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Patterns of *hobo* elements and their effects in natural populations of *Drosophila melanogaster* in Japan

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We studied the dynamics of *hobo* elements of *Drosophila melanogaster* in Japan with the goal of better understanding the invasion and evolution of transposons in natural populations. One hundred and twenty-six isofemale lines and 11 older stocks were tested for the presence and genetic phenotype of *hobo* elements. The oldest H strain, containing complete and deleted *hobo* elements, is Hikone-H (1957), but Hikone-R (1952) has no *hobo*-homologous sequences. The findings suggest that the *hobo* element invaded Japanese populations in the mid-1950s, at about the same time as the *P* element invasion in Japan. This chronology is consistent with the hypothesis of a recent worldwide *hobo* element invasion into *D. melanogaster* in the mid-1950s. In recently collected populations, H$^{\circ}$ strains (low *hobo* activity and high repression potency) are predominant, whereas H^{+} strains (high *hobo* activity and high repression potency) are predominant in the Sakishima Islands, the most south-western islands of the Japanese archipelago. H′ strains (high *hobo* activity and low repression potency) were first found in limited island populations. Japanese populations have not only full-size *hobo* elements and 1.5 kb *Th* elements but also characteristic deletion derivatives (1.6 and 1.8 kb *Xho*I fragments) that we have named *Jh* elements. These results are consistent with transgenic experiments with complete *hobo* elements, in which populations evolved to H^{+} or H$^{\circ}$ via H′, and in which 1.8 kb fragments appeared. We conclude that *hobo* elements invaded the central region of Japan, spread to the far islands, and that the invasion is currently at an intermediate, nonequilibrium stage.
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Introduction

The spread of selfish genetic elements and parasites, such as transposons and *Wolbachia* (rickettsial endosymbiont; Boyle *et al*, 1993), has been described for a number of systems, especially in *Drosophila melanogaster* populations. In *D. melanogaster*, about 50 families of transposable elements are known, divisible into at least three groups according to their DNA structure and putative mechanisms of transposition (Finnegan, 1992). Type II elements that have short terminal inverted repeats and transpose directly from DNA to DNA include *hobo*, *P*, *pogo*, and *HB* elements. Active *hobo* elements are known to induce a suite of anomalies, termed hybrid dysgenesis, as are *P* and *I* elements (Kidwell, 1979). The symptoms of *hobo* hybrid dysgenesis include gonadal dysgenesis (GD), chromosomal aberrations, high mutation rates, and male recombination in the germ line of the hybrid progeny from a dysgenic cross (Lim, 1979; Yannopoulos *et al*, 1983, 1987; Blackman *et al*, 1987). The *hobo* element was first cloned and characterized by McGinnis *et al* (1983). Calvi *et al* (1991) sequenced a functional *hobo* element (HFL1) that was 2959 bp long, with a 2.0 kb ORF. The large ORF (ORF1) polypeptide sequence has strong similarity to the *Ac* (*Zea mays*) and *Tam3* (*Anthirrinum majus*) transposases; this similarity is the basis for describing the *hobo-Ac-Tam* (hAT) family (Calvi *et al*, 1991). The family has representatives in other drosophilid and nondrosophilid insects (Warren *et al*, 1994) and in *Caenorhabditis elegans* (Bigot *et al*, 1996).

The distributions of *hobo* elements in natural populations have been examined previously (Yannopoulos *et al*, 1983, 1987; Streck *et al*, 1986; Periquet *et al*, 1989a, b). Strains collected before the mid-1950s lack *hobo* elements (E strains), while recently collected ones have complete and defective derivatives (H strains). These surveys revealed the presence of locally characteristic defective *hobo*s, for example, the 1.5 kb *Th* elements, first found in Eurasian populations (Periquet *et al*, 1989a), the 1.9 kb *Oh* element in the Oregon-R^{s} strain (Pascual and Periquet, 1991), and the 1.7 kb *Kh* elements in Korean strains (Kim and Kim, 1999). Also shown in all strains tested was the presence of *hobo*-hybridizing sequences that generate high molecular weight (>3 kb) *Xho*I fragments (Streck *et al*, 1986; Boussy and Daniels, 1991; Pascual

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and Periquet, 1991; Boussy and Itoh, 2004). Several *hobo*-homologous sequences were identified in the euchromatin (Galindo *et al*, 2001) and heterochromatin of E strains (Boussy and Itoh, 2004). It is hypothesized that *hobo* elements were introduced into the melanogaster group ancestor twice (Daniels *et al*, 1990; Boussy and Itoh, 2004). In order to understand how the canonical *hobo* elements evolves and how they can influence the *hobo* activity, several transgenic experiments with complete *hobo* elements have carried out (Galindo *et al*, 1995; Ladeveze *et al*, 1998, 2001; Souames *et al*, 2003a, b). After complete *hobo* elements are introduced into an E strain, the populations are E′ (low *hobo* activity and low *hobo* repression potency), become H′ (high *hobo* activity and low repression potency), until evolving an H^+ (high *hobo* activity and high repression potency) or an H° (low *hobo* activity and high repression potency) phenotype (Galindo *et al*, 1995). *Xho*I fragments (1.8 kb), indicating internal deletions in *hobo* elements, appeared independently in transgenic lines of complete *hobo* elements at different generations. Lines were found to evolve to one of three patterns of *hobo*-homologous sequences: containing only full-size elements; containing only deleted elements; or containing both full-size and deleted sequences (Souames *et al*, 2003a, b). It is assumed that similar dynamics occur when *hobo* elements invade natural populations.

We have examined the dynamics of *hobo* elements in Japanese natural populations of laboratory-maintained strains of *D. melanogaster* collected in various parts of Japan from 1952 to 1989 (Gamo *et al*, 1990) and recently collected from 1997 to 2001 (Itoh *et al*, 2001, 2004). Besides the E, H°, and H^+ strains, we have found several E′ strains that contain full-size *hobo* and/or deleted derivatives in older stocks and recently collected populations. We hypothesize about the date of the invasion and spread of *hobo* elements into Japanese *Drosophila* population. A novel phenotypic class (H′ strain) was found in current Japanese populations. The locally characteristic defective *hobo* derivatives, yielding 1.6 and 1.8 kb *Xho*I fragments, that we call *Jh* elements may play regulatory role in the *hobo* system.

Materials and methods

Flies

Japanese natural populations of *D. melanogaster* collected between 1952 and 1989 were represented by eight isofemale lines (Hikone-R, Hikone-H, Eth-29 (Mino-H), Ashidakayama, Okaya, Asuka-1, Asuka-7, and Komagatani-30; Gamo *et al*, 1990) and three isogenic lines (Hikone-N2-1-1, -N4-3-3, and -N9-2-2; Nishino *et al*, 1993) maintained in our laboratory (Table 1). In 1997–2001, we established 126 isofemale lines of *D. melanogaster* that were collected from 11 localities in Honshu, the main island of Japan (KN (Katsunuma), FK (Fukui), MS (Mishima), IN (Inakadate), OS (Ohasama), HG (Higashine), NG (Niigata), OB (Obuse), OM (Okumura, Chichijima), TZ (Tozukawa), and TT (Tottori)); two localities in Hokkaido, the northernmost island of Japan (TY (Toyotomi) and SP (Sapporo); Itoh *et al*, 2004); and four localities in the Sakishima Islands, Okinawa, the southwesternmost islands of Japan (IS (Ishigaki), IR (Iriomote), HT (Hateruma), and YN (Yonaguni); Itoh *et al*, 2001), as shown in Table 2. The P–M status was known from previous work for most of these lines (Tables 1 and 2). Canton-S and Oregon-R (both M in the P–M system) were used as E strain controls, and Harwich (strong P in the P–M system) was used as the reference E strain in the *hobo* system. 23.5*/Cy was used as the reference H strain. 23.5*/Cy was provided by Dr G Yannopoulos; it bears one or more active *hobo* element on the second chromosome (Yannopoulos *et al*, 1987).

Table 1 Phenotypic characteristics in *hobo* system in older Japanese populations

	hobo-*activity potential*[a]	hobo-*repression potential*[b]	hobo *status*	*P–M status*[c]
Controls				
23.5*/Cy (Greek)	23	0	H^+	Q
Harwich (USA)	0	23	E′	P
Oregon-R (USA)	0	36	E	M
Canton-S (USA)	0	32	E	M
Older Japanese population				
1952 Hikone-R	0	70	E	M
1957 Hikone-H	2	0	H°	Q
1957 Eth-29 (Mino-H)	0	0	H°	M
1961 Ashidakayama	0	5	E	M′
1961 Okaya	4	0	H°	M
1972 Asuka-1	2	0	H°	M′
1972 Asuka-7	2	0	H°	M′
1984 Komagatani-30	0	12	E′	Q
1989 Hikone-N 2-1-1[d]	0	0	H°	Q, P
1989 Hikone-N 4-3-3[d]	0	0	H°	Q
1989 Hikone-N 9-2-2[d]	2	0	H°	Q

[a]GD score in cross A.
[b]GD score in cross A*.
[c]Data of P–M status come from Gamo *et al* (1990).
[d]There are isogenic lines and data of P–M status come from Nishino *et al* (1993).

D. melanogaster strains were cultured at 25°C under standard laboratory conditions by mass culture on a cornmeal–glucose–yeast–agar medium (5% cornmeal, 7% glucose, 5% dead yeast, 1% agar, and 0.35% propionic acid).

GD sterility tests

Harwich and 23.5*/Cy were used as the E and H reference strains, respectively, in the GD assays (Stamatis *et al*, 1989). Fifteen virgin individuals of each of the tested and reference strains were mated at 25°C to make two sets of A cross (Harwich♀♀ × tested♂♂) and A* cross (tested♀♀ × 23.5*/Cy♂♂). The F1 female flies were collected, allowed to mature for 3 days at 25°C, and then dissected; at least 50 ovaries were scored. The percent of dysgenic ovaries (100 × (number of dysgenic ovaries/total number of dissected ovaries)) was calculated for each cross. In the A* cross, the GD score was estimated by subtraction of the percent dysgenesis of Cy/tested F1 females from that of 23.5*/tested ones ((GD percent of 23.5*/tested)−(GD percent of Cy/tested)). Cross A measures *hobo*-activity potential of a tested strain and cross A* measures *hobo*-repression potential (Figure 1a).

Southern blot analysis

Genomic DNA was extracted from 50 flies of each strain and then digested with the restriction enzyme *Xho*I.

Table 2 Phenotypic characteristics of *hobo* system in current Japanese populations. (a) Honshu and Hokkaido and (b) Sakishima Islands, Okinawa

Year	*Place*	*Phenotypes of* hobo *element*					*P–M status*[a]
		H^+	H°	E'	H'	*Total*	
Locality in Japan (North to South)							
(a) Honshu and Hokkaido							
2000	TY (Hokkaido)	2	7	1		10	P, Q, M′
2000	SP (Hokkaido)	4	5	1		10	P, Q, M′, P′
2000	IN (Aomori)	2	12	1		15	P, Q, M′
2000	OS (Iwate)		9			9	P, Q, P′
2000	HG (Yamagata)	3	6			9	P, Q, M′
2000	NG (Niigata)	1	8			9	Q, M′
1997	KN (Yamanashi)		1	1		2	Q, M′
1999	FK (Fukui)	2	5			7	Q, M′
1999	MS (Shizuoka)		2			2	ND
2000	OB (Nagano)	1	3			4	ND
2000	TZ (Nara)	2	8			10	Q, M′
2001	TT (Tottori)		9			9	P, Q, P′
2000	OM (Chichi-jima, Tokyo)	2	4	1	1	8	Q, M′
	Subtotal	19	79	5	0	104	
(b) Sakishima Islands, Okinawa							
1997	IS (Ishigaki)	3	1			4	P, Q
1998	IR (Iriomote)	7	1			8	P, Q, P′
1998	HT (Hateruma)	1			4	5	P, Q
1998	YN (Yonaguni)	3	2			5	P, Q, M′
	Subtotal	14	4	0	4	22	
Total		33	83	4	6	126	
Percent		26	66	3	5	100	

[a]Data of P–M status come from Itoh *et al* (2004) and ND represents no data.

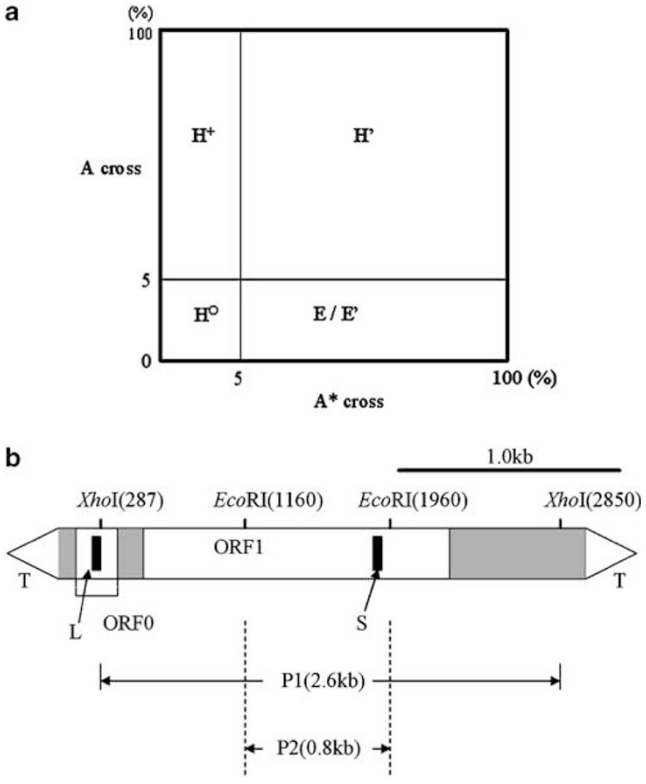

Figure 1 (**a**) Classes of *hobo* strains based on GD sterility tests. (**b**) Structure of a functional *hobo* element, HFL1 (Calvi *et al*, 1991). *Xho*I and *Eco*RI endonuclease cleavage sites and the fragments they generate are indicated.

About 5 μg of digested DNA were electrophoresed in a 1% agarose gel, capillary blotted onto a nitrocellulose membrane (Schleicher and Schuell), hybridized to a labeled probe consisting of the 2.6 kb *Xho*I fragment or the 0.86 kb *Eco*RI fragment of the pH108 *hobo* element (Streck *et al*, 1986), provided by Dr Eiji Nitasaka (Figure 1b), and detected by ECL direct nucleic acid labeling and detection systems (Amersham Pharmacia Biotech UK limited).

Results

Genetic phenotypes of the *hobo* system in Japan

Table 1 shows the genetic phenotypes from GD sterility tests of the *hobo* system in older Japanese populations. Based on the percentage of GD sterility (Figure 1a), Hikone-R, Ashidakayama, and Komagatani-30 were classified as E strains (low *hobo* activity and low repression potency). The rest of the strains established in Japan in 1952–1989 were H° (Table 1). Hikone-H (1957) is the oldest H strain from a Japanese population.

In the current populations from 13 localities of Honshu and Hokkaido, H° strains are predominant (76%), as shown in Table 2a. In five localities (HG, NG, FK, OB, and TZ), only H^+ and H° strains were found. In KN, an E' strain accompanied an H° strain. H^+, H°, and E' strains were mixed in TY, SP, IN, and OM, and only H° strains were found in OS, MS, and TT. One H' strain was found in OM (Chichijima, Tokyo) with H^+, H°, and E' strains.

Twenty-two strains from the Sakishima Islands, southwest of Japan (Table 2b), are classified as 14 H^+ strains, four H° strains, and no E′ strains. In addition, four HT isofemale lines demonstrate a novel phenotype, which is designated as H′ (Figure 1a). H′ strains have high *hobo*-activity potential and low *hobo*-repression potential, like the P′ strains hypothesized in the P–M system (Quesneville and Anxolabehere, 1998) and found in a natural population by Itoh *et al* (2001).

hobo elements in Japanese populations

To determine the temporal distribution of *hobo* elements in older Japanese populations of *D. melanogaster*, Southern blot analysis was carried out by using the 2.6 kb *Xho*I fragment of the complete *hobo* element as a probe (Figure 2). We found two E strains, Ashidakayama (lane 1) and Hikone-R (lane 8), with no *hobo* elements, and one E′ strain, Komogatani-30 (lane 13), having full-size *hobo* elements and deletion derivatives, as did Harwich (lane 10). Almost all H° strains had both complete (2.6 kb) and defective *hobo* sequences (1.8, 1.6, and 1.1 kb), but Eth-29 (lane 2) and Asuka-1 (lane 6) had only defective *hobo* derivatives, and Okaya (lane 5) contained only complete *hobo* elements. Hikone-H (lane 9), established in 1957, is the oldest H strain in Japanese populations. Among three isogenic strains (Hikone-N2-1-1 (lane 3), -N4-3-3 (lane 11), and -N9-2-2 (lane 12) (Nishino *et al*, 1993), there are differences in the patterns of *hobo*-hybridizing bands, and all three are different from the older line, Hikone-H. Hikone-H contains a majority of bands that are found in the three, suggesting that they were derived either from Hikone-H or from the same population as Hikone-H.

Southern blots analysis was also carried out in 126 recently established isofemale lines, also using the 2.6 kb *Xho*I fragment of the complete *hobo* element as a probe (Figure 1b). All lines examined had many *hobo* copies in their genomes (Figure 3). Besides the 2.6 kb *Xho*I fragment of full-sized *hobo* elements and the 1.1 kb fragments of the *Th* elements (Periquet *et al*, 1989a), lines from Honshu and Hokkaido also show 1.6 and 1.8 kb *Xho*I fragments (Figure 3). Furthermore, most of isofemale lines showed a 1.3 kb *Xho*I fragment apparently corresponding to the 1.7 kb *Kh* element, described as characteristic of Korean populations (Kim and Kim, 1999).

Five E′ strains (from TY, SP, IN, OM, and KN) and one H′ strain (OM) have many *hobo*-related sequences, as do H° and H^+ strains (see TY-10 in Figure 3 and IN-9 in Figure 5; others not shown). No specific fragments were observed to be typical for E′, H°, or H^+ strains. No clear relationship was found between *hobo* element profiles and phenotypic characteristics.

In Southern blots of the Sakishima Islands populations, all isofemale lines except one contained full-size *hobo* elements (Figure 4). Among the various defective *hobo* elements, 1.1 and 1.8 kb *Xho*I fragments were found in all strains. A 1.6 kb *Xho*I fragment was also observed in almost all isofemale lines from the Sakishima Islands. Twelve out of 22 Sakishima Islands isofemale lines, and lines from IS (lanes 1, 2, and 4), IR (lanes 5, 7, 8, and 10), HT (lanes 15–17), and YN (lanes 19 and 22), showed a 1.3 kb *Xho*I fragment corresponding to *Kh* elements (Kim and Kim, 1999). The H° strains in IS (lane 3) and YN (lanes 18 and 19) have both complete and defective *hobo* elements, but IR (lane 7) has only defective *hobo* elements, as did Asuka-1 (Figure 2). Four H′ strains from HT (lanes 13 and 15–17) also have both complete and various defective *hobo* elements, as do 14 H^+ strains from the Sakishima Islands.

Locally characteristic deletion-derivative *Jh* elements in Japanese populations

We detected 1.6 and 1.8 kb *Xho*I fragments in some older and almost all current Japanese populations

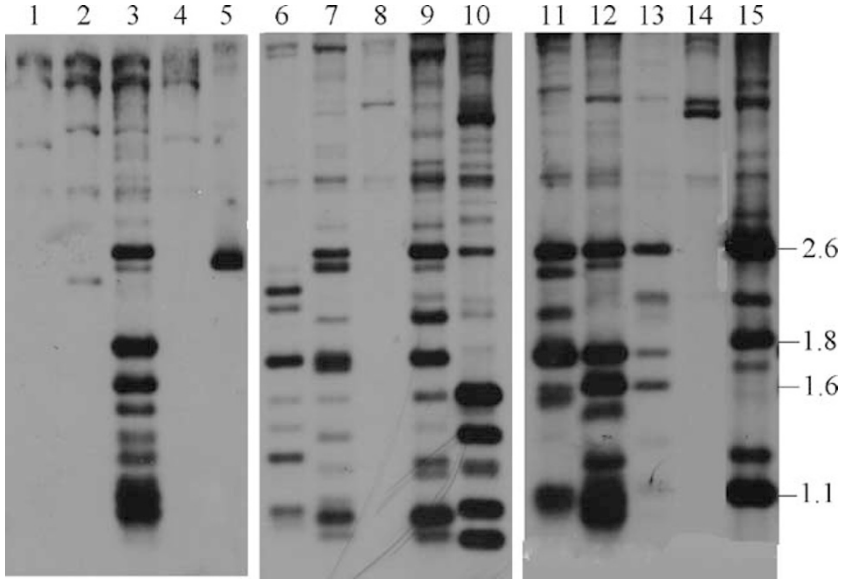

Figure 2 Southern blots of genomic DNA from older Japanese populations, probed with the *Xho*I fragment of the *hobo* element. The bands corresponding to full-length *hobo* elements (2.6 kb *Xho*I fragment), *Th* derivatives (1.1 kb), and *Jh* elements (1.6 and 1.8 kb) are indicated to the right. The strains are the following: lane 1, Ashidakayama; lane 2, Eth-29; lane 3, Hikone-N2-1-1; lane 4, Canton-S; lane 5, Okaya; lane 6, Asuka-1; lane 7, Asuka-7; lane 8, Hikone-R; lane 9, Hikone-H; lane 10, Harwich; lane 11, Hikone-N4-3-3; lane 12, Hikone-N9-2-2; lane 13, Komagatani-30; lane 14, Oregon-R; and lane 15, 23.5*/Cy.

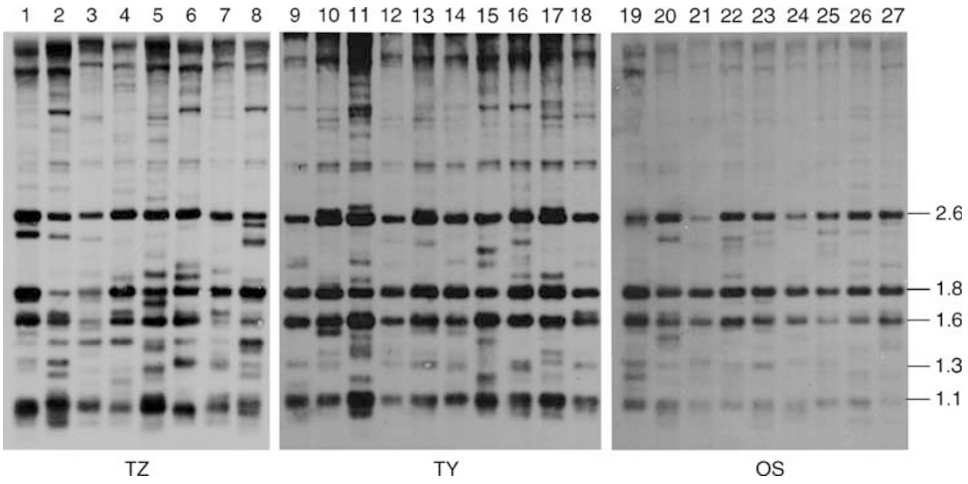

Figure 3 Southern blots of genomic DNA from current populations in Honshu and Hokkaido of Japan, probed with the *Xho*I fragment of the *hobo* element. The isofemale lines are: from Tozukawa (TZ, Nara) – lane 1, TZ-1 (H°); lane 2, TZ-2 (H°); lane 3, TZ-3 (H$^+$); lane 4, TZ-6 (H$^+$); lane 5, TZ-7 (H°); lane 6, TZ-8 (H°); lane 7, TZ-9 (H°); lane 8, T-11 (H°); from Toyotomi (TY, Hokkaido) – lane 9, TY-1 (H$^+$); lane 10, TY-2 (H$^+$); lane 11, TY-3 (H°); lane 12, TY-4 (H°); lane 13, TY-5 (H°); lane 14, TY-6 (H°); lane 15, TY-7 (H°); lane 16, TY-8 (H°); lane 17, TY-9 (H°); lane 18, TY-10 (E′); and from Ohasama (OS, Iwate) – all isofemale lines are H°; lane 19, OS-1; lane 20 OS-3; lane 21, OS-4; lane 22, OS-8; lane 23, OS-9; lane 24, OS-11; lane 25, OS-12; lane 26, OS-13; and lane 27, OS-14. Bands corresponding to *Kh* (1.3 kb) and other elements are indicated to the right, as in Figure 2.

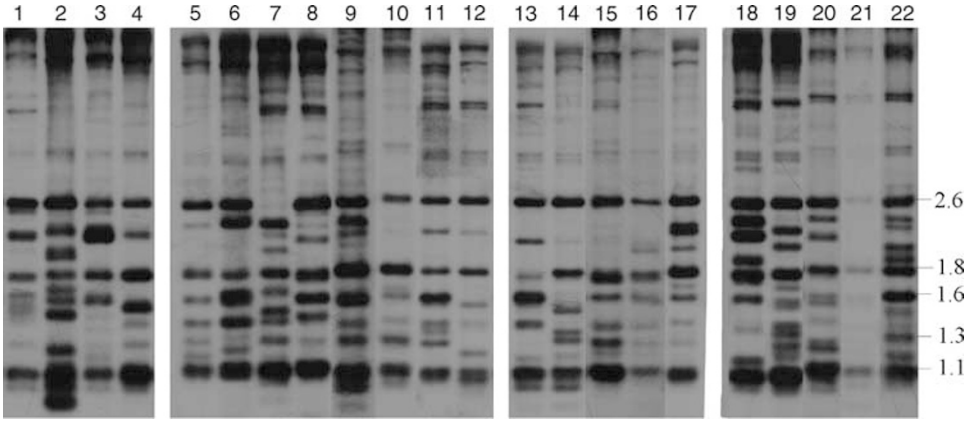

Figure 4 Southern blots of genomic DNA from the Sakishima Islands populations, probed with the *Xho*I fragment of the *hobo* element. The isofemale lines are: from Ishigaki (IS, Okinawa) – lane 1, IS-29 (H$^+$); lane 2, IS-32 (H$^+$); lane 3, IS-53 (H°); lane 4, IS-55 (H$^+$); from Iriomote (IR, Okinawa) – lane 5, IR-1 (H$^+$); lane 6, IR-2 (H$^+$); lane 7, IR-3 (H°); lane 8, IR-4 (H$^+$); lane 9, IR-12 (H$^+$); lane 10, IR-13 (H$^+$); lane 11, IR-14 (H$^+$); lane 12, IR-15 (H$^+$); from Hateruma (HT, Okinawa) – lane 13, HT-25 (H′); lane 14, HT-26 (H$^+$); lane 15, HT-27 (H′); lane 16 HT-28 (H′); lane 17, HT-29 (H′); and from Yonaguni (YN, Okinawa) – lane 18, YN-1 (H°); lane 19, YN-2 (H°); lane 20, YN-3 (H$^+$); lane 21, YN-4 (H$^+$); and lane 22, YN-5 (H$^+$). Bands corresponding to *Kh* (1.3 kb) and other elements are indicated to the right, as in Figure 2.

(Figures 2–4). The 1.6 and 1.8 kb fragments, predicted to correspond to 2.0 and 2.2 kb internally deleted *hobo* derivatives, seem to be characteristic of Japanese populations, and we designate them as *Jh* elements. To characterize the deleted regions in these elements, Southern blot analysis was carried out using the 0.8 kb *Eco*RI fragment of the central region of a complete *hobo* element as a probe (Figure 1b). Nearly all the fragments smaller than the 1.6 kb bands almost disappeared, but the molecular fragments larger than the 1.8 kb bands remained clear (Figure 5b) in comparison with the Southern blot probed with the 2.6 kb of *Xho*I fragment (Figure 5a). This indicates that the 1.6 kb fragments have lost almost the entire *Eco*RI fragment of the central region of the complete *hobo* element, but that the 1.8 kb bands can hybridize to the probe because their defects are smaller than the *Eco*RI fragment.

Higher molecular weight *Xho*I fragments (>2.6 kb) of *hobo*-hybridizing sequences were detected in all tested strains, including E strains (Figures 2 and 5a). These fragments have also lost almost the entire *Eco*RI fragment since most such bands disappeared when probed with the 0.8 kb *Eco*RI fragment of a complete *hobo* element (Figure 5b).

Discussion

In this study, we describe two aspects of *hobo* elements in Japanese *D. melanogaster* populations. We first identify when *hobo* elements invaded and how they spread through the populations, and second, we have identified two deleted forms of *hobo* unique to Japanese populations. We found no *hobo* elements in the Hikone-R strain, collected in 1952, but we did detect them in the Hikone-

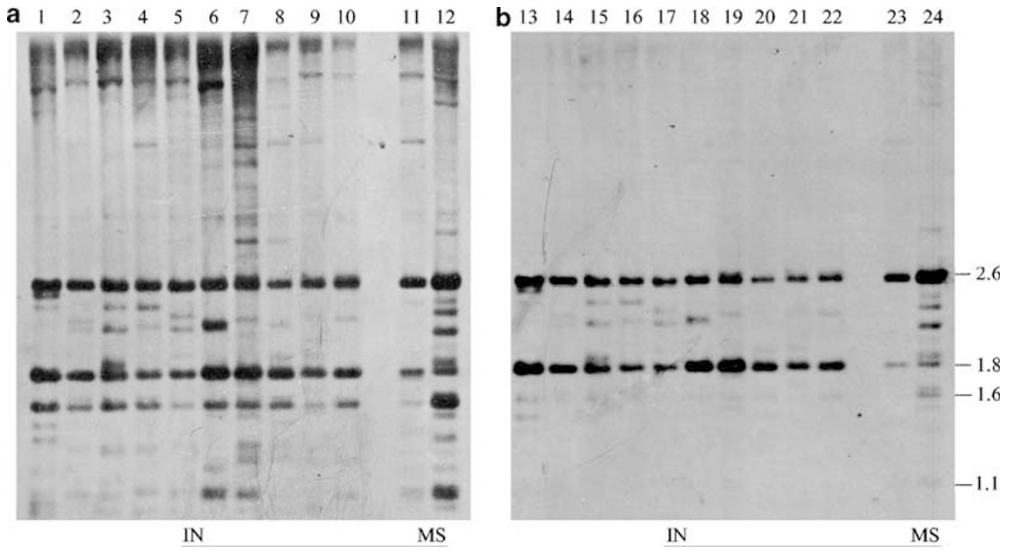

Figure 5 Southern blots of genomic DNA from Inakadate (IN, Aomori) and Mishima (MS, Shizuoka) populations. (**a**) The *Xho*I fragment of the *hobo* element is used as a probe from lanes 1 to 12. (**b**) The *Eco*RI fragment is used as a probe (Figure 1b) from lanes 13 to 24. The isofemale lines are: lane 1, IN-1 (H°); lane 2, IN-3 (H°); lane 3, IN-4 (H°); lane 4, IN-6 (H^+); lane 5, IN-8 (H^+); lane 6, IN-9 (E′); lane 7, IN-11 (H°); lane 8, IN-14 (H°); lane 9, IN-15 (H°); lane 10, IN-17 (H°); lane 11, MS-7 (H°); and lane 12, MS-53 (H°). Lanes 13–24 are the same samples as lanes 1–12. Fragment sizes are indicated as in Figure 2.

H and Mino-H strains, collected in 1957. This is consistent with the hypothesis that *hobo* transposable elements invaded the *D. melanogaster* genome in the mid-1950s in America, France, USSR, China, and Africa (Periquet *et al*, 1989a, b, 1990; Boussy and Daniels, 1991; Pascual and Periquet, 1991). Around 1950, in Hikone-City large quantities of DDT and pyrethrum were used for several years to kill mosquitoes for malaria control, and the insecticide-resistant strain, Hikone-R (E), was isolated (Ogaki and Tsukamoto, 1953). On the hottest days of the summer, the heat tolerant H° strains, Hikone-H and Mino-H, were collected (Ogaki, 1962). We imagine that environmental changes could contribute to the invasion and spread of *hobo* elements, although exactly how is an open question. When complete *hobo* elements were introduced into the Hikone (E) strain, most of the transgenic strains at 14 generations showed a certain degree of *hobo*-activity and -repression potential, and these plateaued by generation 50 at 25°C. The spread of *hobo* elements was dependent on culture temperature (Galindo *et al*, 1995). Gamo *et al* (1990) showed that Hikone-R and Mino-H both lack *P* elements but that Hikone-H has *P* elements. Both *P* and *hobo* transposable elements seem to have invaded the genome in the mid-1950s in at least the central region of Japan.

This study also revealed that H° strains are predominant in current Japanese populations of *D. melanogaster*, but that a high proportion of H^+ strains occurs in the Sakishima Islands, Okinawa. The first discoveries of H′ strains in natural populations were from Hateruma (the Sakishima Islands) and Chichijima (1000 km south of Tokyo metropolitan). This novel type of *hobo* strain, H′, was described from transgenic experiments (Galindo *et al*, 1995). Following introduction of a full-size *hobo* element, the experimental populations were E′ until 14 generations, became H′, and eventually evolved an H^+ or an H° phenotype. If a similar pattern occurs in natural populations, *hobo* elements may have recently invaded *D. melanogaster* populations in Hateruma and Chichijima, and these populations would be expected to evolve to H^+ and H°. Therefore, these populations appear to be in an evolutionarily early phase of *hobo* invasion. We speculate that *hobo* elements invade to the central region of Japan and subsequently spread to the far islands, and that *hobo* elements in Japanese populations are at intermediate, nonequilibrium, evolutionary stages.

The second remarkable character of the *hobo* system in Japanese populations is the presence of 1.8 and 1.6 kb *Xho*I fragments in Southern blots, corresponding to 2.2 and 2.0 kb *hobo* sequences. The deletion of the 1.6 kb fragments is the central portion of the full-sized element. The 2.2 and 2.0 kb deleted *hobo* elements have not been obviously present in the surveys of populations from other countries (Periquet *et al*, 1989a, b, 1990; Boussy and Daniels, 1991; Pascual and Periquet, 1991; Kim and Kim, 1999). Therefore, they seem to be defective *hobo* derivatives unique to Japanese *Drosophila* populations. We call them *Jh* elements. Souames *et al* (2003b) reported that 1.8 kb *Xho*I fragments appeared independently at different generations in lines transformed with complete *hobo* elements. In two lines, the introduction of a *hobo* element did not trigger a multiplicative invasion of the genome, but 1.8 kb fragments appeared (Souames *et al*, 2003b). This study also gives evidence that 1.8 kb deleted elements may be involved in regulating the activity of the *hobo* elements in natural population. H°, H^+ H′, and E′ strains in the current Japanese natural populations have predominantly *Th* elements (1.5 kb; Periquet *et al*, 1989a; Pascual and Periquet, 1991) as well as full-size *hobo* elements and *Jh* elements (1.6 and 1.8 kb *Xho*I fragments). *Th* elements are present in most strains from different continents (Periquet *et al*, 1990). The wide distribution and predominance of the *Th* elements suggest that they might be analogous to the *KP* elements

of the P–M system (Black *et al*, 1987; Jackson *et al*, 1988; Itoh *et al*, 2001, 2004), perhaps even to having a regulatory role. Another defect *hobo* element, the *Kh* element of 1.7 kb, a specific defective element described from Korean strains (Kim and Kim, 1996, 1999), was found in most Japanese populations. When complete *hobo* elements invaded E genomes in experimental populations, they evolved to one of three patterns: only full-size *hobo* sequences; only deleted derivatives; or both full-size and deleted *hobo* sequences (Souames *et al*, 2003a). The first pattern is seen in Okaya (1961) in Figure 2 (lane 5), the second pattern is seen in Eth-29 (Mino-H, 1957) and Asuka-1 (1972) in Figure 2 (lanes 2 and 6) and IR (1998) in Figure 4 (lane 6), and the third pattern is seen in almost all the rest of the tested strains. The presence of a full-length *hobo* element capable of producing active transposase would seem to be required for transposition and GD sterility, but the presence of repression ability in some strains that lack full-length *hobo* sequences suggests that internally deleted elements may be able to play a role in repression.

When genomic DNAs are analyzed by Southern blots, high molecular weight *Xho*I fragments of *hobo*-hybridizing sequences are detected in all current strains of *D. melanogaster* (Boussy and Daniels, 1991; Boussy and Itoh, 2004; this study). We note that they generally seem to lack the homologous sequences in the central region of complete *hobo* elements.

Pascual and Periquet (1991) reported that the *hobo* elements are very variable in laboratory stocks and can be lost in a few decades. In our GD sterility test, the 23% GD sterility we observed for the control cross, ♀♀Harwich × 23.5*/Cy♂♂, is lower than that found by others (Yannopoulos *et al*, 1987; Pascual and Periquet, 1991). They used Harwich^{Y} (an E′ strain) and we used Harwich (also an E′ strain) maintained in our laboratory since 1986, but the original Harwich strain (USA) is described as an H$^{\circ}$ strain (Pascual and Periquet, 1991). Both Harwich^{Y} and our Harwich appear to represent changes of phenotype over time from H to E′.

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