The effect of number of founder females on inversion polymorphisms in laboratory populations of *Drosophila melanogaster.*

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Natural populations of *Drosophila melanogaster* are known to be chromosomally polymorphic, the paracentric inversions being the most common. When these populations were transferred to laboratory cages with several thousand flies, all kinds of polymorphic inversions found at high frequencies even more than 50% of average inversion frequencies in nature, were rapidly eliminated in less than three years (Inoue and Watanabe, 1992). The first several generations were the most critical period for the prediction of inversion extinction in the cage. This "cage effect" was observed irrespective of the geographical origin of the population or the initial frequency of each inversion. The decrease and elimination of inversions in the cage was not overcome by changing conditions such as medium, temperature, or the number (40-600) of isofemale lines initially introduced (Inoue, l979). On the other hand, isofemale lines derived from the same geographical origins as the cage populations kept inversion polymorphisms at significantly high levels, though some of the isofemale lines lost inversions. Moreover, inversion polymorphisms were maintained in individual vials of isofemale lines in most cases. Isofemale lines were established by individually transferring the females inseminated in nature to the glass vial cultures (3 cm diameter × 10 cm height), and they were allowed to lay eggs. These lines were maintained by mass transfer of about 30-60 flies every generation. The cage populations established with one or two isofemale lines also maintained the inversion polymorphisms as high as vial cultures (Inoue and Watanabe, 1992). So, the maintenance or elimination of inversions in the laboratory populations may depend on the number of isofemale lines used. In the present study, we examined inversion polymorphisms in the vial culture established with 2-60 isofemale lines, and in the cage populations established with 2-20 isofemale lines. These lines were sampled from Ishigaki-jima of 1976, one of the Japanese South-west Islands, and maintained for 14 years in the laboratory. The laboratory populations started by mixing flies of equal numbers of females and males from each isofemale lines. The laboratory cages were bucket type (22 cm diameter × 20 cm height) with 12 food cups of the standard medium. Three old food cups were replaced with new ones every four days. The number of generations on the cage population was calculated according to Crow and Chung (1967); a period of 15 days at 25ºC was estimated to represent one generation. For cytological analysis, the salivary gland chromosomes from third instar larvae were stained with lactic-acetic orcein. One larva was considered as analysis of two genomes from each sampled females in the experimental populations. All experiments were carried out at 25ºC. The designations of polymorphic inversions followed the nomenclature of Lindley and Grell (l968). The frequency of each inversion was recorded as frequency per chromosome arm, and the average frequency of polymorphic inversions per major autosome arm was also calculated for each sampling.

In the first preliminary experiment, the inversion polymorphisms in each of 35 isofemale vial cultures were examined individually. A total of 59 chromosome arms among 140 arms (35 vial cultures × 4 chromosome arms) were confirmed to be heterozygous for the inversions and standard chromosomes in the beginning of the experiments, and only one arm among them was found to lose the inversion after 24 generations of laboratory maintenance. Almost all arms were found to

maintain inversions in the balanced condition with the standard chromosomes. These results were the same as those of Inoue and Watanabe (1992). In the second preliminary experiment, the four glass bottle cultures (5 cm diameter × 14 cm height) established with one hundred isofemale lines showed significantly lower frequencies than the initial frequencies after 45 generations of laboratory maintenance. The number of flies or space of laboratory maintenance may not suppress the inversion decrease (data not shown).

Table 1. Number of homozygous chromosome arms in vial cultures initiated by mixing 2-60 isofemale lines and maintained for 24 generations in the laboratory; the 16 replication cultures were made for each class*.

Classes for the used line number	2L	2R	3L	3R	Total
2	2	0	0	0	2
5	9	3	2	0	14
10	14	5	3	0	22
20	11	12	5	0	28
40	8	15	6	0	29
60	9	13	10	1	33

*Final homozygous arms were all found to be with the standard gene arrangement.

Table 1 shows inversion polymorphisms after 24 generations of the vial cultures established with 2, 5, 10, 20, 40, and 60 isofemale lines. The left and right arms of the second and third chromosomes were abbreviated as 2L, 2R, 3L, and 3R, respectively. Sixteen replications of vial cultures were made in each class. In the beginning of the experiment, all vial cultures were confirmed to be heterozygous conditions with the inversion and standard arrangement for all four autosome arms. Among all observed 384 chromosome arms (6 classes × 16 cultures × 4 arms), a total of 128 arms were found to be homozygous after 24 generations. The homozygous conditions were decided by the analyses of 20 larvae (40 genomes) per vial culture. The homozygous cases were found to be all with the standard arrangement. The class of two lines vial culture showed two homozygous chromosome arms, and the class of 60 lines vial culture showed 33 homozygous chromosome arms. The number of homozygous chromosome arms increased gradually in relation to the number of the initially used isofemale lines. When the vial cultures were established with a few number of isofemale lines, they seemed to suppress the inversion decrease.

Table 2. Inversion frequencies in cage populations established with 2, 5, 10 and 20 isofemale lines. All cages were maintained for 48 generations in the laboratory. See text for explanation.

Cage Class	Generation	2Lt	2RNS	3LP	3RP	Average
2 lines cage	Initial	0.335	0.450	0.470	0.495	0.438
	Cage A	0.150	0.650	0.475	0.510	0.446
	Cage B	0.075	0.545	0.365	0.405	0.348
5 lines cage	Initial	0.155	0.290	0.290	0.525	0.315
	Cage A	0.025	0.270	0.085	0.320	0.175
	Cage B	0.015	0.250	0.015	0.280	0.140
10 lines cage	Initial	0.255	0.265	0.140	0.425	0.271
	Cage A	0.010	0.180	0.150	0.275	0.154
	Cage B	0.010	0.145	0.065	0.320	0.135
20 lines cage	Initial	0.350	0.230	0.105	0.575	0.315
	Cage A	0.085	0.165	0.010	0.395	0.164
	Cage B	0.070	0.155	0	0.505	0.184

The cage populations established by mixing many isofemale lines lost the inversions, while the cages established by one or two isofemale lines basically maintained inversions throughout generations. We examined frequencies of inversions from the cages established with 2, 5, 10, and 20 isofemale lines and maintained for 48 generations. Each inversion frequency was obtained by the 200 genomes analysis and was calculated per chromosome arm. The results of cage experiments are shown in Table 2. Four inversions, In(2L)t, (In2R)NS, In(3L)P, and In(3R)P, are categorized as Common Cosmopolitans, each of which exists in the right and left arms of two major autosomes, respectively. They are usually maintained in most natural populations all over the world with high frequencies. The inversions of other categories (Rare Cosmopolitans, Quasi Cosmopolitans, Endemics, and Uniques) were generally in much lower frequencies than Common Cosmopolitans (Inoue and Igarashi, 1994). The number of inversions in each population sample was thus represented by frequencies of the four Common Cosmopolitans and their average frequency. Two replication cages (A and B) were made for each class. In two lines, cages of the initial average frequency of 0.438, In(2L)t decreased and instead In(2R)NS increased, and final average frequencies of A and B were 0.446 and 0.348, respectively. Similar results were reported by Inoue and Watanabe (1992), where one of two cages established with two isofemale lines maintained the initial inversion frequency on the average and the other cage showed lower average frequency than the initial. In both cage experiments established with two isofemale lines, some inversions increased or decreased compared with each initial frequency. All four inversions were decreased in both A and B, especially In(2L)t and In(3L)P in the five lines cages of the initial average frequency of 0.315. The final average frequencies of In(2L)t and In(3L)P were 0.175 and 0.140, respectively. In the 10 lines cages of the initial average frequency of 0.271, all cases showed the inversion decreases other than In(3L)P of cage A. The final average frequencies were 0.154 and 0.134, respectively. In the 20 lines cages of the initial average frequency of 0.315, all cases showed the inversion decreases other than In(3R)P of cage B. The final average frequencies of A and B of 20 lines cages were 0.164 and 0.184, respectively. All six cages other than the two lines cages did not suppress the inversion decreases, showing similar final average frequencies ranging between 0.135 and 0.184.

In the present study, the vial cultures established with a few number of isofemale lines kept more heterozygous condition than those established with many number of isofemale lines. In addition, laboratory cage populations started with over five isofemale lines decreased inversion polymorphisms after 24 generations. These results confirmed the importance of initial number of the founder females on inversion polymorphisms in laboratory populations. It is interesting that observation of the cellar habitat population showed lower frequencies of the third chromosome inversions than those of vineyard population outside (Gonzalez and Mensua, 1987). The behavior of inversions in the cellar may be analogous to that of the laboratory populations with the mixture of many isofemale lines. However, a mechanism for high inversion polymorphisms in cage populations established with one or two isofemale lines has not been clear by the studies of inversions only. If one type of inversion, such as In(2L)t, has several different genetic constitutions in a population, one of the mechanisms we supposed is that vial cultures and cage populations established with a few number of isofemale lines can keep more heterozygous condition by over dominance effect without any breakdown of genetic constitution of inversions. On the other hand, laboratory populations established with a large number of isofemale lines will decrease inversion polymorphisms by low fitness of heterogeneous genetic constitution after recombination between the same inversions type with different genetic constitution. The above hypothesis will be confirmed by the combinational studies of inversions and their DNA sequences on effects of initial number of the founder females of inversion polymorphisms in laboratory populations.

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Studies on *Drosophila* biodiversity of Harangi Forest: Coorg District, Karnataka.

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Drosophila has been used as a model organism for research for almost a century. It has richly contributed to our understanding of the pattern of eco-distribution, biodiversity (Guru Prasad *et al.,* 2010), and altitudinal variation (Guru Prasad and Hegde, 2006). The studies have also been made on the population genetics of different species of this genus. The *Drosophilid* family is composed by 65 genera and more than 3500 described species that occur in a number of ecosystems all over the world (Bachli, 1998). However, various studies have been carried out in the laboratory and field by many workers. Though early studies on *Drosophila* in India were mainly concerned with taxonomy, 1970 onwards studies in other fields have also been initiated such as biodiversity. The taxonomical and population genetical studies have progressed little due to lack of interest of people in it. Although many workers feel that taxonomical work shall not be neglected, people show little interest because of the hardship during work and lack of opportunity in the field. To fill up this gap at least partially, we took this work for the study *Drosophila* population and altitudinal variation of *Drosophila* and their species diversity in give Harangi hill. Three months survey was conducted to analyze the altitudinal variation in diversity of *Drosophila* in Harangi hill of Kushalnagar, Coorg district Karnataka state, India.

To study the altitudinal variation of *Drosophila* and their community, the collection was done in the Harangi hill during June-Aug 2009. The Harangi hill is a famous tourist spot with altitude 900 meters, 8 km from the Kushalnagar City, Coorg, Karnataka, India. The altitude of the hill from the foot (base) is 500 meters, the temperature ranges from 16°C to 35°C, and relative humidity varies from 19% to 75%. The collections of flies were made during monsoon season (June to August once in 15 days of the months). For this method flies were collected by using sweeping and bottle trapping methods from the altitudes 500m, 600m, 700m, and 800m (500m base of the hill) such as lower altitude of Harangi hill. 1) Bottle trapping method, 2) Net sweeping method. In bottle trapping method regular banana baits in quarter pint 250 ml milk bottles sprayed with yeast were tied to the twigs of trees at two and half feet above the ground in cool shaded areas covered by scrubs. Next day flies were attracted by the bait and thus the bottles were collected during early morning by plugging with cotton to the mouth of the bottles.

In net sweeping methods rotting fruits are spread usually beneath shaded areas of the bushes of plantation various fruits such as *Musca paradisca* (banana), *Ananas comuses* (pineapple), *Vitas vanifera* (grape), *Artcarpus hetrophylles* (jack fruit), *Pyrus malus* (Apple), *Carica papaya* (papaya), *Arthras* (guava), and *Citrous auranthium* (lime) are mixed and used for spreading. After one day of spreading, the flies are swept using fine net. This is done in all the altitudes, 500m, 600m, and 700m, and 800m height of the hill. The flies are transferred to the bottles containing wheat cream–agar medium and then brought to the laboratory isolated, sexed, and identified according to the Texas Publication 1975 records. Then they were examined under microscopy.