CONTRIBUTIONS TO THE EVOLUTIONARY GENETICS OF THE LADY-BEETLE, HARMONIA. I. GEOGRAPHIC AND TEMPORAL VARIATIONS IN THE RELATIVE FREQUENCIES OF THE ELYTRAL PATTERN TYPES AND IN THE FREQUENCY OF ELYTRAL RIDGE*

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Received February 15, 1950

REVIEW OF GENETICS OF ELYTRAL PATTERN IN LADY-BEETLES

THE lady-beetles have been used by many workers as materials for genetic study, because of the high variability of their elytral and pronotal patterns. The previous papers on this subject were thoroughly reviewed by SHULL (1943). The differences in these markings in all cases so far worked out are due to multiple allelic genes. The expression of these genes is much dependent on external conditions to which the larva and pupa are subjected, high temperature and low humidity tending to decrease the extension of melanic pigment, especially tn the elytra, while low temperature and high humidity tend to increase it (JOHNSON 1910, ZIMMERMANN 1931, HOSINO 1942). Besides, even differences in details in size, shape and interrelation of individual spots are often under control of genic activity. These genes may belong to the same allelic series as the genes determining the major pattern types, or they may be entirely different ones (JOHNSON 1910, ZARAPKIN and TIMOFFÉEF-RESSOVSKY 1932, TENENBAUM 1933, HOSINO 1940–1943, SHULL 1944, 1945, 1948, 1949, TAN 1949). And for such variations, the presence of a directive tendency is often maintained (KELLOGG and BELL 1904, JOHNSON 1910, ZARAPKIN 1930, 1931, 1933, 1938, MODEREGGER 1933). The relative frequency of the genes controlling the elytral pattern, as well as the frequency of the genes governing their minute details, is geographically variable (JOHNSON 1910, DOBZHANSKY 1924, 1933, 1941, DOBZHANSKY and SIVERTZEW-DOBZHANSKY 1928, TIMOFFÉEF-RESSOVSKY 1932, KURISAKI 1927, ZIMMERMANN 1931, ZARAPKIN 1938, KOMAI, CHINO and HOSINO 1946, 1948, SHULL 1949). Furthermore, it has been demonstrated in some cases that such frequency shows seasonal fluctuations (TIMOFFÉEF-RESSOVSKY 1939, 1940, TAN 1949).

GENETICS OF ELYTRAL PATTERN IN *Harmonia*

HOSINO has been working on the genetics of this variable lady-beetle since 1933, and published his findings in his serial papers in the Japanese Journal of Genetics (1936–1948) and also in the Journal of Genetics (1940a). He held the view from the beginning that all differences among the major elytral pattern types are due to genes belonging to the same allelic series. TAN and

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LI (1934), who studied the same problem on Chinese material, once expressed the opinion that these patterns were governed by several different genes. In more recent papers, however, TAN (1946, 1949) admits the validity of the view of multiple allelism. HOSINO has further confirmed that even small variations within each major type, such as difference in size and shape, mode of appearance or disappearance, fusion or separation of individual spots, often have genetic bases. Thus, he has been able to distinguish the following numbers of subtypes within each major type:—*succinea*—10, *axyridis*—6, *aulica*—2, *conspicua*—4. All these differences are to be assigned to different alleles of one and the same locus. TAN also has arrived at practically identical results in his study on the Chinese brood of the same species, and he distinguishes the following subtypes:—*conspicua*—2, *spectabils*—2, *transversifacsia*—2. These two authors' classifications apparently have some coincidence. But, as far as can be judged from TAN's figures, the light areas in the elytral markings in most Chinese samples look considerably larger than in Japanese samples of the corresponding types. This difference probably has some relation to the difference in climatic conditions of the two countries.

GEOGRAPHIC VARIATION IN THE RELATIVE FREQUENCIES OF ELYTRAL PATTERN TYPES

DOBZHANSKY (1924, 1941) found very striking geographic variation in the relative frequency of the elytral pattern types of this lady-beetle collected at various localities in the Asiatic Continent and in Japan. His generalization on this subject is that "West-central Siberia (Altai, Yeniseisk) is occupied by a race manifesting nearly always the pattern *axyridis*. In central Siberia the yellow forms appear and rapidly displace *axyridis*, which on the Pacific Coast of Siberia and in China is very rare or is absent. *Spectabilis* and *conspicua* are found in the Far East only, the latter apparently reaching a high frequency in Japan. *Aulica* is nowhere frequent, but is found almost everywhere in the Far East" (DOBZHANSKY 1941, p. 69). Previous to DOBZHANSKY'S study, CHINO and KOMAI compared their specimens of this species collected at two localities in Japan, Suwa in Nagano Prefecture and Tondabayasi near Osaka, and recognized a significant difference in the composition of the two samples (CHINO 1912). This material was supplemented by further collections of the beetles from the same localities, and also by those from several other localities (CHINO 1918). In 1927, KURISAKI carried out a similar study on the materials collected at Sapporo in Hokkaido, Tokyo, Gihu(Gifu) and Okayama in Honsyu, Matuyama in Sikoku, and at Hukuoka (Fukuoka) and Miyazaki in Kyusyu, and also Suigen in Korea. He found considerable variations among these materials. He noticed a general tendency in these variations that the light colored type, *succinea*, is relatively more abundant in the northern localities (Hokkaido and Korea), whereas the dark-colored types, *conspicua* and *spectabilis*, are relatively commoner in the southern localities (Sikoku and Kyusyu). This tendency, he remarks, is in reverse to the tendency observed in pupal coloration which becomes darker under low temperature.

TABLE 1

Percentages of different pattern individuals, and also percentage of the individuals provided with elytral ridge, among the materials from various localities. When more than two data are available from the same locality, the most recent datum is shown.

LOCALITY	YEAR	*succinea*	*axyridis*	*spectabilis*	*conspicua*	ELYTRAL RIDGE	TOTAL
Sapporo	'43, '44	42.9	1.0	21.6	34.3	99.5	1,184
Simamatu	'44	43.1	0.0	26.4	30.6	100.0	72
Akita	'44	60.0	2.2	9.6	28.2	42.4	135
Yamagata	'44, '45	34.0	5.9	10.0	50.2	53.8	253
Nikko	'14	47.3	3.5	8.2	41.0	—	451
Tutiura	'14	52.4	3.9	8.7	35.1	—	231
Takasino	'45	36.9	3.5	11.8	47.7	58.6	5,758
Tokyo	'42	34.3	3.9	7.3	54.5	55.6	178
Suwa	'42, '43	32.0	5.0	13.0	49.8	53.2	823
Matumoto	'13, '14	41.2	4.8	12.3	41.6	—	693
Nakatugawa	'16	30.3	5.9	16.4	47.3	—	152
Gihu	'40	19.0	4.8	11.7	64.4	41.6	272
Sanagé	'48, '49	23.1	14.5	25.9	36.2	34.7	24,443
Nagoya	'40	26.0	6.7	9.6	57.8	36.3	135
Terazu	'46	29.8	3.1	15.3	51.9	—	131
Kyoto	'40–'43	15.3	5.1	15.8	63.7	24.4	2,494
Osaka	'39	16.7	3.3	13.8	66.1	24.9	181
Tondabayasi	'43	16.0	5.7	10.3	68.0	23.8	194
Amagasaki	'40	13.7	3.4	10.9	71.9	20.5	386
Akasi	'44	13.3	5.4	15.0	66.3	18.8	240
Okayama*	'25	10.5	—	—	—	24.0	200
Kurasiki	'47	12.7	11.4	19.0	56.7	—	79
Onomiti	'44	12.3	4.1	13.7	69.9	18.8	219
Hirosima	'44	4.3	2.2	26.1	67.4	10.9	46
Matuyama	'44	10.7	5.8	19.1	64.1	11.2	534
Koti	'44, '45	9.4	8.6	20.1	61.9	8.0	673
Hukuoka	'44	2.3	2.2	11.1	83.6	12.1	995
Miyazaki*	'25	—	—	—	—	17.4	350
Suigen*	'25	85.8	—	—	—	97.0	402
Mukden	'44	90.7	0.0	4.5	4.6	96.5	1,865
Chihfeng	'44	82.4	0.0	12.1	4.4	98.9	91
Peiping†	?	83.3	0.0	8.9	7.3	—	9,635

In cases when the total percentage is smaller than 100, the deficient fraction is occupied by *aulica* and other rare types.

* KURISAKI's data.

† TAN & LI's data.

Recently, we obtained by the kindness of our friend biologists rather extensive materials of this beetle from various localities in Japan as well as in Manchuria. These specimens were collected mainly in the active season of this beetle from late spring to early summer. Only the materials from Suwa, Kyoto, Takasino in Japan, and those from Chihfeng and Mukden in Manchuria are hibernating groups. TAN (1949) states that he has observed in a population of this species near Hanchow a rather considerable seasonal change in its composition. We have not noticed any such change so far in Japanese broods. If it is ascertained that such striking seasonal change as that found in the Chinese brood actually occurs in Japanese broods, our findings may require re-examination by taking this fact in consideration. Our findings on these materials are summarized in table 1, which includes KURISAKI'S, and TAN and LI'S data as well.

In this table it is shown that throughout Japan *succinea* (in which all forms with light-colored elytra are included) never exceeds 50 percent, and also

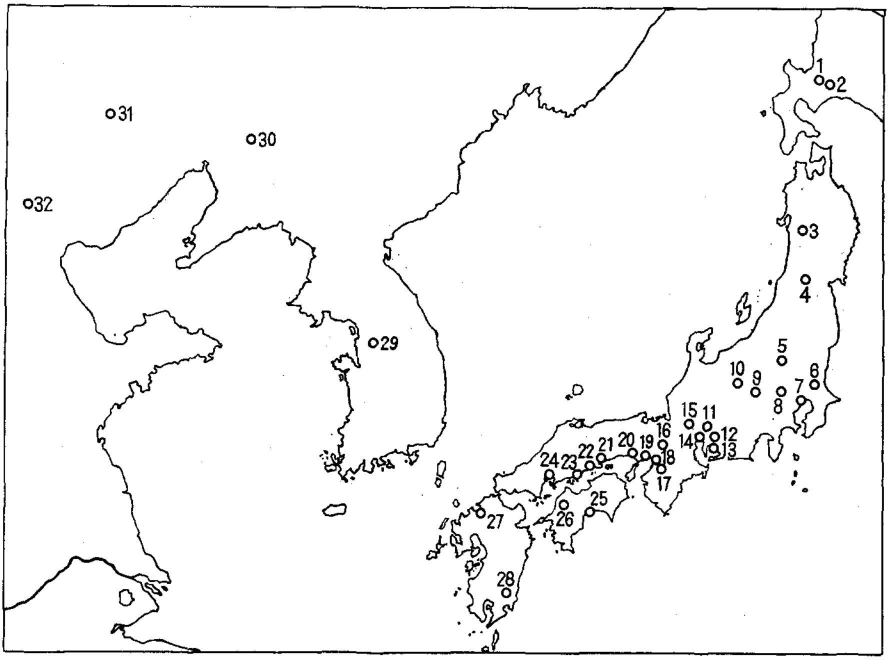

FIGURE 1.—The localities where the samples were collected: 1. Sapporo, 2. Simamatu, 3. Akita, 4. Yamagata, 5. Nikko, 6. Tutiura, 7. Tokyo, 8. Takasino, 9. Suwa, 10. Matumoto, 11. Nakatugawa, 12. Sanagé, 13. Terazu, 14. Nagoya, 15. Gihu, 16. Kyoto, 17. Tondabayasi, 18. Osaka, 19. Amagasaki, 20. Akasi, 21. Okayama, 22. Kurasiki, 23. Onomiti, 24. Hirosima, 25. Koti, 26. Matuyama, 27. Hukuoka, 28. Miyazaki, 29. Suigen, 30. Mukden, 31. Chihfeng, 32. Peiping.

that there is a gradual decrease in the relative frequency of *succinea* along the Japanese island chain from north-east to south-west. Thus, in Hokkaido the percentage of *succinea* exceeds 40, it decreases to 30–40 percent in Kanto District including Tokyo, and in Nagoya-Gihu District it comes down to 20–30 percent; in Kyoto-Osaka District the percentage becomes 15±, and in Tyugoku District (Okayama-Kurasiki-Onomiti-Hirosima) it is 10 percent; about the same frequency is found in Sikoku, whereas in Hukuoka in Kyusyu the frequency is as low as 2.3 percent. But, when one leaves the Japanese islands and goes across the strait to the Korean Peninsula, the percentage of *succinea* suddenly rises to 80+ and this high frequency is maintained through the localities in Manchuria to North China (fig. 1).

The corresponding gradient, though less marked, may be recognized in the relative frequencies of *spectabilis* and *conspicua*. Thus, *spectabilis* occupies more than 20 percent of the Hokkaido populations; it is about 10 percent of Tohoku (Akita-Yamagata) and Kanto populations; it is 10–15 percent of Aiti (Nagoya and its vicinity) and Kyoto-Osaka populations, while in Tyugoku and Sikoku Districts it is about 20 percent, and the frequency decreases again to about 10 percent in the continent. Next, as for *conspicua*, 30 percent of the specimens of this species from Hokkaido have this pattern; this percentage is exceeded by the specimens from Nagoya-Gihu District; the percentage still rises in Kyoto-Osaka District to nearly 70. This frequency is maintained throughout Tyugoku and Sikoku, and the frequency attains its highest value, 80 percent in Kyusyu. On the continent *conspicua* is rare, its frequency is lower than 10 percent of the whole population. Designated by the incidence of the recessive gene for *succinea*, this gene is present in 65 percent of the gametes in the Hokkaido population; the value decreases in Kanto to 55–60 percent; it is 45–55 percent in Nagoya-Gihu, 40 percent in Kyoto-Osaka, 30 percent in Tyugoku and Sikoku, and in Kyusyu it is lower than 20 percent, whereas on the continent nearly 95 percent of all the elytral pattern genes are of *succinea*.

As for the type *axyridis*, such geographic variation is not found, its frequency remains uniformly at 5± percent throughout Japan. It may be noticed that this type is almost entirely missing from the Hokkaido populations, as well as from the continental populations. Another peculiar population is that of Sanagé, a village located about 30 kilometers east of Nagoya, which includes unusually many *axyridis* individuals. This population will be more crucially examined in another paper.

GEOGRAPHIC VARIATION IN THE FREQUENCY OF ELYTRAL RIDGE

Some individuals of this species have near the end of each elytron a small transverse ridge which can be recognized with a hand lens. This ridge is not found in other individuals, and its presence or absence is a clear-cut distinction. This fact was first noticed by KURISAKI (1927), who also has found that the frequency of the individuals having this ridge is geographically variable like the relative frequency of elytral patterns. Later, HOSINO (1936) confirmed that the presence or absence of this ridge is inherited on a monogenic basis, the gene for its presence being dominant over the gene for its absence. This gene is inherited independently of the gene controlling the elytral pattern. We have examined our materials in reference to this structural character also, and obtained the results presented in table 1 under the heading of "elytral ridge." One can see in this table that the beetles from Hokkaido are provided with this ridge almost without exception; the ridge is present in more than half of the individuals coming from the northern half of Honsyu, and in about 40 percent of those from Nagoya-Gihu District. The frequency comes down to 20–25 percent near Kyoto and Osaka, and goes on decreasing westward to become 10± in Sikoku and Kyusyu; but it suddenly rises to almost 100 percent in Korea, Manchuria and Jehol. Thus, we find in the frequency of the gene controlling this character, a geographic gradient parallel with the gradient in the relative frequency of elytral pattern genes. Designated by the frequency of the recessive gene for the absence of this ridge, less than

20 percent of the gametes of the individuals from Hokkaido carry this gene; the frequency increases to 70 percent in the northern districts of Honsyu, and goes on increasing westward to become 80 percent in Nagoya-Gihu District, nearly 90 percent in Kyoto-Osaka District and 95 percent in Sikoku and Kyusyu Districts.

TEMPORAL VARIATION IN THE RELATIVE FREQUENCIES OF ELYTRAL PATTERN TYPES

We have also some data on the relative frequencies of the elytral pattern types in samples collected at the same locality on different occasions between which there is sometimes a 15–30 year interval. These data are presented in table 2. Of these, the samples from Tondabayasi were collected by KOMAI, first in June 1911 and June 1913 and later in 1943 from wheat fields near the town. These samples indicate that there has been scarcely any change in this 30-year period in the composition of the population of this beetle at this locality. Next, the populations occurring near Tokyo were examined five times in the 28 years beginning from 1914. They were from different places in the northern suburbs of the city, a few kilometers apart from one another. These samples do not show any significant difference from one another, except the sample of 1917. This sample and also the one of 1914 were collected by KOMAI from wheat fields at Zyuzyo and at Sugamo respectively, which were then in the suburb of the city of Tokyo, and only 3 kilometers apart from each other. The sample of Sugamo is in composition much like the samples from other places near Tokyo. The sample of Zyuzyo, on the other hand, is somewhat peculiar in having more *spectabilis* and less *conspicua* than that of Sugamo, and the difference is statistically significant, thus:—

	SUGAMO SAMPLE	ZYUZYO SAMPLE	DIFFERENCE
Spectabilis	9.6±1.7%	23.1±3.9%	13.5±4.25%
Conspicua	52.1±2.9%	30.8±4.3%	21.3±5.14%

This, perhaps, is to be taken as a case of microgeographic variation rather than of temporal variation.

Next, both the samples of Matuyama and the samples of Hukuoka have a 19 year interval between 1925 and 1944 when they were collected. The composition of the earlier 1925 samples is not given besides the percentages of *succinea*. However, as far as it can be judged from these percentages as compared with the corresponding percentages in the samples of 1944, the populations of these two localities do not seem to have changed significantly in this period. The same is apparently true of the population of Gihu, because the difference between the samples of 1925 and 1940 from there is insufficient for being statistically significant.

In contrast to these samples, the samples of Sapporo collected in 1923 and in 1943–1944 respectively, show a sharp distinction in the relative frequency of *succinea*. While this type occupied 83.9 percent of the 1923 sample, it makes only 42.9 percent of the 1943–1944 sample. The former sample was examined by KURISAKI who gives the figure for *succinea* only. However, since he is a specialist on the taxonomy of Coccinellidae, there is no room for doubting

TABLE 2

*Temporal variation in the relative frequency of different pattern types and in the frequency of elytral ridge.**

LOCALITY	YEAR	*succinea*	*axyridis*	*spectabilis*	*conspicua*	ELYTRAL RIDGE	TOTAL
Sapporo	'23*	83.9	—	—	—	99.5	398
	'44	42.9	1.0	21.6	34.3	99.5	1,184
Suwa	'12, '13	42.6	4.6	9.5	42.3	—	2,005
	'14	41.7	5.6	10.9	41.8	—	1,413
	'15, '17	43.4	4.8	10.7	41.1	—	2,059
	'20	42.4	4.4	10.6	42.4	—	4,512
	'30	37.5	3.9	10.2	48.4	—	13,157
	'42, '43	32.0	5.0	13.0	49.8	53.2	823
Tokyo	'14	36.0	2.3	9.6	52.1	—	303
	'17	45.3	0.9	23.1	30.8	—	117
	'24	45.2	—	—	—	58.0	200
	'30, '31	39.6	3.7	11.0	45.8	52.0	2,283
	'42	34.3	3.9	7.3	54.5	55.6	178
Gihu	'23*	26.4	—	—	—	49.0	239
	'40	19.0	4.8	11.7	64.4	41.6	272
Tondabayasi	'11, '12	11.9	6.2	10.6	71.3	—	672
	'43	16.0	5.7	10.3	68.0	23.8	194
Matuyama	'25*	17.1	—	—	—	4.0	158
	'44	10.7	5.8	19.1	64.1	11.2	534
Hukuoka	'25*	3.1	—	—	—	25.0	229
	'44	2.3	2.2	11.1	83.6	12.1	995

* KURISAKI's data.

the accuracy of his classification of the four types in this species. Thus, it seems safe to conclude that there has been in these 20 years a significant change in the composition of the population of this beetle inhabiting Sapporo. This change implies a corresponding change in the frequency of the recessive gene for *succinea*, which decreased from circa 90 percent to 65 percent.

It is to be noticed that this figure for *succinea* in the older Sapporo sample resembles the one in the samples of Korea and Manchuria of the more recent date. The similarity of the fauna of Hokkaido to the fauna of the Asiatic Continent is known for various groups of land animals, and the Strait of Tugaru which separates Hokkaido from Honsyu is universally recognized as a zoogeographically important demarcation under the name of "Blakinston's Line." The composition of the recent sample of Sapporo, on the other hand, resembles the composition of the samples of the northern districts of Honsyū. The small sample of Simamatu, a village located about 20 kilometers southeast of Sapporo collected in 1944, is in composition nearly identical to the Sapporo sample in 1943–1944. When, however, these recent Hokkaido samples are more crucially examined, a remnant of their continental character becomes apparent, which is shown by the scarcity of *axyridis* and by the relatively high percentage of *spectabilis*.

A more distinct temporal change in the composition of population may be found in the six samples of hibernating colonies collected at Suwa by CHINO in years ranging from 1912 to 1943. As shown in table 2, the population of this beetle in this locality has undergone hardly any change from 1912 to 1920. In the succeeding 10 years, however, it has changed somewhat, namely, the relative frequency of *succinea* has decreased, and that of *conspicua* has increased to a degree statistically significant in either case. This tendency persisted in the next 12 years, from 1930 to 1942 or 1943. During this period *succinea* lost 5.55±1.68 percent, while *conspicua* gained 1.47±1.80 percent; and of these changes that of *succinea* is statistically significant, thus:—

TYPE	YEAR			DIFFERENCE
	1912–1920 (A)	1930 (B)	1942, 1943 (C)	
Succinea	42.66±0.49%	37.51±0.43%	31.96±1.62%	$A-B=5.15\pm0.65\%$ $B-C=5.55\pm1.68\%$
Conspicua	42.04±0.49%	48.35±0.42%	49.82±1.74%	$B-A=6.31\pm0.66\%$ $C-B=1.47\pm1.80\%$

This change in the population of Suwa may be understood to mean that this population which formerly resembled the populations of Kanto District, has come in these 20 years nearer the populations of the Nagoya-Gihu District. Designated by the frequency of the gene for *succinea*, this change corresponds with circa 10 percent decrease of the gene.

TEMPORAL VARIATION IN THE FREQUENCY OF ELYTRAL RIDGE

A temporal variation similar to that found in the elytral pattern may be seen in the frequency of the elytral ridge as well. Thus, of the specimens from Hukuoka, 25 percent possessed this ridge in 1925, but it is found in only 12 percent of the specimens of 1944, and the difference is statistically significant. Among the specimens from Matuyama, on the other hand, the individuals provided with this ridge have increased in the same period from 4.0 percent to 11.2 percent, and the difference is also statistically significant, thus:

LOCALITY	YEAR		DIFFERENCE
	1925	1944	
Hukuoka	25.0±2.86%	12.1±1.72%	25.0−12.1=12.9±3.40%
Matuyama	4.0±1.55%	11.2±1.37%	11.2− 4.0= 7.2±2.06%

Designated by the frequency of the recessive gene for the absence of the ridge, the change in the Hukuoka population corresponds to about 7 percent decrease, and the change in the Matuyama population corresponds to about 5 percent increase.

The population of Gihu likewise appears to have changed somewhat in 17 years, but the difference is statistically insignificant. Such a change can not be recognized in other populations presented in the table. It may be added that, for all the older samples, KURISAKI's data are referred to, to be compared with our own data. But, the presence or absence of this ridge is such a clear-cut distinction that scarcely any subjective judgement could appreciably influence the results of the examinations by different workers. It may also be noticed for the Sapporo population, that, in spite of the significant change in the relative frequencies of pattern type genes, no change has occurred in the frequency of the elytral ridge gene.

DISCUSSION

The geographic gradient observed in the proportion of the elytral pattern types and in the frequency of the elytral ridge in this beetle undoubtedly belong to the category of HUXLEY's "cline." The gradient passes along the Japanese island chain from north-east to south-west, undergoing a gradual change. A similar cline is recognized by GOLDSCHMIDT (1934, 1938, 1940) concerning various characters in Lymantria. These characters, according to him, vary in accordance with the local climatic conditions, especially with the difference in temperature. The gradient found in the relative frequencies of the pattern types in this beetle, however, proceeds apparently with little relation to the local temperature condition. It is a common experience of the students in the biology of lady-beetles that the size of the elytral markings is dependent on temperature and humidity to a great extent. The melanic marking is reduced under high temperature and low humidity and enlarged under low temperature and high humidity. The cline observed in the pattern types of Harmonia inhabiting Japan indicates that, as a general tendency, light-colored forms are found in smaller proportion in warmer southern districts than in colder northern districts.

A similar gradient is also found in the beetles of this species occurring on the Asiatic Continent, as well as in Korea. Thus, in KURISAKI's sample of Suigen, Korea, *succinea* occupies its 86 percent which resembles the figure 81.3 percent given by DOBZHANSKY (1927, 1933) for his sample of "Korea." Such high frequency of *succinea* is observed throughout Manchuria, Jehol and North China, as shown in TAN and LI's sample and ours. The frequency decreases southwards to become 66.6 percent in Soochow, and 42.6 percent in Szechwan (DOBZHANSKY 1941). TAN (1949) gives 48.6 percent for an April population and 58.5 percent for a November population of Hanchow in 1947. Thus, on the Asiatic Continent also, the cline goes rather in reverse direction to the temperature gradient. When, however, the humidity is considered, we find that the cline largely accords with the climatic gradient. DOBZHANSKY (1933, 1941), who studied the geographic variations in the elytral markings of several species of lady-beetles, has arrived at the conclusion that there is a tendency in this variation common to all these species, and that the variation largely accords with GLOGER-ALLEN's rule, the humidity being more effective than temperature on this variation.

To see if any differential vitality is found in relation to pattern types, the following observation was performed in the winters of 1942 and 1943. Certain numbers of beetles with different pattern types were selected from among four hibernating colonies collected in Kyoto or in Suwa, and they were kept in an unheated room in Kyoto through winter. The room temperature in this season in Kyoto sometimes comes down a few degrees below 0°C. The proportions of the individuals with different pattern types which died during winter before the end of April were compared with one another (table 3). The total number of beetles used was rather small. The general tendency, however, is obvious: the death rate of *axyridis* was significantly higher than that of other types among which no such difference was recognized.

TABLE 3

Death rate of individuals with different elytral pattern types during winter (%).

SOURCE OF SAMPLE	*succinea*	*axyridis*	*spectabilis*	*conspicua*	TOTAL NUMBER TESTED
Kyoto	14.0	39.1	21.0	21.8	378
Kyoto	22.4	40.0	16.7	24.6	496
Suwa	17.9	33.3	12.3	19.4	228
Suwa	16.0	35.7	23.5	19.8	255
Average	17.5±2.3	38.0±5.7	19.1±2.9	22.2±1.5	1,357

Axyridis–succinea =38.0−17.5=20.5±6.1
Axyridis–spectabilis=38.0−19.1=18.9±6.3
Axyridis–conspicua =38.0−22.2=15.8±5.9.

This finding, though meager, seems to have ascertained that selective mortality existed in connection with the *axyridis* pattern only, which is just in the middle in the grade of extension of melanic pigment, and that neither for the types with greater extension of the pigment, *conspicua* and *spectabilis*, nor for the type with smaller extension, *succinea*, was there any differential mortality during winter. TAN (1949) records his experience similar to this on a hibernating colony in Hanchow. His observation, however, seems to have substantiated that there was a selective mortality during the active season. If this seasonal variation is proved to be a regular one, it is perhaps due to the selective influence by humidity which shows considerable seasonal change in South China. This observation of TAN's recalls TIMOFFÉEF-RESSOVSKY's statement (1939, 1940, 1940b) on *Adalia bipunctata*, another variable lady-beetle. In a colony of this beetle hibernating at a certain place in Buch near Berlin this author found a regular cyclic change in the ratio between the black and red forms. This finding is different from TAN's in that the selective mortality occurred during both the active and hibernating periods. At any rate, we can safely state that no apparent adaptive significance such as found in various characters in Lymantria can be attached to the difference in the elytral pattern of this beetle. The same is undoubtedly true for the presence or absence of the elytral ridge as well. Thus, both the cline in the elytral pattern and the cline in the elytral ridge concern a non-adaptive or neutral character. MAYR (1947) gives several instances of such clines.

In spite of this difference in the clines in Lymantria on the one hand and the clines in Harmonia on the other, one feature is common to both, namely, that Tugaru Strait marks a sharp gap. Of Lymantria, the strongest race in sex character occurs in Tohoku District, whereas the weakest race inhabits Hokkaido. Of Harmonia, nearly all the specimens from Hokkaido have the elytral ridge, while only about half of the specimens from Tohoku possess it. For the elytral pattern, such a gap is not found between the samples of Tohoku and the samples of Hokkaido obtained in 1943–1945. But, if we take the figure of 1923 as representing the original state of the Sapporo population, a similar contrast may be found between the populations of the two districts.

Next, a few previous cases where the composition of the same population of animals was examined on different occasions with a rather long time interval, may be cited. GEROULD (1940) ascertained that the ratio of white and yellow forms of the females of the pierid butterfly, *Colias philodice*, collected at Hanover, N.H. remained unchanged from 1911 to 1940. GORDON (1947) found that the proportion of six genes controlling the tail pattern of the tropical fish, *Platypoechilus maculatus* caught in certain places in Mexico and Guatemala, had remained nearly constant for more than 70 years from 1867 to 1939. One gene, however, increased somewhat from 1902 to 1939, while two genes decreased appreciably in the same period. In the common garden-snails of Europe, *Cepaea nemoralis* and *hortensis*, DIVER (1929) has ascertained that the proportion of the various color types has not changed significantly since the Pleistocene Period.

These are instances where Hardy-Weinberg's law of the constancy of gene composition within a random mating group has prevailed for a number of years. Among the cases where the composition has changed, the following record seems to resemble the present finding. KELLOGG and BELL (1904) observed in a population of a chrysomelid, *Diabrotica soror* inhabiting the campus of Stanford University, that there was a steady increase of the proportion of the individuals with fused elytral spots at the expense of those with separate spots from 1895 to 1904.

The cause of the temporal variation in the relative frequencies of elytral patterns in Harmonia stated above, remains obscure. But, there seems to be no doubt that human agency is responsible after all. It may be assumed for the Sapporo population, that the communication between Hokkaido and Honsyu has recently increased to such an extent that Tugaru Strait has lost its former efficiency of barring the exchange of the genes between the beetles inhabiting these districts. But this conjecture does not accord with the fact that the frequency of the elytral ridge has remained unchanged during the same period. Next, for the Suwa population, perhaps the Kiso Mountain Range had been an effective barrier between the population inhabiting Suwa Basin and that in the Nagoya-Gihu District. This barrier has been lifted by the recent development of traffic across the mountain range, and the gene flux between the populations of the insects inhabiting these two districts has been accelerated, and this may have brought the composition of the Suwa population somewhat nearer the state in the Nagoya-Gihu population. All these, however, are mere conjectures. The real cause remains to be elucidated.

SUMMARY

1. A geographic gradient (cline) is found in the relative frequencies of elytral pattern types and also in the frequency of the elytral ridge in the lady-beetle Harmonia inhabiting the Japanese islands. Neither of these characters seems to have any adaptive significance, and the cline passes apparently without any relation to climatic, especially temperature, gradients.

2. The clines are interrupted by an abrupt change at the Tugaru Strait between Hokkaido and Honsyu, and at the strait between Korea and Kyusyu.

The population of Hokkaido is characterized by its resemblance to the populations in Korea and North China.

3. Sets of samples collected at the same locality on occasions with an interval of from 15 to 30 years between, were secured from several localities in Japan. Some of them show that there was practically no change in its composition during this period; but a few others indicate an obvious change in the composition of the population.

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