Genetics of Ladybeetles*

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I. INTRODUCTION

Many species of ladybeetles, the insects belonging to the family Coccinellidae, show considerable intraspecific variation in the markings of elytra, as well as of the head and pronotum. Naturally, this variation was taken by earlier taxonomists as sufficient ground for subdividing a species into varieties or forms, and for giving them different names. Occasionally, individuals of the same species, especially of rare species, having distinct markings, had been assigned to different species or even to different genera. The variation also has attracted attention of investigators interested in the problems of variation, heredity, and evolution for many years.

The elytral markings in most of the species of this group are formed by combinations of areas of black, reddish, brownish, yellowish, or

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whitish color. The black pigment is melanin and the light ones are carotenoid derivatives. The variation in the pattern depends on the relative extension of the areas of different colors. It is commonly believed that the ground substance of melanin, the so-called chromogen, is distributed on the elytron in a characteristic manner predisposed by the genetic composition of each individual, and the pigmentation is realized by the activity of oxidase contained in the blood in the presence of oxygen supplied by trachea (Tennenbaum, 1934). This view, however, needs elaboration through modern biochemical knowledge.

It is very probable that at least the melanic and brownish or reddish ground color have different physiological significance, and they have different adaptive values under various environmental conditions. Also, it has been assumed that the conspicuous patterns possessed by the insects of this family belong to "warning markings." The insects have the habit of expelling unpleasant fluid when disturbed, and it is thought useful for them to warn their enemies by these markings. It has been found that these patterns vary considerably according to environmental conditions under which the insect develops. In general, high temperature and low humidity reduce the melanic area, while low temperature and high humidity enlarge it (Johnson, 1910; Zimmermann, 1931; Timoféeff-Ressovsky, 1932).

The papers on the genetics of ladybeetles published up to 1943 are briefly reviewed by Shull (1943); the reader is referred to his paper for further information than is presented below. The present review includes some additional data notably from Japanese and Chinese sources.

II. Intraspecific Variation in Elytral Patterns

Bateson (1894) notes the great diversity of the elytral marking of *Coccinella 10-punctata* which is very common in England. He points out that, in spite of this diversity, "there are certain types which are again and again approached, while the intermediates are comparatively scarce" (loc. cit., p. 49). He compares this species with another common species, *C. 7-punctata* which is far less variable, and he expresses his doubt about the adequacy of the opinion which attributes the invariability of a character to its usefulness for the life of the owner. Schroeder (1901–1902) has studied the variability of the elytral pattern in *Adalia bipunctata*. He endeavors to follow up the phylogeny of the different patterns in this species. Kellogg and Bell (1904, also Kellogg, 1909), in their paper dealing with the variations in several characters of various insects, describe the variation in the elytral pattern among a population of *Hippodamia convergens* collected at Palo Alto, California. They were impressed by the great diversity of the pattern, and also by that these

varieties "will apparently go safely through life despite the malevolent search of all-powerful Inquisitor, Rigour of Selection" (Kellogg, 1909, p. 104).

A far more extensive study of the variation in ladybeetles were carried out by Johnson (1910). He examined nearly all of the species of this family occurring in North America. His method was to arrange different patterns according to the degree of apparent similarity or dissimilarity into a system much like a phylogenic tree. When the material was sufficient, he estimated the relative frequencies of different patterns. Unfortunately, the outcome of this laborious study is meager, and his conclusions about the heredity, variation, and evolution of the character in question are vague and inconsistent with the knowledge of modern genetics. He firmly advocates the view of "determinate evolution" as the title of his paper indicates. He means by the determinate that the variation in the elytral patterns of these insects has some definite tendency or direction instead of being fortuitous. "The progeny differ in the long run from the parents in some one direction, instead of offering indifferently plus (+) and minus (−) variations in all the characteristics and having new characteristics in all directions" (loc. cit., p. 81). He also doubts the efficacy of natural selection in determining these patterns. Because, " . . . since the pattern is for the purpose of an association with the bad taste, if it has a purpose, its highest utility would lie in constancy and idiosyncracy. The spotted pattern being in these beetles the commonest and characteristic one, natural selection, in so far as it is operative, should favor this pattern. If, then, we find any evolution away from this pattern, it must have taken place either without the aid of natural selection or in opposition to it" (loc. cit., p. 95). Thus, his view is much similar to that of the believers in orthogenesis. However, Johnson emphasizes the difference on the ground that the term orthogenesis is generally used for a change operating unswervingly and in one direction for a long time, while in this case the line of evolution is much diversified. The author endeavors to make a point of this view by means of the knowledge of genetics current in his day. Thus, his paper is of some interest to modern geneticists in reviewing the development of our knowledge of these problems.

The variation and heredity of markings of some species of ladybeetles was intensively studied in the third decade of this century by the group of workers headed by N. W. Timoféeff-Ressovsky, including Tennenbaum, H. A. Timoféeff-Ressovsky, Zarapkin, Zimmermann, and Moderegger. The species worked on were *Adalia frigida*, *A. bothnica*, *Subcoccinella* 24-*punctata*, *Coccinella* 10-*punctata*, *Propyrea* 14-*punctata* (Zarapkin, 1930a,b; 1938a,b), *Epilachna chrysomelina* (Timoféeff-

Ressovsky, 1932; Tennenbaum, 1933; Zarapkin, 1933, 1937; Zarapkin and Timoféeff-Ressovsky, 1932; Zimmermann, 1931, 1934, 1936), and *Coccinella* 14-*pustulata* (Moderegger, 1933). The findings of these authors may be summarized as follows: The appearance or disappearance of individual spots is not fortuitous, but seems to follow some definite order of sequence. The presence of some spots is very irregular, while other spots are more persistent, and there are many grades in between. For instance, *Coccinella* 10-*punctata* may have spots on each elytron varying in number from zero to eight. If these spots had equal chance of presence, then we should expect the frequency of each spot number as shown on the second line of Table 1. The actual numbers of different combinations of spots among the 3928 individuals examined are presented on the third line of the same table.

TABLE 1

Expected and Observed Numbers of Different Combinations of Elytral Spots among 3928 Individuals of *Epilachna chrysomelina**

Number of spots	0	1	2	3	4	5	6	7	8
Expected number of different combinations	1	8	28	56	70	56	28	8	1
Observed number of different combinations	1	6	8	12	19	14	12	6	1

* From Zarapkin (1938b).

This table shows that the distribution of the actual combinations is largely symmetrical, and apparently conforms to the rule of binominal expansion. But the curve is more flattened than the normal curve. Next, assuming equality of chance of appearance of different combinations among each spot-number class, the frequency of each combination should be equal. Actually this is far from the case. For instance, among the 254 individuals belonging to the one-spot class examined, 152 had the post-humeral, 64 the mid-sutural, and 33 the humeral spot, while 2 had the scutellar, 2 the apical and only 1 the marginal spot. Such striking unevenness in the distribution of spots may be found throughout the material (Zarapkin, 1930a, 1938b).

Much the same tendency in the appearance or disappearance of spots has been recognized in other variable species having many spots, such as *Adalia frigida* (Zarapkin, 1930a), *A. bothnica* (Zarapkin, 1930a), *Subcoccinella* 24-*punctata* (Zarapkin, 1930a) and *Coccinella* 14-*pustulata* (Moderegger, 1933). Furthermore, in most of these species the spots may be independent or confluent with one another. This confluence is often present to such an extent as to unite all spots into a definite pattern, and finally to turn the ground of the elytra into a melanic field, leaving

the light areas as mere spots or freckles. This fusion of spots and the advancement of melanic change do not occur in fortuitous manner; it is apparently prefixed which spots should be united first, and which pair should remain always independent, and also to which direction a particular spot is to be extended.

Thus, all these authors emphasize the presence of a strong "determinate" or "eunomic" tendency in the variation of spots. This tendency may be correlated with the development of spots in each beetle after emergence. All spots are never formed simultaneously, they appear in succession, following a definite order which largely accords with the order of frequency of each spot among the population—the spots more commonly occurring appear first and develop better than those rarely found (Zarapkin, 1938a).

Smirnov's study (1927) differs from all other authors' in that he uses geometrical techniques in estimating the extension and location of the spots on the elytra. Such an approach may help to solve some fundamental problem of genetics, yet the data presented by him do not contain anything of our interest.

The question of correlation existing between the variation in elytral pattern and pronotal pattern has been worked on in various species, by Dobzhansky (1924), Schilder (1928), Lus (1928), and Strouhal (1939). The existence of a certain degree of such correlation was recognized by all these authors. However, nothing has been elucidated about this problem on a sound basis of breeding data.

III. Geographic Variation in Elytral Patterns

The variation in the elytral pattern in correlation with geography has been examined by a number of investigators in various species of this family. Meissner (1907, 1908, 1909, 1910, 1911) compared the samples of *Adalia bipunctata* derived from several localities in Germany and Switzerland. He found among other facts that the sample of Potsdam contained about 10% less typical form and about 10% more var. 4-*maculata* as compared with the samples of other localities. Zarapkin (1933, 1937) compared samples of *Epilachna chrysomelina* collected at various localities on the Mediterranean coast including Corfu, Palestine, Algeria, and Corsica. He found some peculiarities of each sample in respect to the mode of distribution of pigment among the six elytral spots. A similar study was made by Kawabé (1947, 1948) and Inoué (1952) on *Epilachna* 28-*maculata*. They found some differences among local populations of this species in regard to the disappearance and the fusion of spots.

Rather distinct correlation between the size, appearance or dis-

appearance of spots, and climatic conditions has been pointed out by some authors. Johnson (1910) found that the colonies of certain species inhabiting dry localities on the Pacific Coast of North America present a common tendency to have smaller or fewer spots than the colonies occurring in humid localities. Landis and Mason (1938) compared samples of *Epilachna varivestis* of Ohio and of Mexico, and confirmed that individuals with fused spots were more abundant among the former, while those with reduced spots were commoner among the latter.

Shull (1944) examined population samples of *Hippodamia convergens* derived from various localities in the United States. He found that the incidence of the dominant gene for spotless was about 6 or 7% in the samples from the localities in Colorado and Michigan, while it was 2–3.5% in the samples from the localities in California.

The most extensive and thorough studies on this line were conducted by Dobzhansky (1933). He used specimens of this family kept in the museums in Russia and in the United States, as well as the specimens possessed by some individual collectors. Special attention was paid to highly variable species, such as: *Harmonia axyridis*, *Adonia variegata*, *Hippodamia convergens*, *H.* 13-*punctata*, *Coccinella nivicola*, *C.* 9-*notata*, *C.* 5-*punctata*, *C. transversoguttata*, *C. trifasciata*, *C. divaricata*, *Anatis ocellata*, and *Synharmonia conglobata*. He recognized in many of these species striking variability in pattern types among individuals of the same species, and also marked geographic variation in the proportion of different pattern types. This geographic variation seemed to be rather intimately correlated with climatic conditions, and accorded with Allen's or Gloger's rule of animal geography implying that arid regions are generally inhabited by light-colored varieties or races, while humid regions are predominated by darkly pigmented varieties or races. He located the centers of light forms, one in the southern part of Turkestan and in Persia, and the other in the region in North America covering California, Arizona, and probably North Mexico. The center of dark forms was found in northeastern Asia including Maritime Province, Kamchatka, and Japan. This correlation between climatic condition and pigmentation, however, was not necessarily very close. For instance, the light-form area in North America extends as far as British Columbia the climatic condition of which is very different from Arizona or Mexico. Also, different species did not always behave similarly in geographic variation.

The general tendency mentioned above is well manifested by the common *Coccinella* 7-*punctata* studied by Dobzhansky and Sivertzev-Dobzhansky (1927). The individuals of this species invariably have 7 well-defined spots. The size of these spots varies to a rather considerable

extent. The authors made a biometric study of this variation and found significant geographic variation which conformed well with the general rule governing all the species of the above mentioned family. The specimens of this species derived from the eastern region of Central Asia have spots of smallest size. The spots become gradually larger the greater the distance from this center. This tendency is especially marked toward the east and northeast direction. The local races in Siberia, Korea, Kamchatka, and Sachalin have increasingly larger spots. The peak is found in the Japanese local race.

In most of these works on the variation of ladybeetles, only phenotypes are studied. It is clear that, in order to examine this phenomenon more critically, exact knowledge of the genetics of these phenotypes is indispensable.

IV. Breeding Experiments

1. *Adalia bipunctata*

Of this species we have works by Palmer (1911, 1917) on the American material, and by Lus (1928) on materials coming from the neighborhood of Leningrad and from Semirjetchje in Central Asia. The results obtained by these authors are conflicting with each other regarding rather important points, as already pointed out by Shull (1943). The names of varieties used by these authors also disagree. However, they seem to be coordinated as follows:

Palmer	*bipunctata*	*humeralis*	*annectans* *coloradensis*	*melanopleura*
Lus	*typica*	4-*maculata*	*simulatrix* *annulata* *unifasciata*	*impunctata*

Sublunata, 6-*pustulata* and 12-*punctata* among Lus' material are missing among Palmer's material. Lus' study is relatively more extensive and thorough than Palmer's, so our attention will be directed primarily to the former. Based on the results of his breeding experiments, Lus concludes that the genes for the markings *sublunata* (S^l), 4-*maculata* (S^m), 6-*pustulata* (S^p), *impunctata* (S^i), *typica* (S^t), *simulatrix* (S^s), *annulata* (S^a) and 12-*pustulata* (S^d) form a multiple-allelic set with the dominance order $S^l > S^m > S^p > S^i > S^t > S^s > S^a > S^d$. It is to be noticed that the first four steps, $S^l > S^m > S^p > S^i$, indicate a gradual decrease of melanic areas, while the latter five steps, $S^i > S^t > S^s > S^a > S^d$, follow the order of gradual increase of melanic areas. Probably, the various light-ground forms of this series like *impunctata*, *typica*, and *simulatrix*, can be classified into a single type, like *succinea* in *Harmonia*

axyridis described below. It seems very natural that they are recessive to the melanic types, *sublunata*, 4-*maculata*, and 6-*pustulata*. What appears more striking is their dominance over 12-*pustulata*. However, as far as Lus' data indicate, this seems to be a reasonable view, inasmuch as the breeding of two 12-*pustulata* together invariably produced this type only. According to Palmer, *humeralis* which apparently corresponds with Lus' 4-*maculata* behaves as a recessive.

2. *Adalia* (*Coccinella*) 10-*punctata*

Adalia (*Coccinella*) 10-*punctata* was worked on by Hawkes (1927a) and Lus (1928), who named the different types of this species as follows:

Hawkes	testaceous	black	humeral
Lus	*typica*	10-*pustulata*	*bimaculata*

According to Lus, these three varieties also form a multiple-allelic series, with the dominance order $S^t > S^d > S^b$. Hawkes' results agree with this view, with the exception that some *typica* were produced from the mating of blacks (10-*pustulata*) together. This black or 10-*pustulata* appears much like 12-*pustulata* in *Adalia bipunctata*, while the testaceous (*typica*) and humeral (*bimaculata*) in 10-*punctata* resemble *simulatrix* (*annectans*) and *sublunata* in *bipuctata*, respectively. The dominance order in these two species disagrees as pointed out above. It is to be hoped that further careful research will clarify this apparent inconformity.

Lus also examined the inheritance of pronotal patterns in *Adalia* 10-*punctata*. The pronotum may be nearly black, or with 5 dark dots on a whitish ground, or with a figure M on the whitish ground. These types often coexist each with a particular elytral pattern, for instance, the black pronotum is associated always with the dark elytral type of *sublunata*, 4-*maculata*, or 6-*pustulata*, and it is dominant over the other pronotal types. The 5-dotted pronotum may be combined with any light-ground elytra, and this pattern seems to be dominant over the M-figured pattern. The exact mode of inheritance of the pronotal pattern in this species, as well as in other species, however, remains to be worked out.

3. *Coelophora inaequalis*

Timberlake (1922) studied the inheritance of different elytral-pattern types in this species. This species has three varieties or types with respect to this pattern, namely, (1) 9-spotted, (2) "normal" with the spots in the former type extended and fused into an irregular dark pattern, and (3) having a universally black elytra. This distinction also is apparently due to multiple-allelic genes, with the dominance order, 9-spotted > normal > black.

4. *Propyrea japonica*

Miyazawa and Itô (1921) worked on this small species which is one of the commonest ladybeetles occurring in Japan. The typical form of this species has yellowish elytra marked with 5 dark spots—1 pair of promixal spots, 1 central (median) spot, and a pair of distal spots. The central and distal spots are connected together by a dark sutural band.

TABLE 2
Data on the Inheritance of *Propylea japonica**

P	F_1			N	χ^2	P
	japonica	4-*maculata*	*feliciae*			
jap. × *jap.* ($pp \times pp$)	191	0	0	191		
fel. × *fel.* ($p^f p^f \times p^f p^f$)	0	0	31	31		
fel. × *fel.* ($p^f p \times p^f p$)	59 (51.75)	0	148 (155.25)	207	1.355	0.2–0.3
4-*mac.* × 4-*mac.* ($p^m p \times p^m p$)	35 (35.5)	107 (106.5)	0	142	0.009	0.90–0.95
4-*mac.* × *jap.* ($p^m p \times pp$)	108 (123.5)	139 (123.5)	0	247	3.89	0.02–0.05
jap. × *fel.* ($pp \times p^f p$)	148 (149.0)	0	150 (149.0)	298	0.014	0.7–0.8
4-*mac.* × *fel.* ($p^m p \times p^f p^m$)	0	58 (55.5)	53 (55.5)	111	0.225	0.5–0.7
4-*mac.* × *fel.* ($p^m p \times p^f p$)	22 (18.0)	20 (18.0)	30 (36.0)	72	2.111	0.3–0.5

p, p^m, and p^f: gene symbol for *japonica*, 4-*maculata*, and *feliciae*, respectively.
* From Miyazawa and Ito (1921).

One variety, 4-*maculata* Kurisaki, has no central spot, while another variety, *feliciae* Mulsant is lacking both the central and distal spots. The authors confirmed by intercrossing these forms that these patterns behaved as Mendelian characters. Their data published only in Japanese are summarized in Table 2. These data show clearly that these elytral pattern types depend on a series of multiple-allelic genes, with the dominance order 2-spotted (*feliciae*) > 4-spotted (4-*maculata*) > 5-spotted (*japonica*).

5. *Harmonia axyridis*

a. *Elytral Patterns.* On this common species occurring in Siberia, Manchuria, China, Korea, and Japan, genetic studies were carried out by Tan and Li (1934), Hosino (1936, 1939, 1940a,b, 1942, 1943a,b, 1948) and Tan (1942a,b, 1946). In Tan's paper (1946) the salient facts of the genetics of this species are clearly and concisely presented. The variation found in the elytral pattern is partly due to environmental effect. This is especially true of the variation in the number and size of the spots in the variety (form) having the light (reddish or brownish) ground color, *succinea*. It has been disclosed, however, that all of the distinctive types of the elytral pattern so far worked out, have a genetic background, and these types are dependent on genes whose effect is manifested according to the principle of "mosaic dominance" (Tan, 1946). By mosaic dominance it is meant that the heterozygote produced by intercrossing of the different types manifests the characteristics of parent types in such a way that the light area on the elytra of the heterozygote invariably coincides with the light area in the darker parent, and if the parents have light areas on different parts of the elytron, the heterozygote will have its light area restricted to the parts common to both parents. One can predict in accordance with this principle, the pattern type of the heterozygote from those of the parents by superimposing the parents' patterns one upon the other. The type *succinea* is largely recessive, because it has the smallest black area. Still the heterozygote carrying the gene for this type can often be identified by the presence of an indentation on the margin of the light area, or of a black spot included in the latter, on the melanic ground. This indentation or dark spot corresponds with one of the spots possessed by *succinea*. The melanic types may be arranged according to the order of dominance which is manifested by the grade of extension of melanic area into a series, *axyridis*, *spectabilis*, and *conspicua*. The gene symbols for these types may be designated as h (*succinea*), h^x (*axyridis*), h^s (*spectabilis*) and h^c (*conspicua*), respectively. I have proposed (Komai, 1954) h as the basic symbol for all these patterns. Hosino uses p instead throughout his papers, while Tan prefers s. However, both p and s commonly appear in biometrical literature with particular usages, and they are preferably to be avoided. As was pointed out first by Hosino and concurred later by Tan, these genes form a multiple-allelic series, with the dominance order $h < h^x < h^s < h^c$. These four genes constitute the main part of the gene pool of this species. In addition, there are some types belonging to the minority which usually occupy a proportion lower than 1% even when all of them are taken together. They

mostly represent some apparently slight modifications of the major types. For instance, *transversifascia* (h^T, Fig. 1, 5–7) resembles *conspicua* in having just one light area in the proximal part of the elytron, but the area extends from margin to margin as a broad transverse band. In *equicolor* (h^E, Fig. 1, 8) this band reaches the proximal margin so as to occupy the whole proximal half of the elytron. In *intermedia* (h^I,

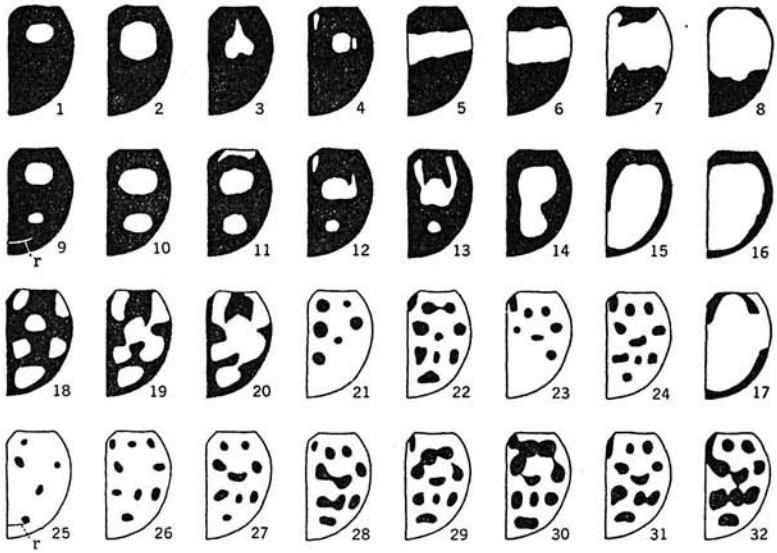


FIG. 1. Diagrammatic figures of the types of elytral pattern in *Harmonia axyridis*. 1. *conspicua*-1 (h^{C1}), 2. *conspicua*-2 (h^{C2}), 3. *gutta* (h^G), 4. *distincta* (h^D), 5. *transversifascia*-2 (h^{T2}), 6. *transversifascia*-3 (h^{T3}), 7. *transversifascia*-1 (h^{T1}), 8. *equicolor* (h^E), 9. *spectabilis*-1 (h^{S1}, r = ridge), 10. *spectabilis*-2 (h^{S2}), 11. *tripunctata* (h^{Tr}), 12. *rostrata* (h^R), 13. *forficula* (h^F), 14. *intermedia* (h^I), 15. *aulica*-2 (h^{A2}), 16. *aulica*-1 (h^{A1}), 17. *uncinata* (h^U), 18. *axyridis*-1 (h^{X1}, heterozygous), 19. *axyridis*-2 (h^{X2}), 20. *axyridis*-3 (h^{X3}, heterozygous), 21, 22. *succinea*-1 (h^1), 23, 24. *succinea*-2 (h^2), 25, 26. *succinea*-3 (h^3, r = ridge), 27, 28. *succinea*-4 (h^4), 29, 30. *succinea*-5 (h^5), 31, 32. *succinea*-6 (h^6). In each *succinea* pair the preceding one shows the pattern under higher temperature. From Hosino (1936, 1940b, 1941, 1942, 1943a,b, 1948) and Tan (1946), modified.

Fig. 1, 14) the two light areas in *spectabilis* are confluent. When this confluent area occupies nearly the whole elytron, leaving a narrow black border on the proximal and external margins, we get *aulica* (h^A, Fig. 1, 15, 16), which is relatively the commonest and most distinctive of these minor types. Tan (1946), further, recognizes *tripunctata* (h^R, Fig. 1, 11) provided with a light cicatrix along the basal margin in addition to the two spots present in *spectabilis*. Hosino (1939) distinguishes *forficula* (h^F, Fig. 1, 13) from *spectabilis* by the scissor-shaped proximal spot,

gutta (h^G, Fig. 1, 3) by a rather long apical process from the middle of the same spot (Hosino, 1940a), and *rostrata* (h^R, Fig. 1, 12) by a short beak-like process on the external side of the proximal spot and a small light freckle near the scutellum (Hosino, 1941). He (1941) also recognizes *distincta* (h^D, Fig. 1, 4) from *conspicua* by the presence of an independent cicatrix on the external side of the proximal spot and a small freckle near the scutellum. Both Tan and Hosino confirmed by breeding experiments that all the peculiarities of these minor types are inherited as distinct Mendelian characters, and all the genes responsible for them belong to the same allelic series.

Furthermore, Tan distinguishes *conspicua*-2 (h^{C2}, Fig. 1, 2) from *conspicua*-1 (h^{C1}, Fig. 1, 1) and *spectabilis*-2 (h^{S2}, Fig. 1, 10) from *spectabilis*-1 (h^{S1}, Fig. 1, 9) by the distinctly larger light spots in the latter type within each group, and *transversifascia*-2 (h^{T2}, Fig. 1, 5) and *transversifascia*-3 (h^{T3}, Fig. 1, 6) from *transversifascia*-1 (h^{T1}, Fig. 1, 7) and *aulica*-2 (h^{A2}, Fig. 1, 15) from *aulica*-1 (h^{A1}, Fig. 1, 16) by the smaller light area in the latter type in each group. Hosino (1942, 1948) also distinguishes among *succinea* obtained from various localities in Japan, as well as in Manchuria and North China, 10 subtypes (h^{1-10}), by the differences in the behavior of individual spots under various temperatures in larval and pupal stages. These subtypes are characterized by relative size, order of disappearance under high temperature, etc. of the spots (Fig. 1, 21–32). He (1943a,b) also distinguishes among *axyridis* 6 subtypes (h^{X1-6}), by minute differences in shape and in the mode of confluence of the spots (Fig. 1, 18–20). Although Hosino's breeding data are not quite sufficient for some of these types, yet, they seem to allow the conclusion that even these minor peculiarities are inherited as distinctive Mendelian characters. Hosino (1943a,b) has found that neither the gene h^8 (*succinea*-8), h^{X1} (*axyridis*-1) nor h^{X3} (*axyridis*-3) can become homozygous, because of the lethal effect of the gene itself or of the presence of a recessive lethal gene intimately linked with that gene. The heterozygote h^{X1}/h^{X3} has been found to be viable.

Thus, if it is admitted that the genes responsible for all these minor characteristics are independent, we have more than 30 different genes for the elytral pattern reported thus far by these authors. It is more than probable that, if further experiments are performed, more genetic types will be isolated. As a matter of fact, Oiwa, a student in the Sanagé Agricultural High School, describes and illustrates in detail, in his manuscript shown to me, his breeding experiment on an apparently new type which I named *uncinata* (Fig. 1, 17). This type resembles *aulica*–1 more than any other, but has a distinctive pattern with a claw-shaped marking on the proximal margin, and the black border restricted to the distal

half of the elytron. Crossing with *aulica* and *succinea* has shown that the character of this apparent new type also breeds true.

b. *Elytral Ridge.* Some individuals of this species have a tiny transverse ridge near the end of the elytra (Fig. 1, 9, 25,*r*). There is apparently no sexual difference in the shape and frequency of this ridge, and nothing is known so far regarding its function. Hosino (1936) has found that the presence or absence of this ridge is determined on a monogenic basis, the gene for the presence being dominant over the gene for the absence. Tan (1942) largely concurs with this view, except that he observed some progeny with ridges which invariably appeared from the matings of ridgeless pairs. This was probably owing to the incomplete penetrance of the effect of the gene rather than reverse mutations as he assumes.

6. *Epilachna chrysomelina*

Tennenbaum (1931, 1933) worked on the genetics of minor characteristics in the elytral marking of this species. He obtained by selection, from among a population sample derived from Palestine, 3 different lines which were distinguishable by the mode and order of disappearance of individual spots. He attributed this difference largely to the difference in genetic makeup among the lines. He also found that the shape and size of the spots was genetically controlled. For example, as for the post-scutellar spots, the elongated shape is dominant over the rounded shape, the oblique setting of the spot with the basal end near the suture is dominant over the oblique setting with the distal end near the suture, the angular and broad shape in contrast to the smooth and slender shape is controlled by two sets of Mendelian genes. Also, one of the genes which reduce the dorsal spot III interferes with viability, acting as a homo-lethal.

Zimmermann (1934) also studied many population samples of the same species derived from various localities. The results obtained may be summarized as follows: Unpredictably large number of genes seemed to be concerned with the variation in this spotted pattern. He isolated, for instance, a gene for the darkening of pronotum and elytra, another for the darkening of elytra alone, also a gene for the fusion of basal spots, another for the fusion of post-scutellar spots, still another for the fusion of apical and marginal spots. Besides, there are several genes affecting the size, shape, and position of the six pairs of spots. This list does not include genes causing pathological changes including lethals and semilethals. The local populations of this species have the store of these genes in different combinations.

Timoféeff-Ressovsky (1932) summarizes these and other findings on

this species by his colleagues as follows: "1. All 'races' are extremely heterogenic and contain many 'foreign' recessive genes in heterozygous condition; 2. the characteristic 'type of race' prevailing in the center of the race area is a combination of more or less independent distribution areas of different single genes; 3. many race characters consist of single, genetically independent elements (such, for instance, as the size, mean form and 'tendency' or 'direction' of form variation of a single spot on the elytrae) and give no Mendelian segregation if considered as a whole; 4. it seems to be very probable (however, not yet exactly analyzed), that not the single genes, but some definite 'harmonic' gene combinations can have a specific selective value under given geographical conditions" (loc. cit., p. 232). Thus, we see that he is primarily interested in the differentiation of local races in this species.

7. *Hippodamia* spp.

Shull (1943, 1944, 1945, 1948) intensively investigated on the genetics of spotted patterns of the ladybeetles belonging to the genus *Hippodamia*, *H. sinuata*, *H. convergens*, and *H. 5-signata*. He discovered that the spotless pattern in both *H. sinuata* and *H. convergens* was different from the spotted on a single gene basis, and that the former was largely dominant over the latter. The phenotypic effect of this major gene in *H. convergens* was influenced in some measure by modifying genes. Shull estimated the number of these genes as 3 or 4, and also the frequency of these genes included in each population. According to this estimation, the modifiers were nearly 3 times as frequent in Colorado and Michigan populations as in California populations.

Shull (1945) also crossed different spotted-pattern varieties of *H. 5-signata*, and concluded that "the great majority of the results can be explained by assuming four main pairs of genes along with supplementary genes which modify the dominance or otherwise affect the expression of some of these four. One of the principal pairs of genes relates to the spotless or spotted pattern. A second pair concerns the anterior band connecting spots 1 and 2; a third determines the presence of absence of spot 3; and a fourth relates to the merger of spots 4 and 5 into the postmedian band" (loc. cit., p. 153).

V. Interspecific and Intersubspecific Hybridization Experiments

Marriner (1926, 1928) maintains that *Adalia bipunctata* may be easily crossed either with *A. variabilis* (= *10-punctata*), or with *Coccinella 14-punctata*, and that he secured from the cross *bipunctata* ×

variabilis a hybrid progeny named by him *C. biabilis*. The latter, however, was suspected by Capra to be nothing but form 10-*pustulata* of *A. variabilis*.

According to Kerkis (1933), crossing experiments between *Adalia bipunctata* and *A. fascio-punctata* were performed by Lus. The hybrid progeny consisted only of females which were either sterile or became fertile long after emergence.

Shull (1945, 1948) crossed *Hippodamia* 5-*signata* and *H. convergens*, and introduced the gene for spotless possessed by the latter species into the former species. He found that this gene was as effective in suppressing the spots in the other species as in the original species. Also, seven other genes affecting the details of the spotted pattern in *H.* 5-*signata* acted similarly on the pattern in *H. convergens*, when they were introduced from the former species into the latter by interspecific crossing (1946b, 1948, 1949).

The same author (1946a) studied the genetics of the differences in the structure of male genitalia between these species. He concludes that the contrasted characters are inherited in polygenic fashion; "the siphonic flaps and the aedeagal keel may be differentiated in the two species each by two pairs of genes, the width of the sipho by five pairs, with a distinct possibility that the number should be three pairs for each of them" (loc. cit., p. 302).

Shull (1948) also found some specimens of *H.* 5-*signata ambigua* hibernating with *H. sinuata spuria*. The former subspecies is distinguished from the typical subspecies *H.* 5-*signata* 5-*signata* by being spotless. These specimens were revealed by breeding to be heterozygous for the spotted gene, they were accordingly assumed to be hybrids between the subspecies. The author thought that this heterozygosity was probably responsible for allowing them to be hibernating as guests in the colony of the different species.

Several Japanese entomologists have shown their interest in the problem of specific or subspecific distinction between *Epilachna* 28-*maculata* Motschulsky (= *nipponica* Lewis) and *E. pustulosa* Kono. The former is the common pest of potato plant in the northeastern regions of Japan, while the latter was described in 1937 based on the specimens obtained at Sorati in Hokkaido. The typical form of *E. pustulosa* is characterized by the distal margin of elytra being angular and tuberculated, and by the highest point of the back of the body being shifted toward the posterior end of the body about ⅓ from the end. In *E.* 28-*maculata* the distal margin of the elytra is thin and evenly curved as is usual in ladybeetles, and the back has its highest point at the middle. The legs are blackish in the former "species," while they are dark brown in the latter. There are

some slight differences between the "species" in the shape of spermatheca (Ebara, 1953). The food preference is also different, *E. 28-maculata* feeds usually on plants of Solanaceae and Cucurbitaceae, while *E. pustulosa* is found on the thistles, *Cirsium boreale* and *C. kamtschaticum*. These distinctive characters are variable to a fairly large extent, and it is problematical whether *E. pustulosa* could really represent a species distinct from *E. 28-maculata*, or whether it is merely a subspecies or variety of the latter (Ebara, 1952, 1953). According to Yasutomi (1954), the cross between these two forms produced fertile F_1 progeny which were intermediate in the shape of the elytra between the parent types. In F_2 this character showed segregation, and the progeny varied considerably in this respect. Yasutomi postulates two incompletely dominant genes for the difference in the shape of elytra between the two forms.

VI. Variations and Evolution in Harmonia and Adalia

1. *Macrogeographic Variation in* Harmonia

Dobzhansky (1924, 1933, 1951) indicated a striking geographic variation in the composition of the populations of this species. The form *axyridis* is most abundant in the populations inhabiting the western region of the range of the species from Altai to Lake Baikal, whereas *succinea* occurs there only as an exception. The proportion of *axyridis* decreases rapidly when one proceeds eastward in Siberia, and it occupies only a small fraction, usually lower than 10%, of the populations inhabiting far-eastern Asia. The proportion of *succinea*, on the other hand, rapidly increases in the eastward direction, and reaches 80% in eastern Siberia including the Maritime Province, and even higher in North China, Manchuria, and Korea. From this region on, the frequency falls southward to 66.6% in Soochow, 48.6–58.5% in Hanchow, and 42.6% in Szechwan (Tan, 1948; Dobzhansky, 1951).

An even more regular gradient may be found in the composition of populations of this species distributed in Japan. This is recorded in detail in Komai, Chino, and Hosino's papers (1946, 1948, 1950, also Chino, 1912, 1918; Kurisaki, 1927, 1932). Additional samples were accumulated more recently, and we have data derived from the samples from 55 localities in Japan, one locality in Korea, and two localities in Manchuria.

On the whole, Japanese populations of this species contain *succinea* in proportions varying from 2 or 3% to 60% and *conspicua* in proportions varying from 30% to 80+%; the percentage of *succinea* decreases gradually from northeast to southwest, while that of *conspicua* increases conversely. This change is so regular as to form a typical gradient, or "cline," along the Japanese main island chain. Thus, in Hokkaido the proportion

of *succinea* is 40–60%, in Tôhoku District (northeastern half of Honsyû) 30–40%, in Kantô and Middle (Nagoya-Gihu) Districts 20–30%, in Kinki (Kyoto-Osaka) District about 15%, in Tyûgoku-Sikoku Districts about 10%, and in Kyûsyû below 5%. The percentage abruptly rises over 80% in Korea, Manchuria, and North China. Conversely, the proportion of *conspicua* shows an ascending gradient, from the 30–40% in Hokkaido, through about 50% in Tôhoku and Kantô, 60–70% in Nagoya-Gihu, as well as in Kinki and Tyûgoku-Sikoku Districts, to above 80% in Kyûsyû. The percentage abruptly falls to about 5% in the populations inhabiting Korea, Manchuria, and North China.

This geographic variation in the relative frequency of the elytral pattern phenotypes may easily be converted into the variation in gene incidence (Fig. 2). The incidence of the recessive gene *h* is higher than 60% in most of Hokkaidô and Tôhoku populations. It gets below 60% in Kantô populations, below 50% in Nagoya-Gihu District populations, below 40% in Kinki, and about 30% in Tûgoku-Sikoku populations. The incidence is the lowest in Kyûsyû populations, being 20% or less. It is the highest, 90% or even more, in Korean and Continental populations. On the other hand, the incidence of the gene for *conspicua* h^c is 10–20% in Hokkaidô and Tôhoku, 30–40% in Kantô, 40% in Nagoya-Gihu, 40–47% in Kinki and Tyûgoku-Sikoku populations, and nearly 60% in Kyûsyû populations.

The proportion of either *axyridis* or of *spectabilis* does not show such a gradient throughout the beetle's range in Japan. However, the populations occurring in certain districts present peculiarities in respect to the proportions of these types. Thus, in the Hokkaidô populations the proportion of *axyridis* is very low, while that of *spectabilis* is relatively high. The Korean and Continental populations are entirely lacking in *axyridis*, and have a few *spectabilis* and *conspicua* (Fig. 2).

A similar geographic gradient is apparent also in the proportion of individuals provided with the elytral ridge. The populations occurring in Hokkaidô, as well as in Korea and Manchuria, have this ridge almost without exception (more than 95%). The percentage abruptly falls to about 50% in Tôhoku, and further decreases to about 40% (Nagoya-Gihu), 20–30% (Kinki), 15–20% (Tyûgoku), and to 10–20% (Kyûsyû). In terms of the incidence of the recessive gene for the absence of the ridge, it is lower than 20% in Hokkaidô and Continental populations; it is 60–70% in Tôhoku and Kantô, about 80% in Nagoya-Gihu, about 90% in Kinki, and about 95% in Sikoku-Kyûsyû populations. Tan (1942b) found that the great majority of the beetles distributed in China had this ridge, but the population occurring in Ishan in Kwangsi Province was entirely lacking it.

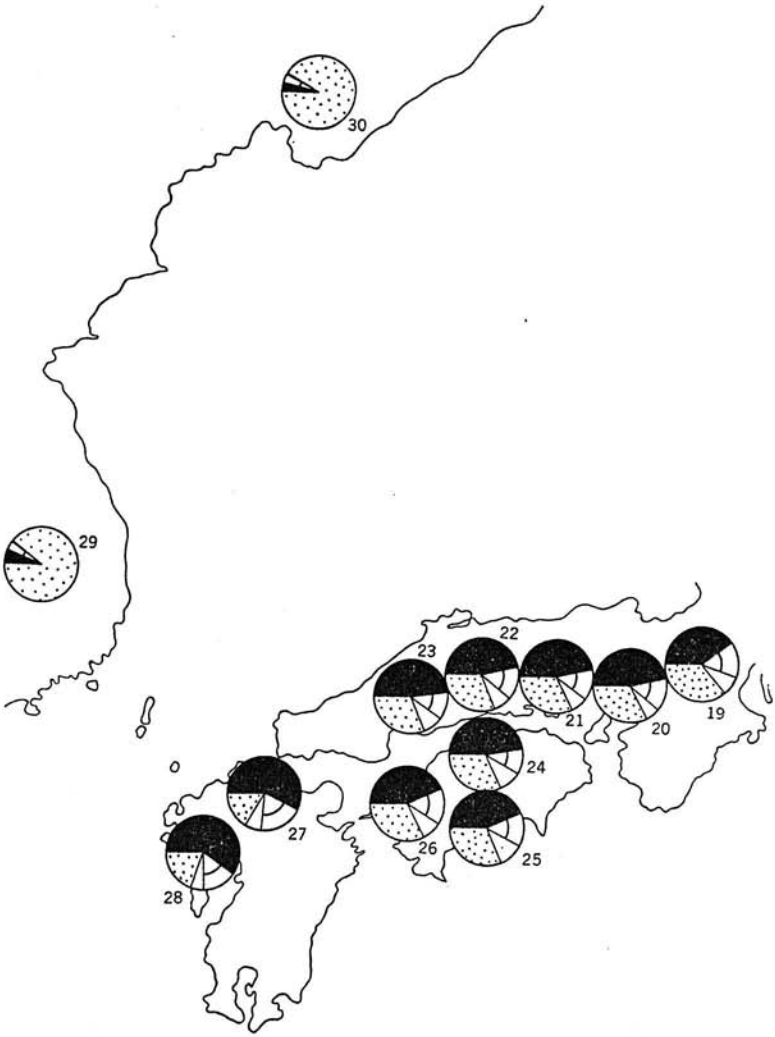
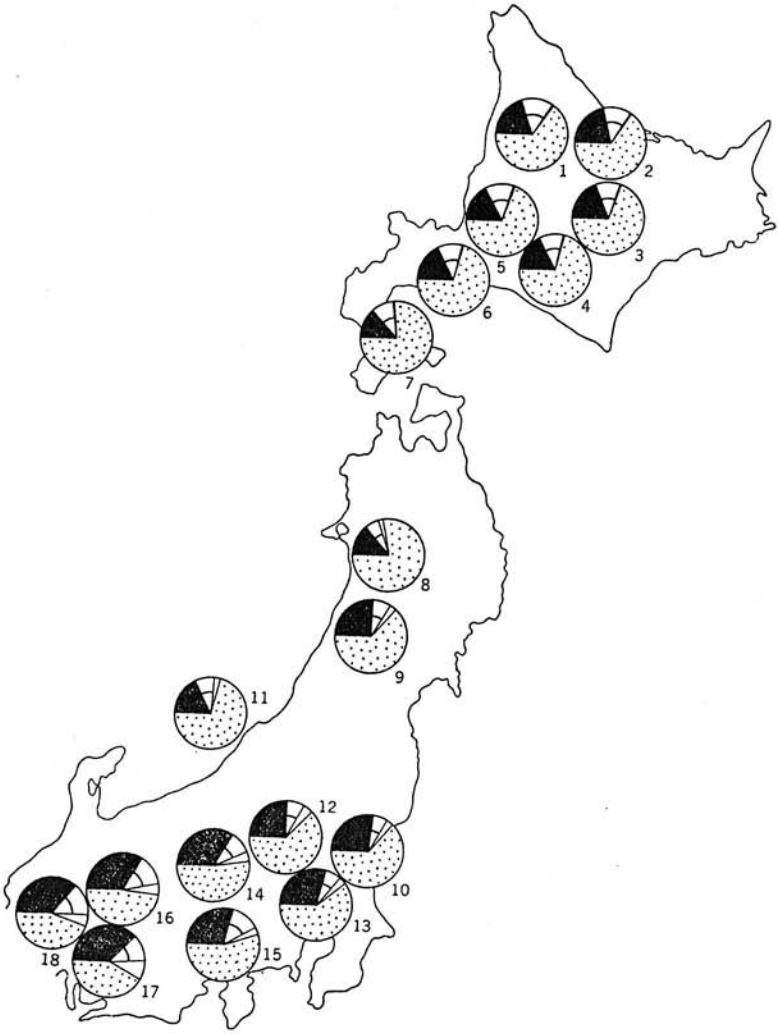


FIG. 2. Map showing the incidence of principal genes for elytral pattern of *Harmonia axyridis* in population samples derived from some localities in Japan and adjacent continent. Black area: h^c, area with arc: h^s, white area: h^x, dotted area: h; in Hokkaido and continental populations the proportion of *axyridis* is very small, so that no white area appears in the diagram. 1. Nakayubetu, 2. Sibetu, 3. Sôunkyô,



4. Yûbari, 5. Sapporo, 6. Muroran, 7. Hakodate, 8. Akita, 9. Yamagata, 10. Tutiura, 11. Akadomari, 12. Takasino, 13. Tokyo, 14. Suwa, 15. Misima, 16. Nakatugawa, 17. Nagoya, 18. Gihu, 19. Kyoto, 20. Amagasaki, 21. Akasi, 22. Onomiti, 23. Hirosima, 24. Tambara, 25. Kôti, 26. Matuyama, 27. Hukuoka, 28. Nagasaki, 29. Suigen, 30. Vladivostok.

2. *Microgeographic Variation in* Harmonia

Ladybeetles have the active power of travelling on wings. The writer does not know whether the exact limit of this power has ever been estimated. However, this seems to be fairly large, considering the enormous number of individuals assembling in shelters for hibernation, even in localities where one experiences difficulty in collecting a few hundred specimens in their active season. In addition, the beetles apparently are favored very often by chances of passive transportation. This mode of distribution is perhaps as important as the distribution by flying. Thus, it is not surprising that many species of them are distributed over wide ranges, and they present geographic variation which is apparent only when distant colonies are compared with each other. The instance of microgeographic variation actually observed is relatively scarce. Komai and Hosino (1951) published their observation of this type of variation in populations occurring at Sanagé, a village near Nagoya.

Rich material of this beetle was secured from this locality in their active seasons in 1948, 1949, and 1950 with the aid of the students in a local agricultural high school. The samples were obtained from various plants, including wheat, fruit trees, and pine trees (Fig. 3). Of these samples, those derived from pine trees had a very different composition from the samples secured from wheat and fruit trees. While the latter were nearly identical in composition to the samples of the populations in adjacent localities, the former was quite unique in containing very many *axyridis* and a few *conspicua* and *spectabilis*. This peculiarity was common to all the samples obtained in these consecutive years. The colonies on the pine trees also had the elytral ridge in such a low frequency as could be found nowhere else in Japan—only 4.2%. These pine trees and fruit trees stood close together often interspersed, and the wheat fields were in close proximity to the hills planted with these trees. The reason for this remarkable peculiarity of the colony attached to the pine trees is entirely unknown. We found that the aphids infecting the pine trees and wheat were different. This difference in food aphids was perhaps correlated somehow with the difference in composition of these populations, since many ladybeetles are known to be very particular about the choice of food.

Dobzhansky (1941) remarks that he once examined in the neighborhood of Kiev several dozen colonies of *Sospita* 20-*guttata* which has two distinct color types, black and yellow. Most of the colonies contained black and yellow specimens in nearly equal ratio. One colony contained only blacks and another only yellows. This condition persisted for at least three years. Hawkes (1927b) records her experience in collecting samples of *Adalia bipunctata* for 6 to 7 successive summers from various localities

within 19–27 miles of the vicinity of Birmingham. At Somerset Road in Birmingham, she found that the black variety formed at least 67.8%, while at Enville, Staffordshire, the *bipunctata* type occupied at least 78.5%. Of the colonies also inhabiting Worcestershire, this type formed 71–81%. At Droitwich, this type and black were about the same in frequency. She suspects that this difference might have been correlated with the difference in the plants which harbored the ladybeetle colonies. In the

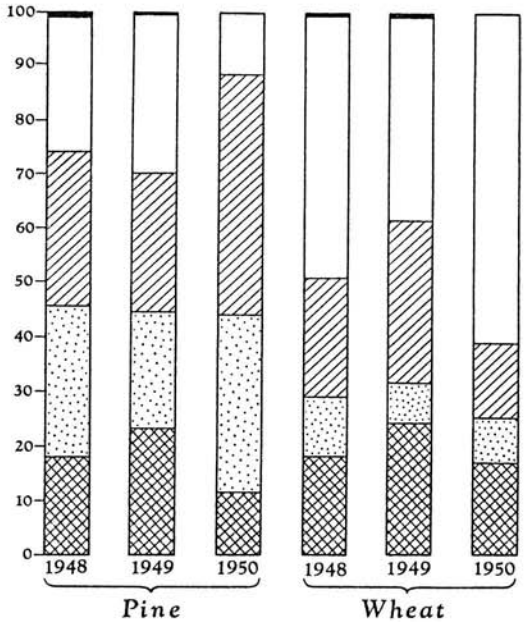


FIG. 3. Diagram showing the relative frequency of the elytral pattern types among the samples collected from pine trees and wheat. Cross-hatched: *succinea*, stippled: *axyridis*, hatched: *spectabilis*, white: *conspicua*, black (at the end): other types. From Komai and Hosino (1951).

summer of 1917, I found a sample of colony of *Harmonia* derived from a wheat field at Zyûzyô in the suburb of Tokyo which was significantly different in composition from the samples derived from other localities within a distance of a few miles (Komai, Chino, and Hosino, 1950).

3. *Seasonal Variation in* Adalia *and* Harmonia

Marriner (1926) records his observations on seasonal variation in the relative frequency of the red (*bipunctata*) and black (4-*maculata* and 6-*pustulata*) forms of *Adalia bipunctata* in certain localities of England. He found that the red forms were more abundant among the spring

brood, while the black forms were prevalent among the late summer brood. He observed in a locality in North England that the black forms occupied 30% of a colony when examined in June, 1921, and 82% of the same colony examined in August of the same year, and that this state lasted for some years after.

Timoféeff-Ressovsky, in a paper often quoted (1939, also 1940b), records his observations on the seasonal variation in a colony of *Adalia bipunctata* occurring in Buch near Berlin. He confirmed by observations extending from 1930 through 1933, that the samples obtained in different seasons, before and after hibernation, were of different composition. Thus, the colony in October contained significantly more black varieties than red ones, while the April population contained more reds than blacks. This apparently means that reds had a higher enduring power against the low temperature during winter, while blacks were more resistant to the high temperature during summer. The selective power of the climate thus acted discriminately on different color types. Timoféeff-Ressovsky considers this alternative preference of selective activity among the different color types is probably the cause of the stable polymorphism in this species.

Tan (1949) reports a somewhat similar seasonal variation found in the composition of a population of *Harmonia axyridis* occurring in the vicinity of Hangchow. His observation apparently indicates that the spring and summer climate was more favorable to the form *conspicua*, whereas the autumn climate was more favorable to the form *succinea*. This is shown by the seasonal variation in relative frequency of these forms. Possibly, the humidity, more than temperature in different seasons in Hanchow, was more responsible for this seasonal variation. Further observations preferably extending over several years are desirable for confirming the validity of this suggestive report.

4. *Temporal Variation in* Harmonia

Komai, Chino, and Hosino (1946, 1948, 1950) and Komai (1954) record temporal variation in a population of *Harmonia axyridis* observed by them during a period extending over 40 years. The material consists of hibernating insects collected from the same spot or from neighboring spots in the town of Suwa in Nagano Prefecture in Middle Japan. They were obtained by Chino or by his friends, and may well be considered to belong to different generations of one and the same population. As shown in Table 3 and Fig. 4, the constitution of this population seems to have remained nearly stationary from 1912 to 1920. Thenceforth it had changed significantly until 1950 by the steady decrease of the proportion of *succinea* and by the concomitant increase of the proportion

of *conspicua*. The grade of this increase and decrease is statistically significant except in the cases marked with asterisk in Table 4. The proportion either of *spectabilis* or of *axyridis* remained nearly constant throughout this period. From 1950 through 1954, the population seems to have remained stationary again.

The proportions of the phenotypes indicated above have been converted into terms of gene incidence and are shown in the Table 5 and Fig. 4 (graph a–d). These figures of gene incidence show essentially the same trend of change as that presented by the proportions of phenotypes.

TABLE 3
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	*N*
Suwa	1912, 1913	42.6	4.6	9.5	42.3	—	2005
	1914	41.7	5.6	10.9	41.8	—	1413
	1915, 1917	43.4	4.8	10.7	41.1	—	2059
	1920	42.4	4.4	10.6	42.4	52.6	4512
	1930	37.5	3.9	10.2	48.4	—	13157
	1942, 1943	32.0	5.0	13.0	49.8	53.2	823
	1950	28.8	3.7	11.2	56.3	57.7	2220
	1954	28.3	3.1	12.0	56.6	57.4	258
Nakatugawa	1916	30.3	5.9	16.4	47.3	—	152
	1950	21.9	4.0	16.0	58.0	42.5	269
Gihu	1923	26.4	—	—	—	49.0	239
	1940	19.0	4.8	11.7	64.4	41.6	272
Sapporo	1923	83.9	—	—	—	99.5	398
	1944	42.9	1.0	21.6	34.3	99.5	1184
	1951	44.9	0.8	20.0	34.3	99.6	501
	1954	45.5	0	21.8	32.7	99.0	101

This temporal variation in the frequency of *succinea*, as well as of *conspicua*, might be interpreted as follows: When the composition of the Suwa population in different periods is compared with the compositions of populations in the neighboring localities, it becomes apparent that the Suwa population previously resembled the populations in the more northeastern localities, such as Tutiura and Nikko, whereas the same population since 1950 has been much like the populations in the localities southwest of Suwa, e.g. Nakatugawa and Gihu. Such an interpretation naturally leads one to suspect that this gradual change might have been

caused by regular immigration of some beetles from the southwestern localities. This possibility, however, has been completely ruled out by the examination of the elytral ridge of the 1918–1920 material which had been preserved in part (420 in number). The proportion of the individuals provided with this ridge among this old material was 52.6%. The ridge was found in 53.2% of the 1942–1943 sample, in 57.7% of the 1950 sample, and in 57.4% of the 1954 sample derived from the same locality.

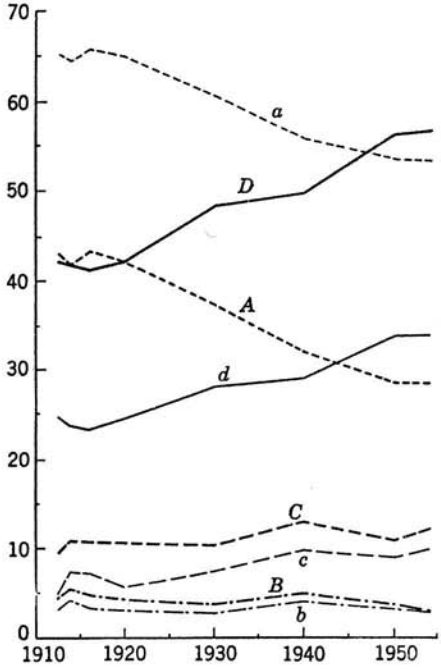


FIG. 4. Graph showing the temporal variation of the frequency of different pattern types in Suwa population. A–D. frequency of phenotypes. A, *succinea;* B, *axyridis;* C, *spectabilis;* D, *conspicua.* a–d. gene incidence: a, *h* (*succinea*); b, h^x (*axyridis*); c, h^s (*spectabilis*); d, h^c (*conspicua*). From Komai (1954).

These figures are closer to the corresponding figures for the populations in the more northeastern localities, such as, Tokyo (55.6%), Takasino in Saitama Prefecture (58.6%), and Nagano (56.6%), than the figures for the populations in the more southwestern localities—Nagatugawa (42.5%), Gihu (41.6%), Sanagé (40.7%), Nagoya (36.3%). This finding clearly indicates that the Suwa population has never received immigrants from the more southwestern populations to an extent that the immigration should have brought about the temporal variation under consideration. This change undoubtedly took place entirely within the Suwa population.

The next possibility which comes to our mind is that this change was due to selection. Based on this idea, the coefficient (s) of the selection which was responsible for the change during the period was estimated according to the formula:

$$\left(\frac{1}{q_n} - \frac{1}{q_0}\right) + \log_e \frac{1 - q_n}{q_n} - \log_e \frac{1 - q_0}{q_0} \text{ (Li, 1948)}$$

where q_0 and q_n denote, respectively, the incidence of the recessive gene h in the initial and nth generation, and e the base of natural logarithms.

TABLE 4

Test of Significance of the Temporal Variation in the Frequency of *succinea* and *conspicua* in Suwa Population

	Year			
Type	1920 (A)	1930 (B)	1942, 1943 (C)	1950 (D)
succinea	42.40 ± 0.73	37.51 ± 0.43	31.96 ± 1.62	28.83 ± 0.94
conspicua	42.40 ± 0.73	48.35 ± 0.42	49.82 ± 1.74	56.31 ± 1.05
succinea	A − B = 4.89 ± 0.85	B − C = 5.55 ± 1.68	C − D = 3.13 ± 1.87*	
conspicua	B − A = 5.95 ± 0.85	C − B = 1.47 ± 1.79*	D − C = 6.49 ± 2.03	

* Not statistically significant.

TABLE 5

Test of Significance of the Temporal Variation in the Frequency of the Genes for *succinea* (h) and *conspicua* (h^c) in Suwa Population

	Year			
Gene	1920 (A)	1930 (B)	1942, 1943 (C)	1950 (D)
h	0.651 ± 0.007	0.612 ± 0.004	0.566 ± 0.017	0.537 ± 0.011
h^c	0.246 ± 0.007	0.282 ± 0.004	0.293 ± 0.017	0.340 ± 0.011
h	A − B = 0.039 ± 0.008	B − C = 0.046 ± 0.018	C − D = 0.029 ± 0.020*	
h^c	B − A = 0.036 ± 0.008	C − B = 0.011 ± 0.017*	D − C = 0.047 ± 0.020	

* Not statistically significant.

The exact figure of n here is unknown. This insect seems to repeat about 4 generations a year under the climatic condition prevailing in that part of Japan, and the value of n is chosen accordingly. As shown in Table 6, rather uniform values of s, 0.0064–0.00706, has been obtained for the apparently continuous change during the period of about 30 years and covering probably about 120 generations of this insect.

From this figure of s, the rate of change of the recessive gene $h(\Delta q)$ has been estimated according to the formula:

$$\Delta q = -sq^2(1 - q)$$

The approximate value of $-(0.00094–0.00098)$ has been found for Δq.

It may be questioned if this change might not be due to natural mutation. A natural mutation from recessive to dominant which we have to postulate in this case may not altogether be impossible. Still, the value of Δq, $-(0.00094–0.00098)$, is much too high, probably nearly 100 times as high as to be expected for any genetic change due to natural mutation which is known to be of the order of 10^{-5} in most of the cases so far estimated.

TABLE 6

Estimation of the Values s and Δq for the Gene for *succinea* (h) in Suwa Population during the Period 1920–1950

Period	*Number of generations* (n)	s	Δq
1920–1930	40	0.00640	0.000947
1930–1942, 1943	50	0.00647	0.000939
1942, 1943–1950	30	0.00706	0.000981

Thus it seems safe to conclude that this change was due to natural selection. The next step is to find out the agent which was responsible for the selection. It seems natural to look for such an agent in environmental condition, especially climate. Now, Suwa is noted for the lake bearing the same name as the town. This lake is frozen in winter and allows skating. At the height of the season in some years, a conspicuous crack appears through the ice stretching across the lake. This crack is considered to be the sign of the thickness of the ice and also of the coldness of the winter. In relatively mild winters no crack is formed through the ice even if the lake is frozen. By consulting old records of the crack of the frozen lake, certain Japanese meteorologists have concluded that the winter climate of Suwa has gradually become milder through ages. This change, however, is detectable only when the chronological unit is taken by centuries. There are no data which suggest any steady change in climate during the past 30 or 40 years. In short, the attempt to correlate the temporal variation observed in the composition of the ladybeetle population in Suwa during the 1920–1950 period with some change in climatic condition has so far been unfruitful, although such possibility seems to remain still.

A similar tendency of the decrease of the proportion of *succinea* in nearly the same period may be recognized in the populations occurring in two other localities in Middle Japan, Nakatugawa and Gihu. In these cases, however, the total number of specimens examined was too small to make this apparent temporal variation statistically significant.

The population in Sapporo in Hokkaido is more suggestive. Kurisaki in 1923 examined a population sample of this species obtained from Sapporo, and recorded the proportion of *succinea* as 83.9%. This is much higher than the figures confirmed for the recent population samples from the same locality (42.9% for 1944 sample, 44.9% for 1951 sample, 45.5% for 1954 sample). The 83.9% for *succinea* is very interesting, because this is similar to the figures found for the populations in the neighboring continental localities (Vladivostock 85.6%, Suigen in Korea 85.8%, Mukden 90.7%, Chihfeng in Jehol 82.4%, Peiping 83.3%). The affinity of the land fauna of Hokkaido to that of the neighboring continent rather than to the fauna of Honsyû is a well-recognized fact. The Tugaru Strait between Hokkaidô and Honsyû is known by the name of Blakiston Line and is a sharp demarcation in the distribution of land animals. So it would not be surprising if the ladybeetle population in Sapporo showed similarity to that of the Continent. Furthermore, in respect to the frequency of the elytral ridge, the Sapporo population samples, both old and new (99.5% for 1923 sample, 99.5% for 1944 sample, 99.6% for 1951 sample, 99.0% for 1954 sample), are nearly identical to the continental samples examined (Suigen in Korea 97.0%, Mukden 96.6%, Chihfeng 98.9%). These data suggest that the Sapporo population had once resembled the continental populations and changed to the present state in some period in the past. The difference between 83.9% and 42.9% is, however, apparently too great for the proportion of the recessive gene for *succinea* lost from the gene pool of the Sapporo population in the period of only 21 years. No change seems to have taken place in this population in the period of 1944–1954, as far as our material indicates.

VII. Conclusions and Remarks

1. *Genes for the Elytral Patterns*

The works on the genetics of various species of ladybeetles reviewed here seem to allow of some generalizations to be presented:

In many species of this group the elytral pattern shows a considerable variation. This depends to some extent on environmental conditions under which the larvae and pupae develop. At the same time it is genetically controlled. The gene controlling this character may be major genes or polygenes. The former determine the major types of the patterns like

succinea, axyridis, etc., in *Harmonia axyridis*, or *typica* (*bipunctata*), 4-*maculata*, etc., in *Adalia bipunctata*. The stable and distinct polymorphism such as found in these species, as well as in many other species of this family, is due to the major genes of this type. It has been demonstrated that these genes behave as multiple-allelic genes. Actually, however, they seem to be "para-allelic" (= pseudoallelic or semiallelic) genes (Laughnan, 1952), namely, allied but distinct genes very closely linked together. This is seen in their behavior since they produce similar but different phenotypes, and since they present "mosaic dominance" in heterozygous combinations.

Tan (1946) records an exceptional specimen of *Harmonia* heterozygous for h^E and h^R genes having a black spot within the light area of the pattern. This spot seemed to indicate the presence of an additional gene for *succinea*. Tan interpretes this peculiarity of the specimen to be due to its being trisomic. It seems equally, or even more, plausible that this specimen was diploid, but had only the gene for pattern in a triplicate state, $h^E/h^R/h$. This is possible, if either parent had been heterozygous (h^E/h or h^R/h), and crossing-over had brought both the h^E (or h^R) and h genes onto the same chromosome.

The stable polymorphism in these insects is probably maintained by the balancing mechanism which favors one type (e.g. the dark type) in one type of environment (e.g. in moist and warm climate), and the other type (e.g. the light type) in the other type of environment (e.g. in dry and cold climate). That such a situation obtains in the cases of polymorphism in ladybeetles is strongly suggested by the observations of Timoféeff-Ressovsky (1939) and of Tan (1948) reviewed above.

On the other hand, the minor peculiarities of spots and pattern such as found in *Epilachna* and *Hippodamia* are apparently controlled by genes which belong to the category of polygenes. The over-all effect of these genes appears as patterns with various constellation and relation of spots. Darlington and Mather, in their book "Elements of Genetics" (1950), cite Baurer and Timoféeff-Ressovsky's figure illustrating the results of inter-racial crossing of *Epilachna chrysomelina* as showing polygenic segregation concerning the shape of spots and the ground color of the elytra.

This type of variation appears not to be fortuitous, but to follow some predetermined order of sequence. This fact has given early investigators a basis for holding the view of "determinate evolution." This "determinateness" in the variation in the number and size of spots does not seem to mean anything more than that the spots are never genetically or physiologically equivalent and behave differently from one another under various external or internal environments, much like the pronotal and

scutellar bristles of *Drosophila* which respond variously to certain genetic or environmental effects. The question, therefore, is concerned primarily with the differentiation of body parts, and it is only remotely relevant to the evolution of species.

As indicated above, Hosino (1942, 1943a,b, 1948) in *Harmonia* distinguishes 10 subtypes among *succinea* and 6 subtypes among *axyridis*, based mainly on the mode of appearance or disappearance, and separation or fusion of individual spots under various temperatures. These observations resemble the findings (reviewed above) by Timoféeff-Ressovsky and his associates and by Shull on the other species, and they seem to be interpreted in the same way. To the reviewer, it seems that at least those subtypes of *succinea* are due to genes which belong to the so-called "isoallelic" type (Stern and Schaeffer, 1943).

The several new types described and studied by Hosino and Tan in the same species are differentiated from the previously known types each by a rather insignificant variation. For instance, *forficula* (Hosino, 1939) is distinguished from *spectabilis* only by the peculiarity of the anterior spots being somewhat scissorlike in shape. So *forficula* appears to represent merely a slight modification of *spectabilis*. The corresponding spots in *conspicua* never take this shape. Tan (1946) distinguishes *conspicua*-2 from *conspicua*-1, and *spectabilis*-2 from *spectabilis*-1 merely by the spots being distinctly larger. All these types thus represent only slight modifications of the corresponding prototypes. It seems natural, in these cases, as well as in similar other cases observed by these authors, to assume that these modifications in phenotype are caused by the corresponding modifications in gene structure. A question arises, then, as to the actual state of these modifications—whether this is a slight change within the structure of the gene, or whether it is due to the presence of another gene closely linked with the prototype gene. At any rate, the genetic data on the elytral pattern of this family suggest to us a composite structure and complicated interrelationship of the genetic material controlling this character, and it seems difficult to understand this on the basis of the orthodox idea of the gene structure.

2. *Geographic Gradient*

Next, we shall discuss the origin of the geographic gradient found relative to the genetic composition of the populations of *Harmonia*. As stated above, more or less marked gradients may be found, one passing from Central Siberia to the Maritime Province, another from North China to Central China, and still another from Hokkaido to Kyûsyû. All these gradients seem to follow largely the gradient of climate. This parallelism is not very close, however. For instance, the Tugaru Straight

between Hokkaidô and Honsyû which is barely 40 kilometers wide sharply demarcates the populations in Hokkaidô and in Honsyû in respect to the incidence of the gene for the elytral ridge, and the Korea Strait between Korea and Kyûsyû which is about 200 kilometers wide, marks the most abrupt change in the composition of populations in the whole range of this species, in respect to both the incidence of the elytral pattern gene and that of the ridge. These findings clearly indicate that the geographic and geologic factors are as important as the climatic factors for the origin and setting of such a gradient. It is very likely that this species was distributed in Japan by way of the Maritime Province → Sakhalin → Hokkaido → Honsyû → Sikoku → Kyûsyû. In the course of this immigration, or/and after the colonies had settled down in the Japanese islands, the composition of the colonies has changed gradually toward the state now found in Kyûsyû populations which contain only a few *succinea* and many *conspicua*. That this trend had continued until only recently is shown by the temporal variation observed in Suwa population.

At any rate, it is almost certain that the climatic factor has operated somehow on the setting of the geographic gradient for the elytral pattern of this species. The same type of gradient may be seen in the geographic variation in genetic characters of some other insects, notably of *Lymantria* which have been intensively studied by Goldschmidt (1934, 1938, 1940). The flying squirrel *Petaurista leucogenys* and the woodpecker *Dryobates leucotos* each have several subspecies within Japan. There is a regular gradient in the degree of darkening of the coat color in these species passing nearly parallel to the gradient of elytral pattern of *Harmonia*. Thus, these vertebrates also represent good examples of Gloger's rule.

It seems more difficult to know if the same climatic factor was also responsible for the gradient of the gene for the elytral ridge, especially since we are quite ignorant of the usage of this ridge for the life of the insect. It is likely that natural selection operated less severely on this character than on the elytral pattern, because, first, the cline of the elytral pattern is steeper than the cline of the elytral ridge, except for the abrupt change at Tugaru Strait in the latter, and second, that the Suwa population significantly changed in the period of 1920 to 1950 concerning the relative incidence of the genes for pattern, while it remained stationary concerning the incidence of the elytral ridge. Certainly, a great deal still remains to be elucidated as to the origin and setting of the geographic gradient for these characters in *Harmonia*. And this seems to be equally true of similar gradients found for genetic characters of other animals and plants.

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