

日本のイエバエの地方集団における移住と連鎖不平衡

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Migration and linkage disequilibrium in local populations of the housefly (*Musca domestica*) in Japan

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ABSTRACT

Housefly populations in Hokkaido, Japan were examined. The frequency of females carrying a female-determining factor (F), which is epistatic to the male-determining factor (M), has been estimated to be very low. This means that most of the chromosomes carrying an M factor are transferred from male parents to their male progeny without recombination. Based on this characteristic situation, the association between the M factor and one of the $6PGD$ alleles in Hokkaido in 1983 and 1984 was studied, and strong linkage disequilibrium was found in the samples from six spots in and around Sapporo. A specific allele, $6PGD^I$, was associated in most of the cases with the M factor-carrying third chromosome (III^M). The $6PGD$ locus was highly polymorphic in the M factor-free third chromosome (III). Based on these experimental results, it is suggested that the frequency of chromosome III^M has recently increased in the population around Sapporo.

1. INTRODUCTION

In the housefly, *Musca domestica*, a necessary condition for maleness in an individual is the presence of a male-determining factor, M (Hiroyoshi, 1964; Rubini and Palenzona, 1967). Although the Y chromosome is basically an M factor-carrier, M factors have been demonstrated in all of the five autosomal linkage groups (Sullivan, 1958; Wagoner, 1969; Hiroyoshi and Inoue, 1979; Inoue et al., 1986). Moreover, natural populations which are hard to detect the Y chromosome have been found in the United States (McDonald et al., 1975), Italy (Franco et al., 1982) and Japan (Tsukamoto et al., 1980). It is postulated that the frequency of M factor-carrying autosomes (A^M) has increased in natural populations (in Japan, Hiroyoshi and Fukumori, 1977; Tomita and Wada, 1989; in Italy, Franco et al., 1982) in the past few decades. There are, however, some difficulties in establishing this postulated phenomenon, due to the limited number of direct comparisons in natural populations.

The present study provides the experimental evidence for the above phe-

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nomenon, using linkage disequilibria between some isozyme marker genes and the M factor. In the populations of Hokkaido examined so far, the F factor, which makes a carrier of M develop into a fertile female, is very rare (Shono et al., 1982; Tomita and Wada, 1989). On the other hand, the frequency of chromosome III^M , which is the most predominant A^M in Japan (Hiroyoshi and Fukumori, 1977; Tomita and Wada, 1989), is high. Therefore, a chromosome III^M in these populations must be transferred from a male parent to his male progeny, and then act as a super-gene complex, since recombination is rare in the male. If the frequency of III^M increased recently from a few founder chromosomes in natural populations, one of its genetic consequences in the F factor-free populations is the existence of linkage disequilibrium between the M factor and its neighboring genes in the third chromosome. On the basis of this finding, we are able to speculate about the timing of the invasion of the III^M chromosome.

2. MATERIALS AND METHODS

Flies were collected at the following 9 spots in 4 areas stretching from west to east in Hokkaido in 1983 and 1984; 6 spots were within a circle of 11 km radius in or near Sapporo, and 1 spot each in Furano, Obihiro and Akkeshi (Fig. 1).

The method of determining the presence or the absence of the F factor in females was described in the accompanying paper (Tomita and Wada, 1989).

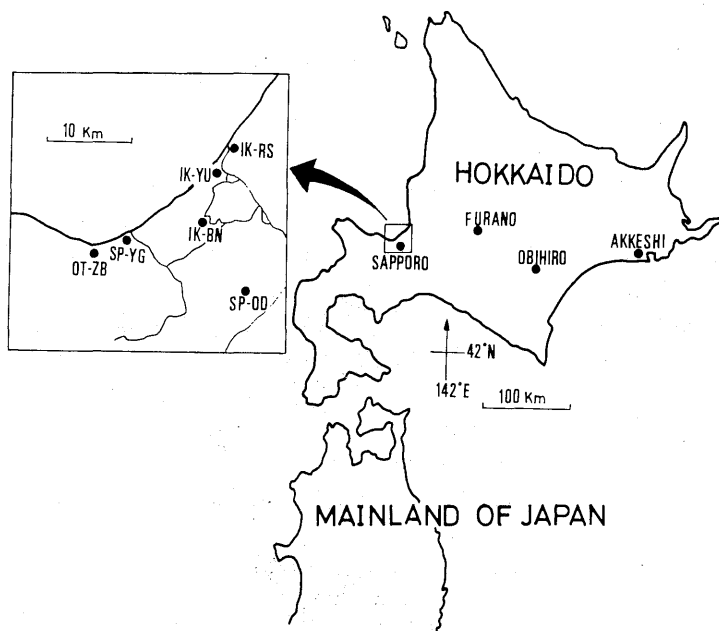


Fig. 1. Geographic locations of the 9 collection spots in Hokkaido, Japan.

Linkage relationships of the *M* factor were determined directly in the males sampled from wild populations, using the *ac; ar; bwb; ye; snp* mating method (Tomita and Hiroyoshi, in preparation). In this process, the F_1 offspring obtained from single male matings with the mutant females were used for the test of the *6-phosphogluconate dehydrogenase (6PGD)* genes on the third chromosome. The electromorphs were tested by starch gel electrophoresis (Stone et al., 1968). If a parental male contained chromosome III^M heterozygously, the *6PGD* gene on this chromosome was tested in the male progeny. If it contained no III^M , the gene on either of the homologous chromosome III s was tested.

The female recombination values among the *w*, *bwb* and *pw* loci were estimated for the chromosome III s and III^M s extracted from the Sapporo sample in 1985, in the same way as genes on chromosome III^M were mapped by Inoue and Hiroyoshi (1984), using the marker strain, *Mk II^M; w bwb pw; Ba F*.

3. RESULTS AND DISCUSSION

The genotypes controlling sex determination and the electromorph of the *6PGD* genes on the third chromosome were surveyed in each of the 9 spots in 4 areas in Hokkaido in 1983 and 1984. The results are summarized in Table 1. The upper 3 rows show the results obtained from Furano, Obihiro and Akkeshi, 1983. The lower 7 rows show those from spots in and around Sapporo, 1983 and 1984.

The *F* factor is sporadically distributed in populations of Japan (Tomita and

Table 1. The frequencies of genotypes and haplotypes for the sex-determining and *6PGD* genes

Sample	Female		Male			Chr. III		Chr. III^M		χ^2 #
	Sample size	<i>F</i> /+	Sample size	Plural <i>M</i> factor-carrying	Chr. III^M -carrying	Sample size	<i>6PGD</i> ^l	Sample size	<i>6PGD</i> ^l	
FR 83	6	0	83	0	0.08	76	0.45	7	0.86	*
OH 83	9	0	38	0	0.21	30	0.47	8	0.50	NS
AK 83	28	0	61	0	0.21	48	0.33	13	0.46	NS
SP-YG 83	23	0	124	0	0.40	75	0.37	49	0.98	**
SP-YG 84	30	0	49	0	0.24	37	0.54	12	1.0	**
SP-OD 84	31	0	52	0	0.33	35	0.49	17	1.0	**
IK-RS 84	30	0.03	51	0.02	0.24	38	0.45	12	1.0	**
IK-YU 84	26	0	50	0	0.30	35	0.57	15	0.93	**
IK-BN 84	28	0	46	0	0.35	30	0.50	16	1.0	**
OT-ZB 84	27	0	55	0	0.29	39	0.41	15	1.0	**

Linkage disequilibrium between *6PGD* genes and *M* factors in the third chromosomes.

* Significant at the 5% level.

** Significant at the 1% level.

Wada, 1989), but the frequencies in the Hokkaido populations are estimated to be very low. This is directly shown from the results of the female test or indirectly suggested by the fact that the males containing plural M factors were rare (Table 1). Chromosome III^M is now widely distributed in Japanese populations (Tomita and Wada, 1989), and it was the second most frequent class of M factor-carriers among 6 pairs of the chromosomes in Hokkaido populations, following the Y chromosome (Tomita and Wada, 1989). These results ensure the holandric inheritance of chromosome III^M in natural populations, which is a necessary condition for the present study.

There are at least three alleles at the $6PGD$ locus, $6PGD^I$, $6PGD^S$, and $6PGD^F$. Only the frequencies of the predominant allele, $6PGD^I$, are shown in Table 1. The $6PGD$ locus on chromosome III was highly polymorphic in every sample. The frequencies of $6PGD^I$ ranged from 0.33 in the Akkeshi sample to about 0.5 in each of the samples from Sapporo and its suburbs. In contrast, the frequencies of the $6PGD$ in chromosome III^M were markedly different among the areas. In the seven samples from 6 spots in and around Sapporo, the $6PGD$ locus on chromosome III^M was consistently monomorphic or nearly so for the $6PGD^I$ allele. However, in each of the Obihiro and Akkeshi samples, the locus was highly polymorphic. Linkage disequilibrium (or non random association) between the $6PGD$ alleles and M factors in the third chromosome was tested by the χ^2 method with the frequencies pooled for $6PGD^F$ and $6PGD^S$ (Table 1). The χ^2 for each of the 7 samples of Sapporo and its suburbs was significant at the 1% level and that of Furono was significant at the 5% level. However, the values of Obihiro and Akkeshi were not significant. Both from the significant linkage disequilibria and from the homogenous values in each of the frequencies in the 5 columns ($F/+$ female, male carrying plural M factors, male carrying chromosome III^M, and the chromosomes III and III^M associated with $6PGD^I$ allele) in the samples from Sapporo and its suburbs, the specific association of the $6PGD^I$ allele with the chromosome III^M is inferred to be generally present in the natural population in and around Sapporo.

The time since the immigration of chromosome III^M to the Sapporo area can be estimated by the application of Nei and Li's (1980) equation,

$$d_n \approx d_0(1-c)^n$$

where c is the recombination value between the $6PGD$ gene and the M factor in males, and d_0 (or d_n) is the difference between the $6PGD^I$ allele frequencies in chromosomes III^M and III at generation 0 (or n). This formula assumes the constancy of the $6PGD$ allele frequencies in chromosome III, which is reasonable in this study. Because the male recombination value, c , was not estimated directly, it was obtained as a product of kc' , where k is the ratio of male to female recombination value and c' is the female recombination value.

Before proceeding to the above estimation, some considerations of chromosomal

polymorphisms that might exist in the natural population of the housefly should be made. At least three types of III^M chromosome maps have been demonstrated by Inoue and Hiroyoshi (1984) in several strains from Japan and elsewhere on the basis of meiotic recombination in *F* factor-carrying females. The three types of maps are identical in the order of the loci (the order is *w bwb pw M*, from distal to proximal), but markedly different in the distances between neighboring loci, except for the *pw-M* interval. On the maps of types 1, 2 and 3, the distances between *w* and *bwb* are 15–22, 35–36 and 0.3, respectively, and the distances between *bwb* and *pw* are 41–47, 4–6 and 3, respectively (Inoue and Hiroyoshi, 1984); these differences may be due to chromosomal rearrangement. In this study, the type of gene arrangement in chromosome IIIs and III^Ms of the Sapporo population was determined by recombination tests, following the same procedure as described in Inoue and Hiroyoshi (1984).

Table 2 shows the recombination frequencies among the *w*, *bwb* and *pw* loci in the *F/+* females, which were made heterozygous with the chromosome III or III^M extracted from the 1985 fly sample in one of the sites of earlier sampling in Sapporo (denoted by SP-YG in Table 1). The per cent recombination frequencies between the *w* and *bwb* loci and between the *bwb* and *pw* loci were within the intervals of 15–22 and 43–49, respectively. The results indicate that the gene arrangements along all the third chromosomes tested correspond quite well with the type 1 map in Inoue and Hiroyoshi (1984) and the standard linkage map in Hiroyoshi (1977). Therefore, assuming monomorphism for the type 1 third chromosome of the housefly in the Sapporo population, the following estimation was made on the basis of recombination in the homokaryotype of the type 1 third chromosome.

Table 2. Female recombination frequencies along the third chromosomes extracted from the Sapporo sample (SP-YG 85)

Third chromosomes tested	No. of chromosomes extracted	Recombination frequencies (%)	
		<i>w-bwb</i>	<i>bwb-pw</i>
III	23	16–22	43–49
III ^M	8	15–21	46–49

On the type 1 map, the distances between *bwb* and *pw* loci and between *pw* and *M* loci are 44.6 and 0.1 cM., respectively (averaged from Inoue et al., 1983 and Inoue and Hiroyoshi, 1984), and the distance between *bwb* and *ge* loci is 25.8 cM. (Hiroyoshi, 1977), where the *ge* locus is located proximal to the *bwb* locus and is within one map unit of the *δPGD* locus (Malacrida et al., 1982). Thus, the value of *c'* was estimated to be $(44.6 - 25.8 + 0.1) \times 10^{-2} = 18.9 \times 10^{-2}$. The rate of male to female recombination frequency, *k*, is estimated to be 0.013 for the region of

w-bwb-pw (Inoue et al., 1983). Using the combined data for the samples from Sapporo and its suburbs in 1984 (Table 1), d_0 and d_n were estimated to be 0.51 and 0.50, respectively.

Thus, the value of n can be estimated by the above equation to be 8, assuming that the third chromosomes employed were type 1. This estimated generation number leads to the simple conclusion that the number of generations since immigration of chromosome III^M into the Sapporo population is extremely small.

The most reasonable interpretation of the linkage disequilibrium found in the natural populations in and around Sapporo is that chromosome III^M was monomorphic for the *6PGD*^I allele when some number of these chromosome were introduced into the population, that the frequency of chromosome III^M increased by unknown mechanism and that the linkage disequilibrium has been retained due to the rarity of recombination events. Green (1980) suggested that the various linkage relationships of the *M* factors found in several dipteran species including the housefly are caused by transposition of these factors. If the transposition of the *M* factor, such as from *Y* to the third chromosome, had been recurrent and the frequency of chromosome III^M had increased, isozyme polymorphisms on chromosome III^M should have been detected in present natural populations, especially in populations with a high frequency of chromosome III^M, such as the Sapporo population. Contrary to the above expectation, the chromosome III^M population was monomorphic. Therefore, the *M* factor seems to be stable in the third chromosome in the natural population of Sapporo. In the samples from Obihiro and Akkeshi, the *6PGD* locus on chromosome III^M was polymorphic, as in chromosome III. These results might be due to the input of polymorphic III^M chromosomes into the populations. If the *F* factor exists in natural populations at a high frequency, linkage disequilibria between the *M* factors and the alleles at the other loci on the same chromosomes would gradually disappear by recombination, since the *F* factor is epistatic to the *M* factor. This may be the actual situation in the populations of the Japanese mainland.

In conclusion, it may be said that some number of chromosome III^M including the *6PGD*^I allele recently immigrated into the Sapporo population from the south.

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