 10 ml of a staining mixture containing 1 g α -naphthylphosphate disodium per 100 ml staining solution (containing 0.2 M sodium acetate, 0.2 M acetic acid, and H₂O at 4:1:5) was poured onto the gel plate. After a 30-min incubation a 10 ml 0.2% Fast Blue Salt B solution was added to the gel. For ODH staining, the staining solution containing 30 ml 0.05 M Tris-HCl buffer (pH 8.5), octyl alcohol 0.5 ml, 0.5 ml 0.05% NAD, 1 ml 0.08% NBT, and 0.005 g PMS was poured onto the gel.

Since these nine loci display no recombination in male flies heterozygous for their loci, they should constitute a linkage group on the 2nd chromosome. As preliminary tests suggested that their loci were separated far apart from each other, we carried out several cross experiments to confirm their locations. Experiments 1 to 5 in Tables 1 and 2 indicate the genotypes for crosses and the results of the mapping.

3. RESULTS

Observations of salivary gland chromosomes

Fig. 1 shows the chromosomes from a *D. virilis* larva heterozygous for *In(2)TY* on the 2nd chromosome. *In(2)TY* is a paracentric inversion on the 2nd chromosome and covers approximately a third of the length of the chromosome. Following Gubenko and Evgen'ev's map (1984) the two breakpoints were located at 24B and 27D, respectively. (On Hsu's (1952) map they probably correspond to K_{d-e} and S_{g-h} , respectively, though some uncertainty is not cleared in establishing the homology between their regions because of the manner of his drawing). This inversion apparently differs in breakpoints from the two inversions reported by Korochkin (1983). Moreover, asynaptic regions can be seen both within the inversion and in the proximal region.

In interspecific hybridizations, a striking feature appeared in hybrids between an *americana* female and a *virilis* male having *In(2)TY*. Although the other chromosomes, X, 3, 4, and 5, showed many asynaptic regions everywhere along their chromosomes and some inversions, the 2nd salivary chromosome indicated a complete pairing from the proximal to the distal region (Fig. 2). Especially this fact contrasts with the appearance of large asynaptic regions within the inversion

Fig. 1. Salivary gland chromosomes of *In(2)TY/ST* from a *D. virilis* larva.

Fig. 2. Salivary gland chromosomes of *D. americana/In(2)TY*-carrying *D. virilis* hybrid. A) All chromosomes except for the 2nd have many regions of asynapsis along their length. B) The 2nd chromosomes show a complete synapsis, as can also be seen for these chromosomes in A).

and on the proximal side of the *In(2)TY*/ST chromosomes (Fig. 1). As shown by Patterson and Stone (1952) in their Fig. 49 (on p. 187), salivary gland specimens of *americana*/ST of *virilis* showed a large inversion on the 2nd chromosome, and we also confirmed it in this study. On the other hand, the 2nd salivary gland chromosomes of *americana*/*texana* showed a complete synapsis over their entire length (Fig. 3), indicating that the 2nd chromosome of *americana* is closely related with that of *texana*. Therefore, these facts strongly suggest that *In(2)TY* has the same chromosomal segment as the 2nd chromosome of *americana* or *texana*.

Fig. 3. Salivary gland chromosomes of an inter-specific hybrid between *D. americana* and *D. texana*. A complete pairing of the 2nd chromosome can be seen.

Mapping of six loci

In Tables 1 and 2, the mean recombination frequency is used to show the map distance between two loci. The distance between the *Est-α* and the *Est-β* locus is a mean of four values including one previously determined (Tsuno, unpublished data). The *Odh*, *tx*, and *cul* loci were comparatively close to each other in the neighboring regions. Whereas the *Acph* locus was far away, at least more than 48.0% from the other loci. The distance between *cul* and *tx* could not be measured directly, because we failed to make a double mutant stock. So, we

Table 1. Recombination experiments on the second chromosome of *D. virilis*

	Exp. 1				Exp. 2				Exp. 3				
	<i>Acph</i> ⁴	<i>cul</i> ⁺	<i>Est-α</i> ⁵	<i>Est-β</i> ^B	<i>Odth</i> ^F	<i>cul</i>	<i>Est-α</i> ⁹	<i>Est-β</i> ^A	<i>Odth</i> ^F	<i>tx</i> ⁺	<i>Est-α</i> ⁹	<i>Est-β</i> ^A	
Female	<i>Acpl</i> ²	<i>cul</i>	<i>Est-α</i> ⁹	<i>Est-β</i> ^A	<i>Odth</i> ^S	<i>cul</i> ⁺	<i>Est-α</i> ⁷	<i>Est-β</i> ^B	<i>Odth</i> ^S	<i>tx</i>	<i>Est-α</i> ⁷	<i>Est-β</i> ^B	
Male	<i>Acpl</i> ²	<i>cul</i>	<i>Est-α</i> ⁹	<i>Est-β</i> ^A	<i>Odth</i> ^F	<i>cul</i>	<i>Est-α</i> ⁹	<i>Est-β</i> ^A	<i>Odth</i> ^S	<i>tx</i>	<i>Est-α</i> ⁷	<i>Est-β</i> ^B	
No. of non-recombinant and recombinant chromosomes in progenies	<i>Acph</i> ²	<i>cul</i>	<i>Est-α</i> ⁹	<i>Est-β</i> ^A	<i>Odth</i> ^F	<i>cul</i>	<i>Est-α</i> ⁹	<i>Est-β</i> ^A	<i>Odth</i> ^S	<i>tx</i>	<i>Est-α</i> ⁷	<i>Est-β</i> ^B	
	<i>Acph</i> ⁴	<i>cul</i> ⁺	<i>Est-α</i> ⁵	<i>Est-β</i> ^B	<i>Odth</i> ^F	<i>cul</i>	<i>Est-α</i> ⁹	<i>Est-β</i> ^A	503	<i>Odth</i> ^F	<i>tx</i> ⁺	<i>Est-α</i> ⁹	
	<i>Acph</i> ²	<i>cul</i>	<i>Est-α</i> ⁹	<i>Est-β</i> ^A	<i>Odth</i> ^S	<i>cul</i> ⁺	<i>Est-α</i> ⁷	<i>Est-β</i> ^B	586	<i>Odth</i> ^S	<i>tx</i>	<i>Est-α</i> ⁷	
	<i>Acph</i> ⁴	<i>cul</i>	<i>Est-α</i> ⁵	<i>Est-β</i> ^B	<i>Odth</i> ^S	<i>cul</i>	<i>Est-α</i> ⁷	<i>Est-β</i> ^B	34	<i>Odth</i> ^S	<i>tx</i> ⁺	<i>Est-α</i> ⁹	
	<i>Acpl</i> ²	<i>cul</i> ⁺	<i>Est-α</i> ⁹	<i>Est-β</i> ^A	<i>Odth</i> ^F	<i>cul</i> ⁺	<i>Est-α</i> ⁹	<i>Est-β</i> ^A	39	<i>Odth</i> ^F	<i>tx</i>	<i>Est-α</i> ⁷	
	<i>Acph</i> ²	<i>cul</i>	<i>Est-α</i> ⁵	<i>Est-β</i> ^B	<i>Odth</i> ^F	<i>cul</i>	<i>Est-α</i> ⁷	<i>Est-β</i> ^B	370	<i>Odth</i> ^S	<i>tx</i>	<i>Est-α</i> ⁹	
	<i>Acph</i> ⁴	<i>cul</i> ⁺	<i>Est-α</i> ⁹	<i>Est-β</i> ^A	<i>Odth</i> ^S	<i>cul</i> ⁺	<i>Est-α</i> ⁹	<i>Est-β</i> ^A	372	<i>Odth</i> ^F	<i>tx</i> ⁺	<i>Est-α</i> ⁷	
	<i>Acpl</i> ²	<i>cul</i>	<i>Est-α</i> ⁹	<i>Est-β</i> ^A	<i>Odth</i> ^F	<i>cul</i>	<i>Est-α</i> ⁷	<i>Est-β</i> ^A	31	<i>Odth</i> ^S	<i>tx</i>	<i>Est-α</i> ⁷	
	<i>Acph</i> ²	<i>cul</i>	<i>Est-α</i> ⁵	<i>Est-β</i> ^B	<i>Odth</i> ^S	<i>cul</i> ⁺	<i>Est-α</i> ⁹	<i>Est-β</i> ^B	44	<i>Odth</i> ^F	<i>tx</i> ⁺	<i>Est-α</i> ⁹	
	<i>Acph</i> ⁴	<i>cul</i>	<i>Est-α</i> ⁹	<i>Est-β</i> ^A	<i>Odth</i> ^S	<i>cul</i>	<i>Est-α</i> ⁷	<i>Est-β</i> ^A	64	<i>Odth</i> ^S	<i>tx</i> ⁺	<i>Est-α</i> ⁷	
	<i>Acpl</i> ²	<i>cul</i> ⁺	<i>Est-α</i> ⁵	<i>Est-β</i> ^B	<i>Odth</i> ^F	<i>cul</i> ⁺	<i>Est-α</i> ⁷	<i>Est-β</i> ^B	69	<i>Odth</i> ^F	<i>tx</i>	<i>Est-α</i> ⁹	
	<i>Acph</i> ²	<i>cul</i>	<i>Est-α</i> ⁵	<i>Est-β</i> ^A	<i>Odth</i> ^S	<i>cul</i>	<i>Est-α</i> ⁹	<i>Est-β</i> ^A	75	<i>Odth</i> ^S	<i>tx</i>	<i>Est-α</i> ⁹	
	<i>Acph</i> ⁴	<i>cul</i> ⁺	<i>Est-α</i> ⁹	<i>Est-β</i> ^B	<i>Odth</i> ^F	<i>cul</i> ⁺	<i>Est-α</i> ⁷	<i>Est-β</i> ^B	92	<i>Odth</i> ^F	<i>tx</i> ⁺	<i>Est-α</i> ⁷	
	<i>Acpl</i> ²	<i>cul</i>	<i>Est-α</i> ⁵	<i>Est-β</i> ^A	<i>Odth</i> ^S	<i>cul</i>	<i>Est-α</i> ⁹	<i>Est-β</i> ^A	8	<i>Odth</i> ^S	<i>tx</i> ⁺	<i>Est-α</i> ⁹	
	<i>Acph</i> ²	<i>cul</i> ⁺	<i>Est-α</i> ⁹	<i>Est-β</i> ^B	<i>Odth</i> ^F	<i>cul</i> ⁺	<i>Est-α</i> ⁷	<i>Est-β</i> ^B	7	<i>Odth</i> ^F	<i>tx</i>	<i>Est-α</i> ⁷	
	<i>Acph</i> ⁴	<i>cul</i>	<i>Est-α</i> ⁵	<i>Est-β</i> ^A	<i>Odth</i> ^S	<i>cul</i>	<i>Est-α</i> ⁹	<i>Est-β</i> ^A	5	<i>Odth</i> ^S	<i>tx</i> ⁺	<i>Est-α</i> ⁷	
	<i>Acpl</i> ²	<i>cul</i> ⁺	<i>Est-α</i> ⁹	<i>Est-β</i> ^B	<i>Odth</i> ^F	<i>cul</i> ⁺	<i>Est-α</i> ⁷	<i>Est-β</i> ^B	5	<i>Odth</i> ^F	<i>tx</i>	<i>Est-α</i> ⁹	
	No. of non-recombinants	Total				1160	Total			2304	Total		2416
	No. of recombinants			310				1089				1182	
	<i>cul</i> ~ <i>Acph</i>		559(48.2)*			<i>cul</i> ~ <i>Odth</i>		231(10.0)*		<i>tx</i> ~ <i>Odth</i>		303(12.5)*	
	<i>cul</i> ~ <i>Est-α</i>		489(42.2)			<i>cul</i> ~ <i>Est-β</i>		997(43.3)		<i>tx</i> ~ <i>Est-α</i>		911(37.7)	
	<i>cul</i> ~ <i>Est-β</i>		514(44.3)			<i>cul</i> ~ <i>Est-α</i>		900(39.1)		<i>tx</i> ~ <i>Est-β</i>		982(40.6)	
	<i>Acph</i> ~ <i>Est-α</i>		560(48.3)			<i>Odth</i> ~ <i>Est-β</i>		1052(45.7)		<i>Odth</i> ~ <i>Est-α</i>		1020(42.2)	
	<i>Acph</i> ~ <i>Est-β</i>		573(49.4)			<i>Odth</i> ~ <i>Est-α</i>		965(41.9)		<i>Odth</i> ~ <i>Est-β</i>		1071(44.3)	
	<i>Est-α</i> ~ <i>Est-β</i>		149(12.8)			<i>Est-α</i> ~ <i>Est-β</i>		267(11.6)		<i>Est-α</i> ~ <i>Est-β</i>		203(8.4)	
			1160				2304					2416	

* The figure in parentheses stands for recombination frequency.

Table 2. Recombination experiments on the second chromosome. *In(2)TY* inversion has genetic markers, *Acph*⁴, *Od**h*^F, *tx*⁺, *cul*⁺, *Est-α*⁵, and *Est-β*^B

	Exp. 4	Exp. 5	Exp. 6	Exp. 7	Exp. 8	Exp. 9
	<i>Acph</i> ² <i>tx</i>	<i>Acph</i> ² <i>Od</i> <i>h</i> ^S	<i>In(2)TY</i>	<i>In(2)TY</i>	<i>In(2)TY</i>	<i>In(2)TY</i>
Female	<i>Acph</i> ¹ <i>tx</i> ⁺	<i>Acph</i> ⁴ <i>Od</i> <i>h</i> ^F	<i>Acph</i> ² <i>Est-α</i> ⁹ <i>Od</i> <i>h</i> ^S <i>tx</i> <i>Est-α</i> ⁷	<i>cul</i> <i>Est-α</i> ⁹ <i>Est-β</i> ^A	<i>Est-α</i> ⁹ <i>Est-β</i> ^A	
Male	<i>Acph</i> ² <i>tx</i>	<i>Acph</i> ² <i>Od</i> <i>h</i> ^S	<i>Acph</i> ² <i>Est-α</i> ⁰ <i>Od</i> <i>h</i> ^S <i>tx</i> <i>Est-α</i> ⁷	<i>cul</i> <i>Est-α</i> ⁹ <i>Est-β</i> ^A	<i>Est-α</i> ⁹ <i>Est-β</i> ^A	
	<i>Acph</i> ² <i>tx</i>	<i>Acph</i> ² <i>Od</i> <i>h</i> ^S	<i>Acph</i> ² <i>Est-α</i> ⁰ <i>Od</i> <i>h</i> ^S <i>tx</i> <i>Est-α</i> ⁷	<i>cul</i> <i>Est-α</i> ⁹ <i>Est-β</i> ^A	<i>Est-α</i> ⁹ <i>Est-β</i> ^A	
No. of non-recombinants	579	319	1305	1123	1072	5302
No. of recombinants	574(49.8)*	301(48.5)*	164(11.2)*	<i>tx</i> ~ <i>Est-α</i> 0 <i>Od</i> <i>h</i> ~ <i>Est-α</i> 0 <i>tx</i> ~ <i>Od</i> <i>h</i> 0	<i>cul</i> ~ <i>Est-α</i> 0 <i>cul</i> ~ <i>Est-β</i> 0 <i>Est-α</i> ~ <i>Est-β</i> 0	
Total chromosomes	1153	620	1469	1123	1072	5302

* The figure in parentheses stands for recombination frequency.

calculated it as the difference between the two distances from the *Od**h* locus. Considering that *Est-β* is located near the centromere on this telocentric chromosome (Korochkin, 1983), these nine loci were mapped as follows:

$$\begin{aligned} &Acph—(>48\%)—Od h—(10\%)—cul—(2.5\%)—tx—(37.7\%)— \\ &—Est-α—(11.9\%)—Est-β—(centromere). \end{aligned}$$

Unfortunately, these loci could not be superimposed onto the genetic map of *D. virilis* drawn by Alexander (1976), because suitable markers are not available. The two esterase loci of *D. virilis*, *esterase-α* and *esterase-β*, however, were mapped by Korochkin (1983). Though we have not closely compared our *Est-α* and *Est-β* loci with his *esterase-α* and *esterase-β* loci, they would appear to be identical ones, because *D. virilis* has only one locus manifesting a major band for the *α*-esterase and the *β*-esterase in homozygotes, respectively. If so, the *Est-α* locus at 2–235.5 and the *Est-β* locus at 2–254.1 (Korochkin, 1983) would correspond to a distance between the loci of 18.6%, a value a little larger than that found by us, 11.9%.

In order to characterize *In(2)TY* we examined some isozyme alleles confined within the chromosome, i.e., the *Est-α*, *Est-β*, and *Od**h* alleles. However, they were within the range of genic variations of *D. virilis* as observed thus far. The linkage type of the *Est-α* and the *Est-β* allele within *In(2)TY* was a low frequency couple in natural populations of *D. virilis*; that is, $\alpha^{1.02}$ in the α^5 group and $\beta^{1.02}$ in the β^B group (see Tsuno et al. 1983), respectively. The other alleles were

identical to the commonest ones at each locus in *virilis* natural populations in Japan.

4. DISCUSSION

Muller's proposal (1940) suggests that the 2nd chromosome of *D. virilis* corresponds to the 3R arm of *D. melanogaster*. Molecular evidence for this, based on *in situ* hybridization techniques, was provided by Whiting et al. (1989). In the map of Ohnishi and Voelker (1979), the region from *Od* to *tx* of *D. virilis* is inversely corresponded to that of *D. melanogaster*. The genetic map of *Drosophila littoralis*, which is a member of *virilis* group, was reported by Gontcharenko et al. (1985) to be as follows:

$$Acp\text{-}1\text{---(centromere)}\text{---}Me\text{---}cu\text{---}dt\text{---}\alpha\text{-}Est\text{---}[Inv.2t]\text{---}\beta\text{-}Est.$$

Though this order could be produced by at least two inversion events from the locus order of *D. virilis* (see a review by Throckmorton, 1979), a linkage type of *cu*, *Est- α* , and *Est- β* is maintained on the chromosome.

In(2)TY and the linkage maps

The proximal breakpoint of *In(2)TY*, 2-27D, is located distal to both the *Est- β* locus (cytologically at 2-29C-D-) and the *Est- α* locus (from 2-28D to 2-28E) on the chromosome map of *D. virilis* by Korochkin (1983). It is a striking feature that the heterozygote for *In(2)TY* displays recombination neither on the inside nor on the outside of the large inversion, ranging over a wide region from a central 2-24B to the proximal *Est- β* locus near the centromere. In general, an inversion heterozygote depresses recombination at the flanking regions near the breakpoints due to chromosomal interference. So, the neighboring regions to the *Est- α* locus cannot recombine between homologous chromosomes due to a close distance to the break point (29C). But within a large inversion a double crossover occurs generally and regions far away from a breakpoint can escape from the effect of chromosomal interference. In fact, recombinations could be observed neither between *Od* and *Est- α* , although their distance would be more than 50%, nor between *Est- α* and *Est- β* (in this region some weak interference may be present). Upon cytological examination, some asynaptic pairings were observed in these regions. Thus, this possibly results in no recombination between them.

On the other hand, females heterozygous for *In(2)TY* and the ST chromosome displayed a recombination frequency of 0.112 between the inversion and the *Acp* locus (Exp. 6 in Table 2). Thus *Acp* is located on the outside of the inversion. As a whole, the map of the 2nd chromosome can be drawn at present as shown in Fig. 4.

Fig. 4. Breakpoints of *In(2)TY* and linkage maps of some loci on the 2nd chromosome of *D. virilis*. CM stands for centromere and 24B and 27D are the breakpoints of the inversion on the salivary chromosome on Gubenko and Evgen'ev's map (1984).

The origin of In(2)TY

Though our isozyme data cannot exclude the possibility that the *In(2)TY* rearrangement is due to a spontaneous occurrence in *virilis* species *per se*, the chromosomal patterns shown in Figs. 1 to 3 strongly suggest that the inversion would be a visitor from a member of closely related species of *virilis* group such as *americana* or other related species. In this paper, however, we could not discover the origin of *In(2)TY* to specify the donor species in the *virilis* group. Between some species of the *virilis* group interspecific hybrids are easily obtained, and both females and males of their hybrids are fertile (see Table 75 in Patterson and Stone, 1952). Although the development of interspecific hybrids would generally be rare in natural populations, the probability of the appearance of interspecific hybrids among members of the *virilis* group would be higher than that of such hybrids among other species groups.

Though we got at least two strong candidates for the donor of the inversion, some other candidate species for it might be found in the species of Japan, where several members of the *virilis* group are known. Meanwhile, if the donor species is *americana*, *texana*, or *montana*, since natural populations of such species have never been found in Japan, interspecific hybridizations to bring about the mixing of genomes into *virilis* would have occurred in some foreign country. In line with this idea, most of the logs landed in Toyama lumberyards, where *In(2)TY* was sampled, are imported mainly from Canada, the northern U.S.A., and the USSR, all regions inhabited by many closely related species of *virilis*. So far data alone from salivary chromosomes cannot simply answer to the question of the donor species, even if many inversion patterns among the *virilis* group are compared with each other.

When we first found the inversion in larvae of a stock crossed several times with some laboratory *virilis* lines, about ten months had passed since the male fly having *In(2)TY* was caught in the Toyama population. Thus the traces indicating the origin of the rearrangement, e.g., *In(X)* or *In(4)* as shown in Fig. 2, might have been lost from the genome in the process of the hybridization. However, the original male *virilis* fly might have had some asynaptic regions or

inversions as a part of its genome, which might have been incorporated by recombination from one of the related species. Through our experiments, however, the *In(2)TY*-carrying flies have exhibited no signs of hybrid breakdown, e.g., few progenies, dull movement of larvae, and developmental abnormality, all of which are frequently observed in interspecific hybrids. So, we have the impression that this *In(2)TY* inversion would already have experienced many generations since it was incorporated as a part of the *virilis* genome.

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