w^+ marked *P* elements which indicate that the *PGawB* insertion tested has not replaced the $lio^{P423.24}$ element. If no [w^-] individual was observed, the *PGawB* strain was considered as a candidate for replacement of the $lio^{P423.24}$. 25 strains showed linkage with lio^1 and were tested by PCR with a GAL4 specific primer and two primers flanking the insertion site of $lio^{P423.24}$. Combination of the GAL4 specific primer with each of the flanking primer allows to assay for replacement of $lio^{P423.24}$ by *PGawB* in one orientation or the other. In 6 cases, no amplification was obtained indicating that the *P* replacement did not succeed. In 19 cases, PCR experiments yielded amplification products indicating that GAL4 sequences were present. This was confirmed by expression pattern (see Table 1). Replacements by *PGawB* were obtained either in the same 5' to 3' orientation as $lio^{P423.24}$ (16 cases) or in the other (3 cases).

References: Brand, A.H., and N. Perrimon 1993, Development 118: 401-415; Callahan, C., M. Muralidhar, S. Lundgren, A. Scully, and J. Thomas 1995, Nature 376:171-174; Dura, J.-M., E. Taillebourg, and T. Préat 1995, FEBS Lett. 370: 250-254; Geyer, P. K., K.L. Richardson, V. Corces, and M.M. Green 1988, PNAS 85: 66455-6459; Gonzy-Tréboul, G., J.-A. Lepesant, and J. Deutsch 1995, Genes Dev. 9: 1137-1148; McCall, K., and W. Bender 1996, EMBO J. 15(3): 569-580; Moreau-Fauvarque, C., E. Taillebourg, E. Boissoneau, J. Mesnard, and J.-M. Dura 1998, MOD 78: 47-61; Peronnet, F., F. San Giorgio, J.-A. Lepesant, J.S. Deutsch, and G. Gonzy-Tréboul 2000, Mol Gen Genet 262: 1123-1131; Sepp, K.J., and V. Auld 1999, Genetics 151: 1093-1101; Taillebourg, E., and J.-M. Dura 1999, PNAS 96: 6856-6861.

Predominance of two colonizing species of *Drosophila* in Ehime Prefecture, Japan.

Watada, Masayoshi,[1] Kazuo Morinaga[1*] and Osamu Ochi[2]. [1]Department of Biology and Earth Sciences, Faculty of Science, Ehime University, Matsuyama, Ehime 790-8577, Japan, and [2]Ehime Women's Junior College, Ibuki-cho, Uwajima 798-0025, Japan. E-mail: watada@sci.ehime-u.ac.jp. *Present address: Division of Water Quality Control, Public Service Bureau of Matsuyama, Mizonobe-cho, Matsuyama 791-0101, Japan.

Introduction

Drosophila simulans, subgenus *Sophophora*, is a sibling species closely related to *D. melanogaster* and distributes throughout the world in association with human habitation. This species, however, was absent from Japan before 1972 except in the Ogasawara (Bonin) Islands, 1000 km south of Tokyo (Okada, 1956; Watanabe and Kawanishi, 1976). The sudden colonization of *D. simulans* in the Japanese mainland was recently reported. In 1976, many individuals of *D. simulans* were collected in the southern and central areas of Japan, but this species was not collected in the intervening area (Watanabe and Kawanishi, 1978). Electrophoretic and morphological analyses suggested that these mainland populations of *D. simulans* shared the same origin, but did not derive from the Ogasawara population (Watada *et al.*, 1986a, b). In addition, Watada *et al.* (1986c) showed that *D. simulans* gradually colonized the intervening area and became abundant in or near the large cities of the area.

Within Japan, some isolated populations of *D. simulans* were found in the Northern (Sapporo, Akita) and Southern areas (Miyakojima). However, *D. simulans* was not always successful in colonization, as shown by Miyakojima (Watada and Itoh, 1999). This species also failed to invade the inland area of Japan. In order for *D. simulans* to expand to such areas of Japan, it must adapt to the climate and niche of new environments and also overcome the competition with other drosophilid species. Invasion of a few individuals does not always result in colonization. Therefore, a long term survey is necessary to determine the expansion and predominance of *D. simulans* to a new environment.

The second species of *Drosophila* to colonize the Japanese mainland is *D. albomicans*, which belongs to the *immigrans* species group of the subgenus *Drosophila*. This species is distributed widely in tropical and subtropical regions in Asia (Kitagawa *et al.*, 1982). In Japan, *D. albomicans* has been reported in the Nansei Islands (Iriomote-jima, Ishigaki-jima, Okinawa, Tokunoshima, and Amami-oshima etc.). However, until recently, despite extensive population surveys on *Drosophila* species, *D. albomicans* has not been recorded from the Japanese mainland (Watanabe and Kawanishi, 1978; Watada *et al.*, 1986c). The northernmost border of its distribution was stated as Amami-Oshima in the Nansei islands (Kitagawa *et al.*, 1982), but in 1982, one of the authors collected many individuals of *D. albomicans* in Yakushima. This result suggested that *D. albomicans* had immigrated into the northern area from the nearest southern island, where *D. albomicans* was already reported. However, the sudden colonization of the Japanese mainland by *D. albomicans* was reported to have occurred on an island of Setonaikai (Inland Sea of Japan), which was far from Yakushima in the Nansei Islands (Asada, 1988; Mikasa, 1991). Studies of chromosomes, allozymes and sex ratio in hybrids of *D. albomicans*, made to identify the origins of the newly colonizing populations of the species in the Setonaikai area, strongly suggested the origins of the Japanese mainland populations to be Taiwan, and not the Nansei islands (Ohsako *et al.*, 1994). After colonization into the Setonaikai area, *D. albomicans* is now spreading throughout western Japan. In 1989, many individuals of this species were reported in Kinki, Chugoku, Shikoku and Kyushu districts (Kitagawa, 1991).

The adaptive strategy of *D. simulans* and *D. albomicans* as newly colonizing species is an interesting evolutionary problem. The sudden colonization of *D. simulans* and *D. albomicans* in Japan is a good example for studying adaptation, interspecific competition and the evolutionary processes of a colonizer in nature. We therefore studied the distribution and seasonal change of the two colonizing species, *D. simulans* and *D. albomicans*, in Ehime, in order to examine the successful colonization and predominance of the these colonizing species in Japan.

Methods

Collections of flies were made in various localities of Ehime in October of 1991 (Figure 1). Ehime prefecture is geographically subdivided into three areas, East Ehime, Middle Ehime and South Ehime. East Ehime includes sample sites 1(Kawanoe) to 7(Omishima), Middle Ehime 8 (Hojyo) to 13 (Kuma) and South Ehime 14 (Ozu) to 21 (Misyo)(see Table 1). Flies near human habitation were collected using banana bait traps, within seven days after trap setting. Several banana traps with baker's yeast were set at each locality. Monthly collections were made in two localities of Matsuyama from April, 1991 to January, 1992. Flies were collected at the end and middle of every month in Matsuyama 1 and Matsuyama 2, respectively. Collected flies were classified under a dissection microscope and grouped into five major species: *D. melanogaster*, *D. simulans*, *D. lutescens*, *D. immigrans*, *D. albomicans*, and others, which included all other drosophilid species. Species grouping

followed Watanabe and Kawanishi (1978). *D. takahashii*, closely related to *D. lutescens*, is here regarded as *D. lutescens*, after Watanabe and Kawanishi (1978).

Results

To facilitate comparison with previous papers, which report the Japanese mainland distribution of the colonizing species of *Drosophila*, we collected flies at various localities in Ehime during October. Table 1 shows the number of drosophilid flies collected at 21 localities in Ehime prefecture in October, 1991. A total of 9371 flies, most of which were domestic or semi-domestic species, was identified as the members of the family *Drosophilidae*. Major five species (*D. melanogster*, *D. simulans*, *D. lutescens*, *D. immigrans* and *D. albomicans*) were collected at all the localities in Ehime, except *D. immigrans* which was absent from Misyo. A further 17 species, described as others in Table 1, are shown in Table 2 with locality number. Typical wild species are not shown in Table 2, since we tried to collect domestic and semi-domestic species of *Drosophila* in the present study. *D. takahashii* and *D. daruma* in this study represent the first record of these species in Ehime prefecture, and *D. takahashii* was collected only in South Ehime (Uwajima and Matsuno).

Table 1. Number of *Drosophila* flies collected at 21 localities in Ehime during October, 1991.

Locality	*simulans*	*melanogaster*	*lutescens*	*immigrans*	*albomicans*	others	total
1. Kawanoe	74	303	121	27	73	16	614
2. Iyomishima	17	78	117	70	202	10	494
3. Niihama	19	199	41	8	17	34	318
4. Saijyo	70	126	20	5	10	12	243
5. Toyo	4	38	57	8	71	16	194
6. Imabari	170	138	22	91	218	56	695
7. Omishima	18	12	13	37	192	35	307
8. Hojyo	63	323	22	38	86	130	662
9. Matsuyama1	61	619	6	6	23	15	730
10. Matsuyama2	47	435	15	5	121	49	672
11. Iyo	12	152	7	35	169	20	395
12. Omogo	6	10	36	26	3	5	86
13. Kuma	124	145	38	106	107	5	525
14. Ozu	23	21	32	25	196	16	313
15. Yawatahama	148	39	7	143	225	2	564
16. Misaki	330	50	47	99	41	1	568
17. Hijikawa	77	28	30	16	153	2	306
18. Hiyoshi	52	95	21	45	47	1	261
19. Matsuno*	51	74	118	7	63	1	314
20. Uwajima*	15	80	70	25	384	8	582
21. Misyo	48	476	1	0	2	1	528
Total	1429	3441	841	822	2403	435	9371

*: *D. lutescens* and *D. takahashii*

Two colonizing species, *D. simulans* and *D. albomicans* were collected at all localities in Ehime. *D. simulans* was numerically the most abundant species in October, followed by *D. albomicans*, *D. melanogaster*, *D. lutescens*, and *D. immigrans*, respectively. As shown in Table 1, the other species comprised only 4.6 percent of the total flies. The two colonizing species, *D. simulans* and *D. albomicans*, comprised over half of the total number of collected flies. This result means that over 50 percent of flies were *D. simulans* and *D. albomicans* in most of the localities in Ehime. In Kuma and Matsuno, these two species respectively comprised 48 and 44 percent of the total flies captured. In Omogo and Misaki, however, these two colonizing species comprised only about 15 percent of all the flies collected.

Both *D. simulans* and *D. albomicans* were the most dominant species in 9 of the 21 localities studied. They were dominant, not only within a particular area but throughout the prefecture. *D.*

Table 2. List of other species of *Drosophila* in Table 1, with the locality numbers.

species name	locality number
D. coracina	3, 5, 7, 8, 10, 12
D. subtilis	8, 10, 11, 17
D. busckii	1, 5, 6, 8, 10-12, 18, 20
D. suzukii	1-8, 10, 12
D. pulchrella	5, 12
D. ficusphila	2, 4 6-8, 10, 11, 14
D. auraria or *D. triauraria*	1-5, 7-10
D. rufa	6, 7, 10, 11, 14, 16, 17, 19, 20
D. virilis	6, 8, 12
D. daruma	4, 7, 11, 20, 21
D. lacertosa	1, 11, 20
D. hydei	1, 3-11, 13-15, 20
D. brachynephros	1, 5, 11
D. nigromaculata	20
D. bizonata	1, 2 0

simulans had a tendency to be abundant in large cities or at localities near trunk roads (Table 1). The two colonizing species were not the most abundant in three localities (Omogo, Misaki and Matsuno) which were inland or relatively far from other large cities. In most cases, the second-most dominant species were these originally present at these sites, such as *D. melanogaster*, *D. lutescens* and *D. immigrans*.

Seasonal collections of *Drosophila* were made in order to study the temporal abundance of the two colonizing species at the two localities in Matsuyama. Matsuyama 1 is a riverside area with a secondary forest, graveyard and a vegetable garden. Table 3 shows monthly collection data of *Drosophila* in Matsuyama 1. A total of 4357 individuals comprising 17 different species, were collected from April 1991 to January 1992. The most abundant species throughout the period was *D. immigrans*, followed by *D. simulans* and *D. albomicans*, which accounted for 39, 37 and 7 percent, respectively. Two colonizing species, *D. simulans* and *D. albomicans*, were second-most and third-most abundant species, but the density of the former species was almost the same as that of the most abundant species.

In the spring collection at Matsuyama 1, *D. immigrans* was the most abundant species, and continued to be so until June, when *D. albomicans* became numerically dominant, and continued through July and August. *D. melanogaster* and the other species were never the most abundant throughout the seasonal collection, but did show a single peak in abundance in September and June, respectively. From September to December, *D. simulans* became the most abundant, and accounted

Table 3. Number of *Drosophila* flies monthly collected at Matsuyama 1.

month	*melanogaster*	*simulans*	*lutescens*	*immigrans*	*albomicans*	others	total
4	2	2	51	367	0	52	474
5	0	1	37	672	0	52	762
6	0	0	10	254	0	107	371
7	28	36	0	61	116	64	305
8	22	47	3	0	108	18	198
9	37	86	0	0	33	16	172
10	61	619	6	6	23	15	730
11	8	565	3	101	33	1	711
12	4	240	13	139	6	8	410
1	0	30	38	117	0	39	224
total	162	1626	161	1717	319	372	4357

Table 4. Number of *Drosophila* flies monthly collected at Matsuyama 2.

month	*melanogaster*	*simulans*	*lutescens*	*immigrans*	*albomicans*	others	total
4	0	0	78	334	0	83	495
5	0	1	6	222	0	472	701
6	1	5	2	169	0	653	830
7	77	91	6	186	26	308	694
8	22	23	1	6	13	41	106
9	18	18	0	1	71	32	140
10	47	435	15	5	121	49	672
11	36	450	4	62	21	7	580
12	1	182	12	163	18	3	379
1	0	3	2	38	0	1	44
total	202	1208	126	1186	270	1649	4641

for more than 75 percent of the total flies collected in October and November. The density of *D. immigrans* also increased from November, and it became the most abundant species during January of 1992. *D. immigrans* was the dominant species from winter to spring, and this numerical change was similar to that of *D. lutescens*, although the amount of *D. lutescens* was 3.7 percent of the total flies collected.

Matsuyama 2 was an area with a citrus orchard and vegetable garden in a semi-urban area, and was surrounded by more domestic environment than Matsuyama 1. A total of 4641 individuals, comprising 15 different species, were collected from April 1991 to January 1992 (Table 4). The seasonal distribution pattern of *Drosophila* species collected at Matsuyama 2 differed from that of Matsuyama 1 mainly in terms of the abundance of the other species. At Matsuyama 1, other species comprised only 9 percent of the total flies collected, whereas at Matsuyama 2, other species comprised 36 percent of the *Drosophila* population. This was mainly due to the high abundance of a single species, *D. hydei*, which is a domestic species, collected from May to July. As at Matsuyama 1, *D. simulans* was the second-most abundant species in the seasonal collection at Matsuyama 2, followed by *D. immigrans* and *D. albomicans*.

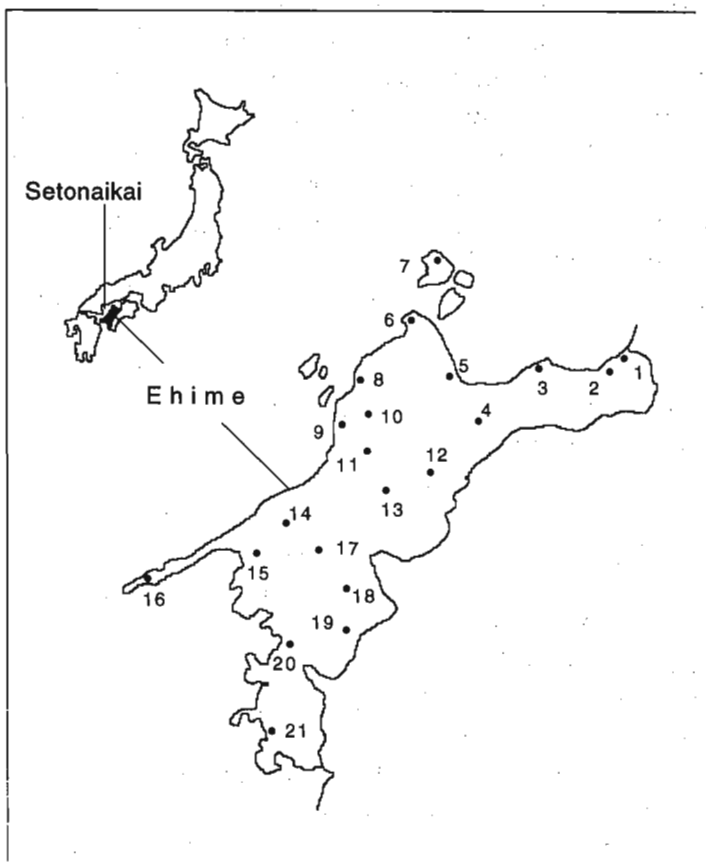

Figure 1. Geographical distribution of sampling localities of *Drosophila* in Ehime. Numbered localities correspond to those in Table 1.

Considering the difference of collection date at Matsuyama 1, the seasonal distribution pattern of drosophilid species abundances at Matsuyama 2 was basically similar to that of Matsuyama 1 except for the abundance of other species. *D. immigrans* was the most abundant in April of 1991 and January of 1992 in Matsuyama 2. *D. albomicans* and *D. melanogaster* had similar abundance pattern with a single peak, but only the former became predominant in September. The abundance pattern of *D. simulans* became bimodal with one peak in August and one in November, although the number of *D. simulans* was much larger in November than in August. *D. simulans* was the most abundant from autumn to winter, and accounted for 65, 78 and 48 percent of the total flies collected in October, November and December, respectively.

Discussion

D. simulans was the most-abundant species in 9 of the 21 collection localities (Table 1). Most of the localities were *D. simulans* abundant sites, except 4 (Omishima, Ozu, Yawatahama and

Hijikawa). *D. albomicans* was also predominant in 9 localities in Ehime, and was abundant in 16 of 21 localities (except Niihama, Saijyo, Omogo, Misaki and Misyo). Only three localities were regarded as being dominated by originally occurring species of *Drosophila* in autumn. Considering the two colonizing species at the same time, within Ehime during autumn they were abundant at all localities. We studied abundance of *D. simulans* and *D. albomicans* only within in Ehime prefecture. However, *D. simulans* is abundantly in the central and western Japanese mainland, and *D. albomicans* is abundant in the Setonaikai area of the western Japanese mainland (Watada *et al.*, 1986c; Shiino, 1993). The two colonizing species have already become numerically abundant in western Japan, as found in Ehime prefecture, and they seem to be spreading to the northern area of Japan.

A comparison of the proportion of collected flies suggested that the expansion of *D. simulans* affected *D. melanogaster* in domestic sites and *D. lutescens* in semi-domestic sites (Watada *et al.*, 1986c). In the seasonal collection at two localities in Matsuyama, the following species basically have a distribution peak in each season: *D. melanogaster* and *D. albomicans* in summer, *D. simulans* in autumn and *D. immigrans* in winter and spring. A similar observation was made by McKenzie and Parsons (1974), who reported that *D. melanogaster* was the most common species during summer, *D. simulans* during autumn and *D. immigrans* during winter in Australia. The seasonal collection data suggested that *D. albomicans* affects the distribution of *D. melanogaster*. However, it should be noted that *D. melanogaster* was only moderately abundant in semi-domestic environments even before the colonization by *D. simulans* (Watada *et al.*, 1986c). The similar distribution peak of *D. melanogaster* and *D. albomicans* in summer probably resulted from the abundance of the two species in domestic and semi-domestic environments, respectively.

Expansion of *D. simulans* affected largely its sibling species, *D. melanogaster*, in semi-domestic environments of Japan. However, laboratory studies have shown *D. simulans* to be weaker than *D. melanogaster* for various physiological characters. For instance, *D. simulans* is less adapted than *D. melanogaster* to both cold and hot temperature stresses (Parsons, 1983). It was difficult to find a condition for which *D. simulans* was superior to *D. melanogaster* (Kawanishi and Watanabe, 1977; McKenzie and Parsons, 1974) and egg to adult developmental time is one of a few physiological characters in which *D. simulans* is superior. Under conditions of low temperature such as autumn, the rapid developmental time of *D. simulans* may be advantageous to compete with other *Drosophila*. A second character of *D. simulans* which may be related to its geographic expansion is a behavioral one. *D. simulans* has a stronger light preference than *D. melanogaster* (Kawanishi and Watanabe, 1978; Fuyama and Watada, 1981). Semi-domestic environments associated with human habitation are usually open field and of a higher light intensity than natural environments. In addition, food preference of *D. simulans* may also play an important role in the rapid expansion of *D. simulans*. Over 400 *D. simulans* and a few *D. lutescens* emerged from a single fruit of the Japanese persimmon from Omiya collection site in 1978 (unpublished). This means that *D. simulans* strongly prefers the Japanese persimmon, which is planted in association with human habitation all over the Japanese mainland. These hypotheses must be tested both in the laboratory and the field.

Although colonization and rapid expansion of *D. simulans* have occurred mainly in suburban areas of Japan, this species has failed to invade inland areas or wild environments (Watanabe *et al.*, 1984; Watada *et al.*, 1986c). However, *D. albomicans* has successfully invaded such localities as Omishima, Ozu and Hijikawa, where *D. simulans* was not abundant (Table 1). The distribution of *Drosophila* was well-studied at Ozu (Watada *et al.*, 1986c). Until recently, *D. simulans* had never been found in Ozu, and never become dominant species to the present time. In contrast, *D. albomicans* easily spread to Ozu and was abundant there. This phenomenon is explained by the observation that *D. albomicans* have been abundant in autumn forests rather than in human habitation in the southern islands of Japan, where *D. albomicans* originally distributes (Watada and Itoh, 1999).

Among the collection localities in Ehime, Omogo has an environment similar to the inland area, but *D. albomicans* was not abundant there because of the relatively cooler conditions than those of other localities. A different situation was found in Misyo, where the sampling site was an urban area around a trunk road, and where over 90 percent of collected flies were *D. simulans* and *D. albomicans* was less than one percent. As a colonizing species, *D. albomicans* seems to adapt to more wild environments than *D. simulans*.

Shiino (1993) found that *D. albomicans* from Japanese mainland populations had acquired resistance to cold temperature as well as those of Taiwan and the Nansei islands populations. This physiological character seems necessary to pass a winter in the Setonaikai area of Japan, and also necessary for immigration into the northern area. Adaptively significant characters appeared to differentiate rapidly among geographical populations of the colonizing species of *Drosophila* (Watada *et al.*, 1986b). Cold resistance of *D. albomicans* seemed to be differentiated extremely rapidly as the species invaded the northern area of Japan. However, for the rapid expansion of the species in the Setonaikai area, the physiological character of cold resistance is not as important as the breeding ability of *D. albomicans* in summer. *D. immigrans* and *D. lutescens* are both semi-domestic or wild species in Ehime, but abundant season of these species was in winter and spring, respectively. There was no strong competitor of *D. albomicans* in semi-domestic area in Ehime during summer, although *D. melanogaster* was dominant at that time in domestic sites.

The seasonal survey of *Drosophila* in the two localities in Matsuyama showed that *D. albomicans* was the most abundant species in August or September. However, this species was dominant in October in 9 localities of Ehime (Table 1). Additional collections of *Drosophila* were made in November of 1991, to survey the abundance of colonizing species in two localities of East and South Ehime (data not shown). Saijyo, Toyo and Uwajima showed similar data with that of Matsuyama 1 and 2 in November of 1991, showing that *D. simulans* was the most abundant and *D. albomicans* was less than 12 percent. However, in November, *D. immigrans* was the dominant species in Ozu, and *D. simulans* and *D. albomicans* comprised 16 and 1.2 percent, respectively. In Ozu, the dominant species changed directly from *D. albomicans* to *D. immigrans*, and *D. simulans* did not become dominant even in autumn. The dominance of *D. albomicans* in autumn seems to occur in localities where *D. simulans* fails to invade and to become abundant, and suggesting that at these localities, strong competition between the two colonizing species did not occur.

D. albomicans was first recorded in Yakushima of the Nansei islands in 1982, two years before it was recorded in the Setonaikai area. The Yakushima population of *D. albomicans* was thought not to be related to the Setonaikai populations. *D. albomicans* was first found in a citrus orchard in Yakushima, agreeing with its documented occurrence near citrus trees in other Nansei Islands. It is interesting to consider the relationship between citrus trees and the abundance of *D. albomicans*, because Ehime is a major producer of Japanese oranges. Citrus trees are planted throughout the prefecture, especially in islands and South Ehime. If *D. albomicans* has a strong food preference for citrus fruits, the rapid expansion of *D. albomicans* in Ehime is easily understood. This preference would also help to avoid severe competition with already occurring species. In addition, there is a possibility, depending on the physiological characters such as cold resistance, that *D. albomicans* will colonize the Tokai district, northmost area of Japan where citrus trees are planted.

We reported predominance and rapid expansion of *D. simulans* and *D. albomicans* in Ehime prefecture. *D. albomicans* invaded the Japanese mainland from the southern area, probably from Taiwan. The invasion from south to north may occur in Ehime prefecture, by the third species, *D. takahashii*, which belongs to *melanogaster* species group of subgenus *Sophophora*. *D. takahashii* is a closely related species to *D. lutescens*, which is one of the originally dominant species on the Japanese mainland. These two species separate their distribution area, *D. lutescens* in northern area and *D. takahashii* in southern area of Japan, at the boundary of 33 degrees North. This boundary

crosses southern Shikoku and has not changed for several decades, suggesting the severe competition between the two closely related species. Prior to the present study, in Shikoku, *D. takahashii* had been reported only from Kochi prefecture, south of Ehime, and had not been recorded in Ehime prefecture. It is extremely difficult to distinguish females of *D. takahashii* and *D. lutescens*, but based on male abundances, these two species occur at a 1:2 ratio in Uwajima and Matsuno, the two localities at which *D. takahashii* has been found in Ehime (Table 1). Further studies on distribution and seasonal change are needed for newly immigrant species of *D. takahashii*, as well as two colonizing species, *D. simulans* and *D. albomicans*, in nature.

Acknowledgments: We are grateful to Drs. T. K. Watanabe and A. Rossiter for critical reading of the manuscript.

References: Asada, N., 1988, Zoological Science 5: 915-918; Fuyama, Y., and M. Watada 1981, Zoological Magazine 90: 62-68; Kawanishi, M., and T. K. Watanabe 1977, Jpn. J. Ecol. 27: 279-283; Kawanishi, M., and W. H. Lee 1978, Jpn. J. Ecol. 28: 231-235; Kitagawa, O., 1991, *Evolution of Populations*. Tokyo University Press, Tokyo (in Japanese); Kitagawa, O., K. I. Wakahama, Y. Fuyama, Y. Shimada, E. Takanashi, M. Hatsumi, M. Uwabo, and Y. Mita 1982, Jpn. J. Genet. 57: 113-141; McKenzie, J. A., and P. A. Parsons 1972, Oecologia 10: 373-388; McKenzie, J. A., and P. A. Parsons 1974, Aust. J. Zool. 22: 175-187; Mikasa, K., 1991, J. Arts Sci. Meikai Univ. 3: 8-18; Okada, T., 1956, *Systematic Study of* Drosophilidae *and Allied Families of Japan*. Gihodo, Tokyo; Ohsako, T., T. Aotsuka, and O. Kitagawa 1994, Jpn. J. Genet. 69: 183-194; Parsons, P. A., 1983, *The Evolutionary Biology of Colonizing Species*. Cambridge Univ. Press, Cambridge; Shiino, T., 1993, *Invasion of the Japan Mainland by* Drosophila albomicans. Ph.D. thesis, Tokyo Metropolitan University; Watada, M., Y. N. Tobari, and S. Ohba 1986a, Jpn. J. Genet. 61: 253-269; Watada, M., S. Ohba, and Y. N. Tobari 1986b, Jpn. J. Genet. 61: 469-480; Watada, M., Y. Inoue, and T. K. Watanabe 1986c, Zoological Science 3: 873-883; Watada, M., and M. Itoh 1999, Dros. Inf. Serv. 82: 50-53; Watanabe, T. K., and M. Kawanishi 1976, Proc. Jpn. Acad. 52: 191-194; Watanabe, T. K., and M. Kawanishi 1978, Zoological Magazine 87: 109-116; Watanabe, T. K., Y. Inoue, and M. Watada 1984, Jpn. J. Genet. 59: 225-235.

Metaphase karyotypes of *Drosophila guayllabambe* and *Drosophila novemaristata* (*repleta* group, *hydei* subgroup).

Morán, I., Tomás,[1] and Ana B. Mafla[2]. Laboratorio de Genética Evolutiva, Departamento de Ciencias Biológicas, Pontificia Universidad Católica del Ecuador. jtmoran@hoy.net; amafla@puceuio.puce.edu.ec

The cytogenetic and molecular analysis can contribute valuable information when species that are closely related are studied, helping thus, toward the knowledge of its phylogenetic relations. Here we present the karyotypes of *D. guayllabambe* (Rafael and Arcos, 1988) and *D. novemaristata* (Dobzhansky and Pavan, 1943), sibling species which have been incorporated in the *hydei* subgroup, *bifurca* complex, based on their morphological characteristics.

D. guayllabambe and *D. novemaristata* are endemic species of the Andes. *D. novemaristata* has been found in four regions of Perú: Ancash, Ayacucho, Lima and Junín (Dobzhansky and Pavan, 1943; Vázquez and Pilares, 1987), while *D. guayllabambe* has been registered in five provinces of Ecuador: Imbabura, Pichincha, Tunguragua, Chimborazo and Loja (Rafael and Arcos, 2000). The two species have never been found to grow together.