CONTRIBUTIONS TO THE EVOLUTIONARY GENETICS OF THE LADY-BEETLE, HARMONIA. II. MICROGEOGRAPHIC VARIATIONS *

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FROM June 1947 to December 1948, HOSINO stayed at Sanagé, a village located about 30 kilometers east of Nagoya, and taught biology in a local high school. In the meantime he succeeded in cultivating interest in the genetics of the lady-beetle among the students, and he organized a group of the students who assisted him in his research works. These boys collected the beetles from various plants in the neighborhood. They also learned to classify the samples according to the elytral pattern, and to do some breeding experiments themselves. Thus extensive materials of this beetle were obtained by HOSINO and these boys in the springs and early summers in 1948 and 1949. In May 1950, both the writers visited the village, and collected the beetles from some of the places where the samples had been obtained in the previous years. The local map of Sanagé and the vicinity is shown in figure 1. Of these places, Area A and Area B are both low hills of the diluvian formation such as commonly found in Middle and West Japan. They are covered by the common pine-trees, *Pinus densiflora,* several meters in height, and among the undergrowths are shrubs like *Eurya japonica, Vaccinium Buergeri, Rhododendron dilatatum, Juniperus rigida, Smilax china* and the fern *Pteridium aquilinum.* Area C is directly continuous to Area B. It is a kind of plateau of which the large part is used for the school ground and experimental farm. It is planted with fruit trees like peaches, pears and plums, as well as with ornamental trees like maples and so on. A part of the ground is used for cultivating wheat. The samples of the lady-beetles used for the present study were obtained mostly from the plants found in these areas. Area D, E and F are all a part of low land scattered with farmers' houses and surrounded by rice fields. A small part of the materials were collected from the plants in these areas.

SAMPLES COLLECTED IN 1948

Table 1 shows the composition of the samples of the beetles collected in 1948. The composition shows a marked difference according to the host plant. This is especially the case in the relative frequency of the type *axyridis,* as well as in the relative frequency of the type *conspicua.* While *axyridis* occupies from 27 to 29 percent of the samples from pine trees, it forms only from 5 to 10 percent of the samples from the wheat as well as the samples

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from the fruit trees. *Conspicua* amounts nearly to 50 percent of the samples from the wheat and fruit trees, but it occupies only 24 percent of the samples from pine trees, thus:

Area	Plant	*Axyridis* %	*Conspicua* %
A	Pine	26.94	24.59
A	Plum	4.71	50.78

Axyridis	Pine - plum	26.94 - 4.71 = 22.23 ± 2.43
Conspicua	Plum - pine	50.78 - 24.59 = 26.19 ± 4.05

Area	Plant	*Axyridis* %	*Conspicua* %
B, C	Pine	28.75	24.18
C	Wheat	10.94	49.26
C	Pear, peach, maple, etc.	10.35	46.27

Axyridis	Pine - wheat	28.75 - 10.94 = 17.81 ± 1.17
	Pine - pear, etc.	28.75 - 10.35 = 18.40 ± 1.28
Conspicua	Wheat - pine	49.26 - 24.18 = 25.08 ± 1.37
	Pear, etc. - pine	46.27 - 24.18 = 22.09 ± 1.62

Less striking but significant difference may be found in the relative frequency of *succinea,* and in the relative frequency of *spectabilis* among the samples collected in Area C, between the samples from the wheat and the samples from fruit trees, thus:

Area	Plant	*Succinea* %	*Spectabilis* %
C	Wheat	17.43	22.08
C	Pear, peach, maple, etc.	25.49	17.74

Succinea	Pear, etc. - wheat	25.49 - 17.43 = 8.06 ± 1.35
Spectabilis	Wheat - pear, etc.	22.08 - 17.74 = 4.34 ± 1.31

SAMPLES COLLECTED IN 1949

A similar tendency may be noticed in the samples collected in 1949 also (table 2). The samples from different kinds of plants show distinct difference in composition which is especially pronounced for the frequency of *axyridis.* This type is relatively far more abundant among the samples from the pine trees than among the samples from other plants. As for *conspicua,* the samples from wheat show a higher relative frequency than the samples from pine trees.

Area	Plant	*Axyridis* %	*Conspicua* %
B, C	Pine	20.27	30.84
C	Wheat	7.28	39.98
C	Pear, etc.	12.64	
C	Soy bean	11.80	

Axyridis	Pine - wheat	20.27 - 7.28 = 12.99 ± 0.59
	Pine - pear, etc.	20.27 - 12.64 = 7.63 ± 0.76
	Pine - soy bean	20.27 - 11.80 = 8.47 ± 1.34
Conspicua	Wheat - pine	39.98 - 30.84 = 9.14 ± 0.83

TABLE 1

The composition of the samples of the beetle collected in 1948 at Sanage and its neighborhood.

Area	Host plant	Succinea	Axyridis	Spectabilis	Conspicua	Other types	Total
B, C	Pine	387 (18.42%)	604 (28.75%)	591 (28.13%)	508 (24.18%)	11 (0.52%)	2101 (100.00%)
A	Pine	99 (17.90%)	149 (26.94%)	162 (29.29%)	136 (24.59%)	7 (1.27%)	553 (99.99%)
C	Wheat	438 (17.43%)	275 (10.94%)	555 (22.08%)	1238 (49.26%)	7 (0.28%)	2513 (99.99%)
A	Plum	69 (36.13%)	9 (4.71%)	16 (8.38%)	97 (50.78%)	0 (0.00%)	191 (100.00%)
C	Pear, peach, maple	362 (25.49%)	147 (10.35%)	252 (17.74%)	657 (46.27%)	2 (0.14%)	1420 (99.99%)
D	Bamboo	24 (25.26%)	16 (16.84%)	16 (16.84%)	38 (40.00%)	1 (1.05%)	95 (99.99%)

SAMPLES COLLECTED IN 1950

The samples collected in May 1950 by the writers assisted by some of HOSINO's former pupils, as well as by the teachers in the school, are much smaller than those in the previous years. Still, they distinctly show the same tendency as the samples of the previous years (table 3). The samples from pine trees contain relatively more *axyridis* and less *conspicua* than the samples from the wheat. Also, the former includes relatively more *spectabilis* than the latter, thus:

Area	Plant	*Axyridis* %	*Spectabilis* %	*Conspicua* %
C	Pine	36.51	41.27	14.29
C	Wheat	8.70	13.77	60.14

Axyridis	Pine − wheat	36.51 − 8.70 = 27.81 ± 6.5
Spectabilis	Pine − wheat	41.27 − 13.77 = 27.50 ± 6.9
Conspicua	Wheat − pine	60.14 − 14.29 = 45.85 ± 6.1

CHANGES IN COMPOSITION FROM YEAR TO YEAR

In looking over tables 1 to 3, we notice some changes in composition of the population of the beetle from year to year. This is especially the case with the populations inhabiting pine trees: Thus, the relative frequency of *axyridis* was 28.4 [1] percent in 1948, it decreased in 1949 to about 20 percent, and increased again in 1950 to nearly 33 percent. The relative frequency of *spectabilis* remained nearly the same in 1948 (ca. 28.3 percent) and in 1949 (ca. 25.6 percent), but it increased significantly in 1950 to 44 percent. The relative frequency of *conspicua* was nearly 24 percent in 1948, and it increased to about 31 percent in 1949, and decreased in 1950 to 12 percent. The rela-

[1] The figures given in this chapter are the averages of all the corresponding values for each kind of plant.

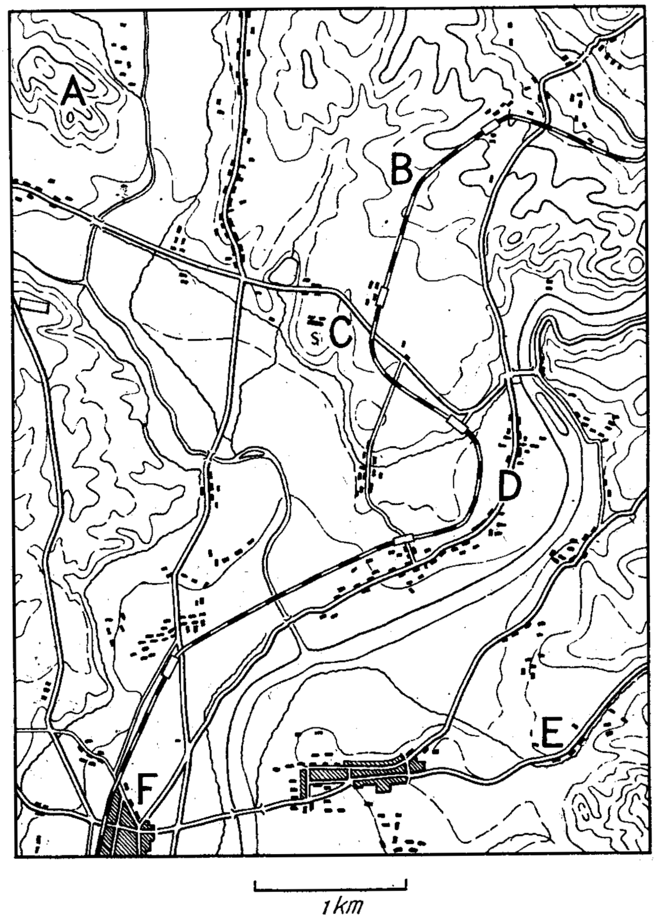

FIGURE 1.—Local map of Sanagé and the neighborhood. S—Sanagé Agricultural High School.

tive frequency of *succinea* increased from 18 percent in 1948 to 23 percent in 1949, and decreased again to about 11 percent in 1950. Similar changes may be found in the population inhabiting the wheat also, for instance, in the relative frequency of *spectabilis*. These changes are graphically shown in figure 2.

VARIATION IN THE FREQUENCY OF ELYTRAL RIDGE

As indicated in our last paper (1950), some individuals of this beetle are provided with a transverse ridge near the distal end of the elytra. The presence or absence of this ridge is due to a single set of Mendelian genes, the gene for its presence being dominant to the gene for its absence. The frequency of this ridge is geographically variable. The frequency among the beetles found in the neighborhood of Sanagé shows a significant difference according to the host plants (table 3). Among the materials obtained in 1950,

TABLE 2

The composition of the samples of the beetle collected in 1949 at Sanagé and its neighborhood.

Area	Host plant	*Succinea*	*Axyridis*	*Spectabilis*	*Conspicua*	Other types	Total
B, C	Pine	1506 (23.09%)	1322 (20.27%)	1669 (25.59%)	2012 (30.84%)	14 (0.21%)	6523 (100.00%)
C	Wheat	1596 (23.62%)	492 (7.28%)	1936 (28.65%)	2702 (39.98%)	32 (0.47%)	6758 (99.99%)
C	Pear, peach, maple	959 (28.93%)	419 (12.64%)	883 (26.63%)	1048 (31.61%)	6 (0.18%)	3315 (99.99%)
C	Soy bean	159 (23.73%)	79 (11.80%)	206 (30.75%)	223 (33.28%)	3 (0.45%)	670 (100.01%)
E	Wheat	26 (22.22%)	11 (9.40%)	38 (32.48%)	42 (35.90%)	0 (0.00%)	117 (100.00%)
F	Maple, Hibiscus	19 (19.79%)	6 (6.25%)	7 (7.29%)	64 (66.67%)	0 (0.00%)	96 (100.00%)

those from the wheat show a much higher frequency of the individuals provided with the ridge than the materials from pine trees. While in the former the individuals provided with the ridge are from 36 to 43 percent of the whole, they form only from 3.7 to 6.4 percent of the latter. Designated by the relative frequency of the recessive gene for the absence of this ridge, the frequency amounts to more than 97 percent of the population on pine trees, it is only about 80 percent of the population on the wheat. The samples of 1948 and 1949 were mixed up, without the frequency of the elytral ridge being examined.

DISCUSSION

First of all, it seems clear from what is given above that the population of this beetle inhabiting pine trees shows a significant difference from the popu-

TABLE 3

The composition of the samples of the beetle collected in 1950 at Sanagé and its neighborhood.

Area	Host plant	*Succinea*	*Axyridis*	*Spectabilis*	*Conspicua*	Elytral ridge+	Total
C	Pine	5 (7.93%)	23 (36.51%)	26 (41.27%)	9 (14.29%)	4 (6.35%)	63 (100.00%)
A	Pine	30 (12.20%)	78 (31.71%)	110 (44.71%)	28 (11.38%)	9 (3.66%)	246 (100.00%)
C	Wheat	24 (17.39%)	12 (8.70%)	19 (13.77%)	83 (60.14%)	59 (42.75%)	138 (100.00%)
F	Wheat	7 (12.50%)	5 (8.93%)	7 (12.50%)	37 (66.07%)	20 (35.71%)	56 (100.00%)

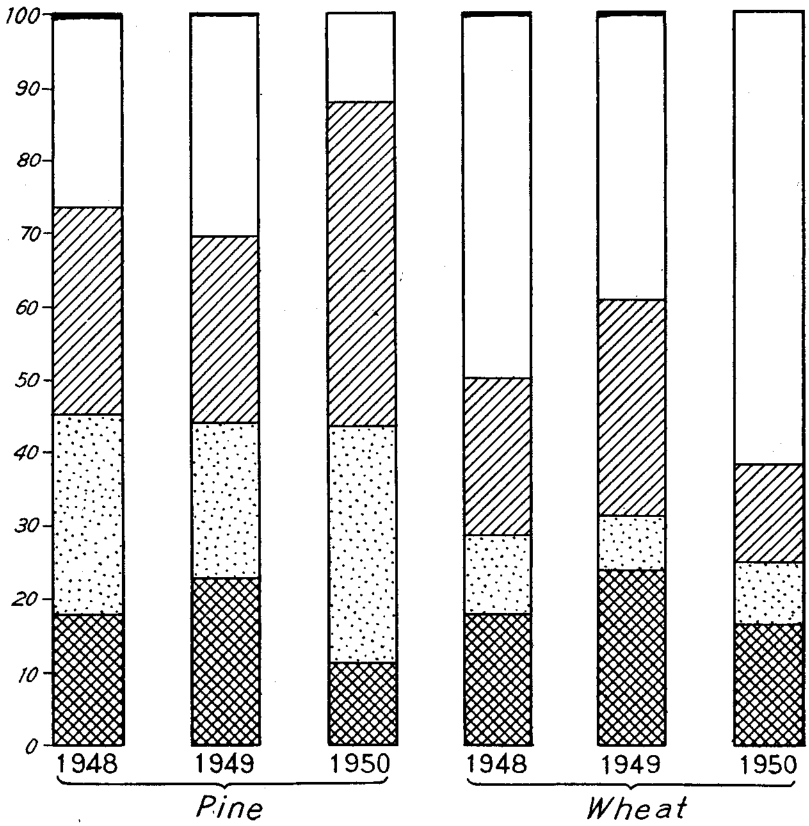

FIGURE 2.—Diagram showing the relative frequency of the elytral pattern types among the samples collected from pine trees and wheat: cross-hatched—*succinea*, stippled—*axyridis*, hatched—*spectabilis*, white—*conspicua*, black (at the end)—other types.

lations found on other plants, wheat and fruit trees. These two populations appear as if they were two distinct local populations geographically very apart from each other. This difference is entirely due to the peculiarity found in the population on pine trees. The populations on other plants, on the contrary, are almost identical in composition with the populations in the neighboring localities. For the sake of comparison, the composition of the samples from Nagoya, Terazu, Gihu and Nakatugawa which are all located within 100 kilometers in diametrical distance from Sanagé, is shown below (cf. KOMAI, CHINO and HOSINO 1950):

Locality	*Succinea* %	*Axyridis* %	*Spectabilis* %	*Conspicua* %	Ridge %
Nakatugawa	21.9	4.0	16.0	58.0	42.5
Gihu	19.0	4.8	11.7	64.4	41.6
Nagoya	26.0	6.7	9.6	57.8	36.3
Terazu	29.8	3.1	15.3	51.9	....
Sanagé (Wheat)	16.0	8.8	13.4	61.9	40.7
" (Pine)	11.3	32.7	44.0	12.0	4.2

In this table the samples taken in 1950 are shown as the representative of the populations in Sanagé, the samples of the previous years being much similar to these samples of 1950. It is clear that, of the samples from Sanagé, the one from the wheat resembles in composition the samples from the neighboring localities, whereas the sample from pine trees is entirely different. The peculiarity of the sample from pine trees is so striking that no sample of this beetle from anywhere else thus far examined, has a similar composition. As pointed out in our previous paper (1950), the relative frequency of *axyridis* is never as high as 10 percent in Japan. So the pine tree population which contains more than 30 percent *axyridis* is quite unique.

Of the elytral ridge, it is present in almost all the beetles collected from the Continent and Hokkaidō; its frequency is nearly 50 percent among the samples from the localities in the northeastern half of Honshū, and the frequency decreases south-westward on Honshū, Sikoku and Kyūshū, but always remaining higher than 10 percent, except in Kōti in Sikoku, where the frequency is 8 percent (KOMAI, CHINO and HOSINO 1950). In the pine-tree sample from Sanagé, however, the frequency is only 4.2 percent. So in this respect also this sample is very peculiar. Thus, the population of this beetle living on the pine trees in Sanagé apparently forms an " island " in the whole range of the distribution of this beetle. Undoubtedly, there must have been some powerful isolating mechanism to bring about such a situation. It is beyond question that this isolate is not due to the presence of some barrier which might hinder the distribution of this beetle, since the host plants of different kinds grow quite close to one another. It is also unlikely that this isolate has developed through the agency of selection, as there seems to be no adaptive significance for the difference in the elytral pattern or for the presence or absence of the elytral ridge. However, the difference in the kind of aphids the beetle feeds on, may somehow come into question. The writers collected in May 1950 from the pine trees and wheat some aphids which were identified by DR. R. TAKAHASHI as follows: from pine trees: *Cinara pineti* Koch, *C. pinidensiflorae* Essig et Kuwana, *C. formosana* Takahashi; from wheat: *Rhopalosiphum prunifoliae* Fitch.

No aphid was collected from fruit trees; undoubtedly, these trees harbored aphids different from those on the pine trees or on wheat. It is also conceivable that ecological conditions other than food aphids peculiar to each kind of plant may have played some role in developing special lady-beetle fauna.

Descriptions of such cases of small isolates found in insects seem to be rather rare in the literature. A case very much similar to the present one is recorded by DOBZHANSKY (1941). He examined in the neighborhood of Kiev several dozen colonies of another kind of variable lady-beetle, *Sospita vigintiguttata,* which has two distinct color types, black and yellow. Most of the colonies " contained black and yellow specimens in ratios approaching equality. One colony contained, however, only blacks and another colony only yellows. This condition persisted for at least three consecutive years " (p. 175). Our colleague DR. M. CHINO remembers that he noticed once about thirty-five years ago in his home in Suwa, Nagano Pref., that, of the three pattern types of *Propylaea japonica*, another variable lady-beetle common in Japan, the intermediate type (*P. japonica* proper) and the whitish type, *P. j. inaequalis*, were commonly found in vegetable gardens, while the melanic type occurred in abundance on plum trees. According to MIYAZAWA and ITO's breeding records on this species (1921), these three color types are undoubtedly due to triple-allelic genes, though these authors do not realize this. CHINO's observation is, however, only a casual one, and no record is preserved. In the well-known chrysomelid, *Gonioctena* (*Phytodecta*) *variabilis*, occurring in Spain, BATESON (1895) noticed the existence of microgeographic variation in the relative frequency of the different pattern-type individuals. He found in the vicinity of Granada rather considerable differences in composition even between the populations of adjacent hill and valley. Ten years later, DONCASTER (1905) visited the same place, and found nearly the same ratio among these pattern types. These types were later demonstrated by ZULUETA (1925, 1929) to be due to multiple-allelic genes. These observations indicate the possibility of the formation of an isolate of a small scale even for insects distributed over a very wide area. The question remains, however, as to whether this kind of variation is to be called "microgeographic" or "ecological."

SUMMARY

1. In this paper the samples of the lady-beetle, *Harmonia axyridis*, obtained from the neighborhood of Sanagé, a village located about 30 kilometers east of Nagoya, are dealt with.

2. Of the materials obtained from this locality, the samples collected from different kinds of plants show a considerable difference in composition among them. The samples from pine trees contain relatively very many *axyridis* and few *conspicua*, as compared with the samples from wheat or the samples from fruit trees. This condition was seen similarly in the collections of the three consecutive years, 1948, 1949 and 1950.

3. The samples from pine trees are also characterized by relatively low frequency of the individuals provided with the elytral ridge as compared with the samples from the wheat or fruit trees.

4. These facts indicate the presence of a small isolate in the range of distribution of this beetle, which perhaps has developed in some relation to the kinds of aphids the beetles feed on.

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