**Further studies on five types of inversions in Japanese and African populations of *Drosophila melanogaster*.**

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Many kinds of inversions have been detected on the four major autosome arms in natural populations of *D. melanogaster* through a number of worldwide studies. Usually these naturally occurring inversions are the paracentric type. Most of them are detected only once in a particular population, but some are nearly always found in most populations. In view of the frequency and geographical distribution, Mettler *et al*. (1977) categorized inversions into four classes (*Common Cosmopolitans*, *Rare Cosmopolitans*, *Recurrent endemics*, and *Unique endemics*). In addition, Inoue and Igarashi (l994) added a new class, *Quasi Cosmopolitans*, to the category and a change from *Recurrent endemics* to *Endemics*. *Common Cosmopolitan* inversions are usually maintained in most populations all over the world with higher frequencies, on occasions being more frequent than standard chromosomes, and show frequency clines over large geographical regions. Four inversions, *In(2L)t*, *In(2R)NS*, *In(3L)P*, and *In(3R)P*, are in this category, each of which exists in the right and left arms of two major autosomes. *Rare Cosmopolitan* inversions are also distributed all over the world, but their frequencies are usually low, not enough to show a geographical cline. *Quasi Cosmopolitan* inversions are also widespread in the world, but just enough to say "cosmopolitan" in their distribution. They appeared with very low frequencies and were absent in many populations. *Endemic* inversions are found more than once in a given population but appear in geographically region-limited manner. Occasionally they show higher frequencies than *Rare Cosmopolitans*. *Unique* inversions are usually observed only in a single individual or its brood from a single population and never found in a different population. However, some of *Common Cosmopolitans* showed the tendency to decrease frequencies, and most were sometimes absent in the recent surveys. On the contrary, some *Quasi Cosmopolitans* or *Endemics* are more frequent than *Common Cosmopolitans*. In the present study, we reported the results of recent surveys of eight Japanese and three African populations (Table 1), and of annual surveys of the Osaka population for 10 years (Table 2). Wild caught females were individually transferred to culture vials and allowed to lay eggs. Established strains were kept at 25°C for examination. Inversions were determined through direct observation of the chromosomes of one larva from each female. This method gives two genomes sampled per each female. Cytological analyses were made on salivary gland chromosomes stained with the lactic-acetic orcein method. Breakpoints and nomenclature of inversions were established by comparing with representations of Bridges' standard map (Lefevre, 1976). Frequency of each inversion was calculated per each chromosome arm. Average frequencies of total *Cosmopolitan* inversions per major autosome arm were also calculated to compare the degree of inversion polymorphisms, because significant positive correlations were observed among all four major autosome arms (Inoue *et al*., 1994).

Table 1 shows the frequencies of the *Cosmopolitan* inversions in Japanese and African populations. Four localities with very low frequency were less than 0.100 on an average among five Japanese northern mainland populations (Locality No. 1-5). The highest frequency found in the mainland was the Kanazawa population (No. 3), being 0.115 on an average. In(2R)NS was absent in Katsunuma, and *In(3L)P*, *In(3R)C*, and *In(3R)Mo* were absent or almost absent in populations other

Table 1. Frequencies of Cosmopolitan inversions in Japanese and African populations.

| Locality (Year) | N* | In(2L)t | In(2R)NS | In(3L)P | In(3R)P | In(3R)C | In(3R)Mo | Arm Average ** |
|---|---|---|---|---|---|---|---|---|
| 1. Yamagata (2006) | 200 | 0.010 | 0.005 | 0 | 0.050 | 0 | 0 | 0.016 |
| 2. Fukushima (2009) | 92 | 0.083 | 0.022 | 0 | 0.065 | 0 | 0 | 0.043 |
| 3. Kanazawa (2009) | 200 | 0.155 | 0.060 | 0.010 | 0.180 | 0.005 | 0.050 | 0.115 |
| 4. Shiojiri (2008) | 200 | 0.070 | 0.015 | 0 | 0.085 | 0 | 0 | 0.043 |
| (2009) | 128 | 0.086 | 0.031 | 0 | 0.094 | 0.008 | 0 | 0.055 |
| 5. Katsunuma (2007) | 200 | 0.080 | 0 | 0 | 0.130 | 0 | 0 | 0.053 |
| 6. Tsushima (2008) | 100 | 0.110 | 0 | 0.070 | 0.060 | 0.040 | 0 | 0.070 |
| 7. Amami-Oshima (2006) | 200 | 0.270 | 0.275 | 0.120 | 0.285 | 0.050 | 0.030 | 0.258 |
| 8. Iriomote-jima(2005) | 186 | 0.388 | 0.293 | 0.303 | 0.723 | 0.090 | 0 | 0.450 |
| 9. Uganda (2010) | 158 | 0.133 | 0 | 0 | 0.057 | 0 | 0 | 0.049 |
| 10. Zambia (2010) | 200 | 0.235 | 0.115 | 0.005 | 0.105 | 0.005 | 0 | 0.116 |
| 11. South Africa (2010) | 189 | 0.328 | 0.167 | 0.010 | 0.061 | 0.131 | 0.005 | 0.176 |

* Number of genomes examined

** Average frequency of inversions per major autosome arm

Table 2. Temporal change in frequencies of Cosmopolitan inversions in the Osaka population.

| Year | N* | In(2L)t | In(2R)NS | In(3L)P | In(3R)P | In(3R)C | In(3R)Mo | Average ** |
|---|---|---|---|---|---|---|---|---|
| 1992*** | 240 | 0.175 | 0.096 | 0.054 | 0.096 | 0.067 | 0.046 | 0.134 |
| 1999 | 292 | 0.236 | 0.106 | 0.014 | 0.158 | 0.059 | 0.096 | 0.167 |
| 2000 | 400 | 0.150 | 0.118 | 0.033 | 0.100 | 0.050 | 0.165 | 0.154 |
| 2001 | 400 | 0.170 | 0.048 | 0.043 | 0.118 | 0.053 | 0.043 | 0.119 |
| 2002 | 400 | 0.243 | 0.133 | 0.125 | 0.095 | 0.130 | 0.045 | 0.193 |
| 2003 | 200 | 0.215 | 0.110 | 0.060 | 0.070 | 0.025 | 0.070 | 0.138 |
| 2004 | 200 | 0.275 | 0.080 | 0.050 | 0.080 | 0.020 | 0.055 | 0.140 |
| 2005 | 200 | 0.225 | 0.100 | 0.045 | 0.105 | 0.030 | 0.050 | 0.139 |
| 2006 | 200 | 0.180 | 0.115 | 0.035 | 0.085 | 0.010 | 0.080 | 0.126 |
| 2007 | 200 | 0.220 | 0.150 | 0.035 | 0.080 | 0.075 | 0.025 | 0.146 |
| 2008 | 200 | 0.215 | 0.105 | 0.040 | 0.060 | 0.020 | 0.090 | 0.133 |
| Average | | 0.209 | 0.105 | 0.049 | 0.095 | 0.049 | 0.070 | 0.144 |

* Number of genomes examined

** Average frequency of inversions per major autosome arm

*** Data from Inoue and Igarashi (1994)

than Kanazawa. The small island Tsushima (No. 6) also showed low degree of polymorphisms with absence of *In(2R)NS* and *In(3R)Mo*. Amami-Oshima (No. 7) and Iriomote-jima (No. 8) in the South-west Islands showed significantly higher polymorphisms, with average frequencies of 0.258 and 0.450, respectively. In this region all four Common Cosmopolitans were observed with high frequencies, which were the same results as 1979, 1982, and 1998's surveys in Inoue *et al*. (2002). In the African populations, Uganda (No. 9) showed similar low frequencies to Japanese mainland populations, lacking *In(3L)P*, *In(3R)C*, and *In(3R)Mo*, being 0.049 on an average. The Zambia population (No. 10) had the same level of polymorphisms as Kanazawa with absence of *In(3R)Mo*.

The degree of polymorphisms of the South Africa population (No. 11) was between Kanazawa and Amami-Oshima, being 0.176 on an average.

Table 2 shows the results of annual surveys in each September of 1999-2008. The collection by the banana traps was carried out in the domestic place in Osaka prefecture, the middle region of Japanese mainland. The yearly average frequencies were relatively stable to be 0.144 on a total average, which was more than the case of Kanazawa (No. 3) and less than Amami-Oshima (No. 7) of Table 1. The data of 1992 from the same place showed the similar results. The characteristics of the Osaka population were that all *Common* and *Rare Cosmopolitans* were observed every year.

*Quasi Cosmopolitan*, *Endemic*, and *Unique* inversions observed in the present survey are listed in Table 3 with their breakpoints, localities, and appearance number. The 17 inversions categorized as *Uniques* were observed in the present study, and they have never been described before. In addition, there are several inversions whose categories are intermediate between *Common Cosmopolitans* and *Uniques*. Five *Endemic* and three *Quasi Cosmopolitan* inversions are as follows:

*In(2R)43D;48B* was observed twice in the Osaka population; most probably these inversions are selected out soon after they were born and dismissed by a stochastic process. *In(2R)44C;54F* and *In(3L)62C;67E* were found only in African populations. The latter appeared a total of 29 times and twice in Zambia and South Africa populations, respectively. This inversion is the typical case of temporal increasing pattern of Endemics.

*In(2R)O 51B;55E* was first reported by Inoue *et al*. (2002) from populations of the South-west Island in Japan. The present study showed that it appeared constantly in the populations of the western region of Japan including the South-west Islands. Recently *In(2R)O* was also observed in Hokkaido, the northern part of Japan of 2004, and Hawaiian population of 2005 (Inoue and Watada unpublished data). The naming of "*In(2R)O*" is derived from Rika Ogoshi, one of the authors.

*In(2L)W 28C;32C* was first found in the middle region of Japan of 1972 (Inoue and Watanabe, 1979). Although its frequencies were always low to be a few percent, the distribution covered all over Japan. Inoue and Igarashi (1994) confirmed its distribution at the five localities in Japanese mainland and the South-west Islands, but *In(2L)W* was found only once in the Osaka population in the present study.

*In(3R)K 86F/87A;96F/97A* was distributed in the worldwide scale (*Quasi Cosmopolitan*). As Inoue and Igarashi (l994) summarized, it was found in many places in Africa, some places in the USA, Wales, Greece, and Mexico. In Japan *In(3R)K* was first found in the South-west Islands (Inoue 1992). Inoue and Watada (2006) and the present study confirmed its distribution in Japanese mainland. *In(3R)K* was also found in Korea (Paik, 1998) and in the Hawaii of 2005 and in Taiwan of 2011 (Inoue and Watada unpublished data). Especially Table 3 showed its high level of polymorphisms in Africa, being the frequencies of 0.184 in Uganda, 0.200 in Zambia, and 0.076 in South Africa, respectively. In these three populations, *In(3R)K* prevailed over the *Common cosmopolitans*, *In(3R)P*, in the right arm of the third chromosome.

*In(3L)Y 68F;75C* was first found in a Japanese mainland population in the 1970's (Inoue, 1979). Although its frequencies were always very low, it was present all over Japan in the 1980's (Inoue, 2000). This inversion is also found in regions other than Japan; in Spain (Roca *et al*., 1982) and North Carolina, USA, in 1974 and 1975 (Inoue and Igarashi, l994). However, *In(3L)Y* could not be found in the present study.

*In(3L)M 66C-71B* showed the worldwide distribution in Florida and Texas in USA, Korea, New Guinea, and Egypt (Inoue and Igarashi, l994). In Japan it was found in the Osaka region of the mainland and the South-west Islands in both 1970's and 1980's with low frequencies (Inoue *et al*., 1994). In the present study, *In(3L)M* was found four times in the Osaka population.

The Katsunuma population (No. 5 in Table 1) in the middle of Japanese mainland has been continuously surveyed for chromosomal polymorphisms since the 1 970's (Inoue and Watanabe,

Table 3. *Quasi Cosmopolitan, Endemic* and *Unique* inversions found in the present study.

| Chromosome Arm and breakpoints | Locality and collection year (observed number) |
|---|---|
| Chromosome Arm 2L | |
| W | Osaka 2007 (3) |
| 23E; 27F | Osaka 2007 (1) |
| 22C; 34 | Osaka 2003 (1) |
| 26A; 31F | Katsunuma 2008 (1) |
| 21E; 27C | Fukushima 2009 (1) |
| 26E; 34B | Shiojiri 2009 (1) |
| 25B; 33F | Zambia 2010 (1) |
| Chromosome Arm 2R | |
| O | Osaka 1999 (17), 2000 (8), 2001 (33), 2002 (33), 2003 (21), 2004 (25), 2005 (20), 2006 (11), 2007 (17), 2008 (15), |
| | Amami-Oshima 2006 (26), Iriomote-jima 2005 (8), |
| | Tsushima 2008 (5), Katsunuma 2007 (1), Kanazawa 2009 (5) |
| 43D; 48B | Osaka 2001 (2) |
| 44F; 54B | Osaka 2007 (1) |
| 44F; 55B | Katsunuma 2007 (1) |
| 45A; 53D | Katsunuma 2007 (1) |
| 44F; 56A | Yamagata 2006 (1) |
| 44C; 54F | Zambia 2010 (4), South Africa 2010 (1) |
| 51F; 55D | Zambia 2010 (1) |
| Chromosome Arm 3L | |
| M | Osaka 1999 (1), 2000 (1), 2005 (1), 2008 (3) |
| 70C; 76D | Osaka 2007 (1) |
| 62C; 67E | Zambia 2010 (29), South Africa 2010 (2) |
| 71D; 76B | South Africa 2010 (1) |
| Chromosome Arm 3R | |
| K | Osaka 1999 (6), 2002 (1), 2004 (4), 2005 (3), 2006 (2), 2008 (1), |
| | Amami-Oshima 2006 (1), Shiojiri 2009 (1), Uganda 2010 (29), Zambia 2010 (40), South Africa 2010 (15) |
| 86F; 96A | Osaka 1999 (1) |
| 85D; 92D | Shiojiri 2008 (1) |
| 87F; 96A | Katsunuma 2007 (1) |
| 89E; 93F | Zambia 2010 (1) |

1979). Among four *Common Cosmopolitans*, *In(2L)t*, *In(2R)NS*, and *In(3R)P* showed the stable higher frequencies, and *In(3L)P* was always in lower frequencies than the other three inversions. In the 1980's, *In(2R)NS* and *In(3R)P* began to decrease in their frequencies and were absent in some collections during the period. Moreover the present study showed lower frequencies of *In(2L)t* and *In(3R)P* with absence of *In(2R)NS* and *In(3L)P*. The Korean populations surveyed from 1978 to 1992 showed similar low frequencies of *In(2R)NS*, *In(3L)P*, and *In(3R)P* (Paik, 1998). Instead of the decreases of *Common Cosmopolitans*, some *Endemics* such as *In(2R)O* and *In(3R)K* increased their frequencies and expanded their distribution. In some cases they overwhelmed the frequencies of

*Common cosmopolitans* in a given chromosome arm. Thus, the long-term surveys over several decades reveal the flexible phase of chromosomal polymorphisms in natural populations of *D. melanogaster*.

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## Is *Zaprionus indianus* invading a preserved Amazon forest?

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In June 2010 *Zaprionus indianus* Gupta, 1970 (Diptera: Drosophilidae) was registered for the first time in the Caxiuanã National Forest in Pará, Brazil. Caxiuanã is a very preserved conservation area, localized in the center of endemism Xingu (1º 57' 37" S; 51º 36' 55" W) (Silva *et al*., 2005). The Caxiuanã correspond to 80% of primary forest, with 1,013 species of plants, classified in 189 families. The most extensive and diverse environment is the dense terra-firme forest occupying 85% of the area; the rest is occupied by forests of inundation (várzea and igapó), savanna formation (hydromorphic field), secondary vegetation, and residual vegetation on sites of orchards (Lisboa *et al*., 1997). The human presence is perceived by small patches of antique fields in recovery areas that are generally close to the river banks and were previously occupied by small proprietors and riverside populations whose current occupation is much more restricted (Praxedes and Martins, in press).

Drosophilidae species have been monitored in Caxiuanã National Forest since 2000, with the specific use of traps (Martins *et al*., 2008) baited with banana exposed into the forest. In this collection five individuals of *Zaprionus indianus* were recorded from two points (1° 57' 38.2" S; 51 ° 36' 57.8" W and 1° 59' 42" S; 51° 37' 03.6" W) in one total of 49,139 drosophilids collected which represented 0.012% of all drosophilids. Since in very low abundance, these finds indicated the higher dispersive potential of these species. The next question is if *Zaprinous* will be well established in this forest.

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