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Drosophila Survey of Hokkaido, XXII. Drosophilid Flies Collected in Breweries¹⁾

By

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(With 3 Text-Figures and 4 Tables)

Lately, a rather broad survey of drosophilid flies in Hokkaido has been going on by Momma and his collaborators, with special regard to natural populations under different conditions in different localities (Momma, 1957, 1965; and some others 1952-1966). Since 1959, Kaneko has had a particular interest in the attractiveness of drosophilid flies to different yeasts and fruits (Kaneko, 1960, 1964). It has been generally accepted that species constituting a *Drosophila* population are different in their habitats and nutritions (Carson and Stalker, 1951; da Cunha *et al.* 1957; Suzuki, 1955; and others). Some species live in association with human habitation, barnyard manures, garbages and lumberyards (Hachiya, 1952; Okada, 1957; Ohmori *et al.* 1960). Certain species may be lured and breed in a brewery where certain kinds of yeasts are practically associated with the production of beer, whisky or other wines. We have undertaken *Drosophila* collections in some breweries. The present paper are presented some accounts on collection records and seasonal fluctuations observed so far in two different breweries.

The authors are very grateful to Professor Sajiro Makino who suggested to take up this project. Further cordial thanks are expressed to Dr. Eizi Momma for valuable advice and criticism given in the course of this work.

Method of Collection: Two different breweries were elected on account of their particular environments. One is a beer factory near the center of the City of Sapporo, and another is a whisky factory in Yoichi, a town about 50 km westward from Sapporo.

Collections in the beer factory were made with the use of small containers about 660 ml in capacity, as traps containing fermented banana, during a period from the middle of September to the beginning of November in 1959, and from the end of May to the beginning of November in 1960. Traps were hanged at about 50 cm above the ground by wire strings, outdoor of the brew-houses in the factory. The factory is surrounded with stout

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fence of brick, bordering home districts. Three stations, St. 1, St. 2 and St. 3, were selected in 1959, and an additional station, St. 4, was settled in 1960.

Collections in the whisky factory were made by means of net sweeping, for a period from the end of August to the end of September in 1958, and from the middle of May to the beginning of July in 1959. The factory is surrounded by human habitats around three sides. In the back side, there is an apple-tree garden across the Yoichi River. Flies were collected once a day at seven stations inside a big house in which whisky and apple wine were bottled.

Results and Remarks

Results of the collections are summarized in Table 1. In the whisky factory, Yoichi, a total of 2,756 flies was collected: they were represented by 10 species belonging 2 genera. In 1958, the majority of species collected was represented by *D. funebris* (24.5% of the total), *D. auraria* race A (23.9%), *D. melanogaster* (21.2%) and *D. virilis* (21.0%). In 1959, *D. virilis* (81.5%) was particularly predominant in

Table 1. Collection records of drosophilid flies in two breweries: a whisky factory in Yoichi (Aug. -Sept. in 1958, May-Jul. in 1959), a beer factory in Sapporo (Sept.-Nov. in 1959, May-Nov. in 1960)

Species	Place Method	Whisky factory Sweeping				Total	Beer factory Trapping				Total
		1958		1959			1959		1960		
		♀	♂	♀	♂		♀	♂	♀	♂	
<i>Chymomyza</i>											
<i>nigrimana</i>		—	—	—	—	—	—	—	1	—	1
<i>Scaptomyza</i>											
<i>concinilis</i>		—	—	—	—	—	—	—	1	—	1
<i>polygonia</i>		—	—	—	1	—	—	—	—	—	—
<i>Drosophila</i>											
<i>histrioides</i>		—	—	—	—	—	—	—	1	—	1
<i>busckii</i>		9	15	1	1	26	55	61	251	312	679
<i>bifasciata</i>		—	—	—	—	—	1	—	3	5	9
<i>lutea</i>		—	—	—	—	—	1	—	—	—	1
<i>melanogaster</i>		82	140	22	35	279	55	115	168	153	491
<i>auraria</i> race A		115	135	93	63	406	2	8	54	66	130
<i>auraria</i> race B		—	—	—	—	—	—	—	2	1	3
<i>brachynephros</i>		—	—	—	—	—	—	—	3	1	4
<i>nigromaculata</i>		—	—	—	—	—	7	2	24	14	47
<i>testacea</i>		—	—	2	—	2	3	5	26	21	55
<i>historio</i>		—	—	—	1	1	—	—	—	—	—
<i>funebris</i>		104	205	33	43	385	28	44	216	460	748
<i>immigrans</i>		9	11	—	—	20	17	36	10	11	74
<i>virilis</i>		100	120	700	694	1614	59	71	317	251	698
<i>ezoana</i>		—	—	—	—	—	—	—	1	—	1
<i>lacertosa</i>		—	1	18	3	22	6	12	7	22	47
Total		419	627	869	841	2756	234	354	1083	1319	2990

occurrence and *D. auraria* race A (9.1%), *D. funebris* (4.4%) and *D. melanogaster* (3.3%) followed. Correlation coefficient of the populations between 1958 and 1959 was obtained as +0.923 based on the formula $r = \frac{\sum(x-\bar{x})(y-\bar{y})}{\sqrt{\sum(x-\bar{x})^2 \sum(y-\bar{y})^2}}$ (see Motomura, 1952). From the above correlation coefficient value, it is apparent that constitutions of the populations between the two years are nearly the same.

In the beer factory, a total of 2,990 specimens was obtained which represented 17 species belonging to 3 genera. Dominant species were *D. melanogaster* (28.9% in total specimens), *D. virilis* (22.1%), *D. busckii* (19.7%) and *D. funebris* (12.2%) in 1959. The order of abundant species in 1960 was *D. funebris* (28.1%), *D. virilis* (23.7%), *D. busckii* (23.4%) and *D. melanogaster* (13.4%). The correlation coefficient between 1959 and 1960 was obtained as +0.698.

In order to compare the *Drosophila* population in the whisky factory (W) with that in the beer factory (B), correlation coefficients between them were calculated in relation to season as follows:

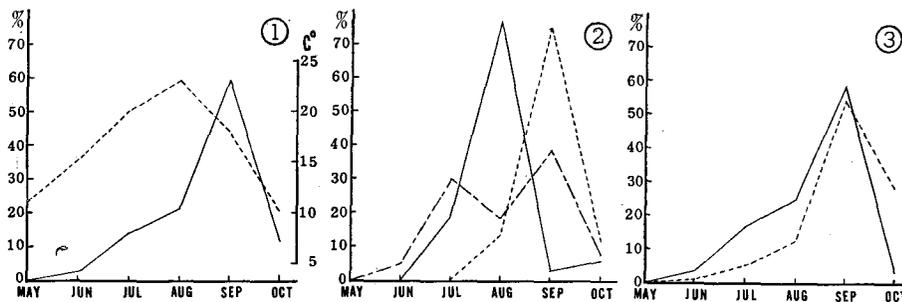
	May - Jul.	Aug. - Sept.	Sept. - Oct.
r	W in 1959 B in 1960	W in 1958 B in 1960	B in 1959 B in 1960
	- 0.073	+ 0.732	+ 0.748

r	W, May - Jul. '59 W, Aug. - Sept. '58	B, Sept. - Oct. '59 B, May - Oct. '60	W, 1958 - 1959 B, 1959 - 1960
	+ 0.923	+ 0.698	+ 0.607

The comparison of the populations between the two breweries during a period from May to July revealed no correlation. It seems likely from the above data that in the whisky factory, *D. virilis* is the most dominant species at that season, while in the beer factory, *D. busckii* and *D. funebris* are predominant in occurrence. The correlation was obtained in a period from August to September. The correlation coefficient of populations was obtained as $r = +0.607$ between the whisky factory in 1958-1959 and the beer factory in 1959-1960. This seems to suggest that the abundant species are approximately the same in the two populations; in all flies pooled at each area, *D. virilis* (58.7%), *D. auraria* race A (14.8), *D. funebris* (14.0) and *D. melanogaster* (10.1) were abundant in the whisky factory, whereas *D. funebris* (25.0), *D. virilis* (23.3), *D. busckii* (22.7), *D. melanogaster* (16.4) and *D. auraria* race A (4.5) were plenty in the beer factory. Two species, *Scaptomyza polygonia*, *Drosophila histrio*, in the whisky factory, and five species, *Chymomyza nigrimana*, *Scaptomyza concimilis*, *Drosophila histrioides*, *D. lutea*, *D. ezoana* in the beer factory, were presented only by single individuals in each area. In spite of different collecting methods, the abundant species in both breweries were so-called "domestic" species associated with human habitation. They might be lured to the breweries from the neighbouring human districts.

The seasonal fluctuation of population was also observed in the beer factory in 1960. The variation was shown by a unimodal curve, and the population-size reached its maximum in September (Fig. 1). Abundant species comprising the population varied in a month (Figs. 2-3). Unimodal curves were represented by *D.*

melanogaster, *D. virilis*, *D. funebris* and *D. auraria* race A. They showed their peaks in September except the last species. Peaks represented by *D. melangoaster* and *D. funebris* in September were reported in U.S.A. by Williams and Miller (1952). Saito *et al.* (1962) described that *D. virilis* showed its seasonal peak in July in Niigata City. It is a well accepted fact that *D. auraria* race A is one of the dominant species in Hokkaido where they distribute over from natural to semi-natural regions in lowlands, and its seasonal peak occurs in summer (Kaneko and Tokumitsu, 1963; Momma, 1964). Only *D. busckii* showed a bimodal curve with peaks in July and September. Williams and Miller (1952) also observed bimodal curves of this species in Nebraska, U.S.A. Wakahama (1961) described that bimodal curves with peaks in spring and autumn were commonly observed in *Drosophila* population in south districts of Honshu.



Figs. 1-3. Graphs showing the monthly mean temperature and seasonal fluctuations of total population-size and of five abundant species observed in a beer factory in Sapporo, 1960. Fig. 1: Dotted line, mean temperature. Solid line, the seasonal fluctuation of total population-size. Fig. 2: Dotted line, *D. melanogaster*. Chain line, *D. busckii*. Solid line, *D. auraria* race A. Fig. 3: Dotted line, *D. virilis*. Solid line, *D. funebris*.

The sex-ratios of the five abundant species were listed in Tables 2 and 3. Percentages of females for each abundant species varied by a year and by a month in two breweries. Two species, *D. funebris* and *D. busckii*, showed male superiority in both breweries, in spite of different collecting methods. The preponderance of males in *D. busckii* was also observed by Mather (1956). In the University Botanical Gardens in Sapporo, the percentage of females of *D. auraria* race A was 48.39 in an average ranging from 44.88 to 55.42 during seven years from 1956 to 1962, and showed relatively high percentages in the beginning and the end of activity seasons (Momma 1964). Sex-ratios of race A obtained in the present survey seem to fall within the range obtained by Momma (1964).

Sample flies collected at each station in the beer factory in the period from September to November are presented in Table 4, in a hope to compare the data between 1959 and 1960. The constitutions of population at each station were

Table 2. Annual percentage frequencies of females in abundant species in two different breweries*

Species	Year	Whisky factory		Beer factory	
		1958	1959	1959	1960
<i>D. busckii</i>		—	—	47.41	44.58
<i>D. melanogaster</i>		36.94	38.60	32.35	52.34
<i>D. auraria</i> race A		46.00	59.62	—	45.00
<i>D. funebris</i>		33.66	43.42	38.89	31.95
<i>D. virilis</i>		45.45	50.22	45.38	55.81

* Female percentages in samples under 50 specimens are omitted.

Table 3. Seasonal percentage of females in five leading species in the beer factory in 1960*

Species	Month	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.
<i>D. busckii</i>		46.88	43.53	49.52	46.54	26.67	20.83
<i>D. melanogaster</i>		—	—	57.14	52.28	47.37	—
<i>D. auraria</i> race A		—	39.13	46.74	—	—	—
<i>D. funebris</i>		47.83	20.83	32.12	33.25	28.57	—
<i>D. virilis</i>		—	55.17	55.56	56.03	55.97	—

* Female percentages in samples under 15 specimens are omitted.

Table 4. Collection records at four different stations in the brewery in Sapporo from September to November

Species	Year	St. 1		St. 2		St. 3		St. 4* 1960	Total	
		1959	1960	1959	1960	1959	1960		1959	1960
<i>Chymomyza nigrimana</i>		—	—	—	—	—	1	—	—	1
<i>Drosophila busckii</i>		23	20	42	3	51	91	142	116	256
<i>bifasciata</i>		—	—	1	—	—	—	—	1	—
<i>lutea</i>		—	—	—	—	1	—	—	1	—
<i>melanogaster</i>		84	31	71	9	15	125	114	170	279
<i>auraria</i> race A		5	2	5	—	—	2	1	10	5
<i>brachynephros</i>		—	1	—	—	—	—	—	—	1
<i>nigromaculata</i>		4	8	4	—	1	1	1	9	10
<i>testacea</i>		—	1	8	2	—	—	1	8	4
<i>funebris</i>		23	3	42	—	7	20	393	72	416
<i>immigrans</i>		8	1	44	—	1	3	11	53	15
<i>virilis</i>		85	219	35	14	10	142	91	130	466
<i>lacertosa</i>		4	5	7	—	7	5	1	18	11
Total		236	291	259	28	93	390	755	588	1464

* This station was taken in 1960.

different between 1959 and 1960. It seems probable that the alteration of the frequency in each year is caused by the change of micro-environments. This evidence is striking at St. 2 and St. 3. Station 2 was wet in 1959 and dry in 1960. On the contrary, the environment of St. 3 showed an inverse change from that of St. 2. Station 1 was very close to the house to wash empty beer barrels returned from various localities. In the light of the above findings, it becomes evident that each species has its specific significant preference for micro-natural biotopes and environments.

In conclusion, it seems possible to conclude that the *Drosophila* populations occurring in the brew-areas consist mostly of domestic species which may originate from human districts and breed there.

Summary

Collections were made in two breweries; a whisky factory in Yoichi, with the use of a net sweeping method, and a beer factory in Sapporo, by means of a trapping method. Dominant species were represented by *D. melanogaster*, *D. auraria* race A, *D. funebris* and *D. vilrilis* in the former, while in the latter *D. busckii* was added to them. The dominant species in each brewery showed more than 90 per cent of the total specimens collected. Based on the correlation coefficient, it is apparent that the constitution of population is approximately the same between the two breweries.

The seasonal variation of the total population-size was studied in the beer factory in 1960. Four of five dominant species showed unimodal curves, and only *D. busckii* was characterized by a bimodal curve.

The sex-ratios of abundant species varied by seasons, years and different breweries. In general the male superiority occurred in *D. busckii* and *D. funebris*.

The distribution of species in the beer factory differed to certain extent at four collecting stations, probably due to the environmental and seasonal changes.

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