

***The genetics of two colour forms of *Chrysolina aurichalcea* (Mannerheim) (Coleoptera : Chrysomelidae) and these gene frequencies around the Utsukushigahara Heights, central Honshu, Japan.***

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**Abstract**

The inheritance pattern of the two colour forms (cyaneus-form and cupreous-form) of adult *Chrysolina aurichalcea* (Mannerheim) was examined by the crossing. The inheritance of these colour forms followed Mendel's law, showing the dominance of cyaneus-form over cupreous-form. The sampling of field populations around the Utsukushigahara Heights, central Honshu, Japan, was carried out at 74 sites. The estimation based on these results showed that the gene frequencies of cupreous-form were high (80-90%) at mountain areas (the sites higher than alt. 1600m) whereas they were low (20-50%) at the basins of both sides of the heights (the Matsumoto and the Ueda basins: the sites lower than alt. 800m). At the boundary of the mountain areas and the two basin areas, the gene frequency showed various rate and in some cases, it changed abruptly between the two sites which were apart from each other by a few hundreds meters. The histogram of gene frequencies among them for 8 years showed that the frequencies were fairly constant at major sites. At some sites, the phenotypic frequency of cupreous-form fluctuated very much.

The causes of differentiation into two groups and of the change at some sites in phenotypic frequency were discussed with possible explanations, especially in relation to natural selection by the environmental factors.

**Introduction**

*Chrysolina aurichalcea* (Mannerheim), which is common all over Japan distributes widely from the Oriental region to the Palearctic region. This species is

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generally univoltine (FUJIYAMA, 1986) and feeds mainly on *Artemisia princeps*. It is well known that this species has two forms according to the dorsal colour of the adults, i. e. cyaneus-form and cupreous-form. Many studies were carried out concerning the frequency distribution of the two colour forms (e. g. OHNO, 1964; KIMURA, 1965; SUZUKI *et al.*, 1975; BABA and KATO, 1978; FUJIYAMA, 1979; HAYASHI, 1980). However, the sampling with a large number of individuals at each habitat has not been begun until by SUZUKI *et al.* (1975).

Thereafter, three main points were established. First, the geographic variation of frequency of the two colour forms has no clear tendency comparable to that of the spotted pattern in *Harmonia axyridis* (KOMAI, 1956). This complex variation was discussed in relation to the restriction of adult migration by reduced wings, though flying populations were recently found by SUZUKI (1978). Second, major populations have a high frequency of cyaneus-form but some population from the Pacific coast (SUZUKI *et al.*, 1976; SUZUKI and SAKURAI, 1977; 1978; SUZUKI and OZAKI, 1980) and from mountain areas (FUJIYAMA, 1979), have a high frequency of the cupreous-form. These trends are discussed in connection to a geological border line (SUZUKI and SAKURAI, 1977) and to the environmental change (BABA and KATO, 1978; FUJIYAMA, 1986). Third, although neighboring populations usually show similar frequencies, drastic changes of the frequencies are seen some cases (e. g. see SUZUKI *et al.*, 1976; BABA and KATO, 1978; FUJIYAMA, 1979).

After that, SUZUKI and OZAKI (1980) proposed the subdivision of the cyaneus-form and cupreous-form containing three and eight colour forms, respectively. As the colour differences between subdivisions are discontinuous but those within subdivisions are continuous, we will treat them as the phenomenon of the colour dimorphism.

However, what determines the two colour forms has not been examined yet. The facts that no change of the colour forms has been detected in rearing experiment under different environmental conditions (FUJIYAMA *et al.*, 1981; FUJIYAMA and MIYACHI, 1985) suggest the colour dimorphism system is determined genetically. If the colour forms are genetically determined, the form of the heredity should be examined to analyse what determines the frequency of the two colour forms. Consequently, we examined the inheritance pattern. Based on this experiment and the sampling data of field populations, the gene frequencies of two colour forms around the Utsukushigahara Heights near Matsumoto City, in central Honshu, Japan were estimated. Furthermore, we discussed on the cause of observed trends in gene frequencies in connection with natural selection.

## Materials and Methods

### *Crossing of cyaneus-form and cupreous-form*

Materials used were progeny of populations collected from Matsumoto City. We followed the same rearing methods as that in a previous paper (FUJIYAMA *et al.*, 1981) except the use of *Artemisia princeps* as the food. Newly-emerged unmated adults were classified into cyaneus-form and cupreous-form according to the dorsal colour. A female and a male of the same colour form were placed inside a plastic container (diameter: 10 cm; height: 5 cm) with a twig of *A. princeps* as food. Two pieces of absorbent gauze about 4 cm square served as oviposition sites inside the container. The eggs were kept at 15°C for 20 days and transferred inside the refrigerator (at about 3°C for three months) in order to terminate diapause. They were then incubated at 15°C for hatching. Newly hatched larvae which were sampled at random were transferred inside the plastic container together with a twig of *A. princeps*. Five to ten larvae were reared until adult emergence by the method of FUJIYAMA and MIYACHI (1985). Hybrid adults classified into four groups by the combination of the dorsal colour and sex were crossed. Details of the crossing experiments are shown in Table 1.

### *Sampling of field populations and estimation of gene frequency*

Sampling was conducted around the vicinity of the Utsukushigahara Heights, central Japan from 1977 to 1984. The stations were shown with code numbers in Fig. 1, and these altitudes were shown in Table 2. In some cases, the stations which are apart from each other by only a few hundreds meters were treated as different populations because *C. aurichalcea's* small dispersal ability due to slight flying ability (see SUZUKI, 1978; 1981), and discontinuous distribution of the host plants, *A. princeps*. The number of individuals collected was usually about 50 to 200 for each station and the least was 20 individuals as shown in Table 2. The colour form was decided by the dorsal colour. Most individuals examined were released to the original habitats after the marking.

The gene frequency was calculated from the phenotypic frequency of the populations in Table 2 by applying the Hardy-Weinberg theorem.

## Results

### *Crossing experiment*

The survival rate of the progeny of the crossing between the different colour forms and of the same colour forms showed normal values, about 40% to 70%. The reduction of the hatchability and fecundity in each F<sub>1</sub> progeny

Table 1 Cross experiment between the cyaneus-form and the cupreous-form in *Chrysolina aurichalcea*.

| Parental phenotype<br>(Number of pairs) |                               | Sex ratio<br>Female/Total | Cyaneus-form   | Cupreous-form | Goodness of fit to<br>expected ratio |                                 |
|---|-------------------------------|---------------------------|----------------|---------------|--------------------------------------|---------------------------------|
| Cyaneus-<br>form                        | ×<br>Cupreous-<br>form<br>(6) | obs.*                     | 64/135         | 135           | 0                                    | —                               |
|   |                               | exp.**                    | 67 or 68/135   | 135           | 0                                    | —                               |
| Cupreous-<br>form                       | ×<br>Cyaneus-<br>form<br>(6)  | obs.                      | 78/162         | 162           | 0                                    | —                               |
|   |                               | exp.                      | 81/162         | 162           | 0                                    | —                               |
| F <sub>1</sub>                          | ×<br>F <sub>1</sub><br>(10)   | obs.                      | 185/358        | 266           | 92                                   | X <sup>2</sup> =0.033, P>0.95   |
|   |                               | exp.                      | 179/358        | 268 or 269    | 89 or 90                             |                                 |
| Cupreous-<br>form                       | ×<br>F <sub>1</sub><br>(5)    | obs.                      | 48/91          | 41            | 50                                   | X <sup>2</sup> =0.89, 0.4>P>0.3 |
|   |                               | exp.                      | 45 or 46/91    | 45 or 46      | 45 or 46                             |                                 |
| F <sub>1</sub>                          | ×<br>Cupreous-<br>form<br>(7) | obs.                      | 75/161         | 81            | 81                                   | —                               |
|   |                               | exp.                      | 85 or 81/161   | 81            | 81                                   | —                               |
| Cyaneus-<br>form                        | ×<br>Cyaneus-<br>form<br>(9)  | obs.                      | 113/233        | 233           | 0                                    | —                               |
|   |                               | exp.                      | 116 or 117/233 | 233           | 0                                    | —                               |
| Cupreous-<br>form                       | ×<br>Cupreous-<br>form<br>(9) | obs.                      | 99/219         | 0             | 219                                  | —                               |
|   |                               | exp.                      | 109 or 110/219 | 0             | 219                                  | —                               |

\* : observed value. \*\* : expected value.

Table 2 Total number of individuals collected, the number of individuals of both colour forms and of both sexes, and the phenotypic frequency of cupreous-form in *Chrysolina aurichalcea*.

| Code number of population* | Altitude (m) | Total number collected | Number of cyaneus-form | Number of cupreous-form | Number of male | Number of female | Phenotypic frequency of cupreous-form (%)** |
|----------------------------|--------------|------------------------|------------------------|-------------------------|----------------|------------------|---|
| 1                          | 690          | 84                     | 81                     | 3                       | 53             | 31               | 3.6   |
| 2                          | 620          | 22                     | 16                     | 5                       | 11             | 11               | 22.8  |
| 3                          | 820          | 58                     | 44                     | 14                      | 27             | 31               | 24.1  |
| 4                          | 720          | 68                     | 67                     | 1                       | 37             | 31               | 1.5   |
| 5                          | 670          | 219                    | 214                    | 5                       | 100            | 119              | 2.3   |
| 6                          | 710          | 22                     | 5                      | 17                      | 15             | 7                | 77.3  |
| 7                          | 720          | 53                     | 45                     | 8                       | 28             | 25               | 15.1  |
| 8                          | 670          | 605                    | 566                    | 39                      | 305            | 300              | 6.5   |
| 9                          | 660          | 46                     | 41                     | 5                       | 23             | 23               | 10.9  |
| 10                         | 690          | 109                    | 89                     | 20                      | 51             | 58               | 18.3  |
| 11                         | 640          | 23                     | 22                     | 1                       | 16             | 7                | 4.3   |
| 12                         | 620          | 84                     | 80                     | 4                       | 51             | 33               | 4.8   |
| 13                         | 620          | 158                    | 149                    | 9                       | 98             | 60               | 5.7   |
| 14                         | 620          | 228                    | 217                    | 11                      | 111            | 117              | 4.8   |
| 15                         | 630          | 142                    | 136                    | 6                       | 66             | 76               | 4.2   |
| 16                         | 620          | 49                     | 43                     | 6                       | 33             | 16               | 12.3  |
| 17                         | 600          | 234                    | 222                    | 12                      | 121            | 113              | 5.1   |
| 18                         | 680          | 82                     | 78                     | 4                       | 41             | 41               | 4.9   |
| 19                         | 620          | 75                     | 69                     | 6                       | 46             | 29               | 8.0   |
| 20                         | 740          | 24                     | 23                     | 1                       | 17             | 7                | 4.2   |
| 21                         | 600          | 38                     | 11                     | 27                      | 22             | 16               | 71.1  |
| 22                         | 860          | 30                     | 9                      | 21                      | 15             | 15               | 70.0  |
| 23                         | 1050         | 91                     | 24                     | 67                      | 40             | 51               | 73.6  |
| 24                         | 1070         | 32                     | 22                     | 10                      | 12             | 20               | 31.3  |
| 25                         | 910          | 52                     | 48                     | 4                       | 21             | 31               | 7.7   |
| 26                         | 900          | 155                    | 139                    | 16                      | 85             | 70               | 10.3  |
| 27                         | 990          | 26                     | 22                     | 4                       | 13             | 13               | 15.4  |
| 28                         | 1080         | 97                     | 59                     | 38                      | 56             | 41               | 39.2  |
| 29                         | 900          | 33                     | 19                     | 14                      | 19             | 14               | 42.4  |
| 30                         | 820          | 110                    | 94                     | 16                      | 64             | 46               | 14.5  |
| 31                         | 870          | 66                     | 49                     | 17                      | 42             | 24               | 25.8  |
| 32                         | 1050         | 46                     | 34                     | 12                      | 19             | 27               | 26.0  |
| 33                         | 1260         | 29                     | 20                     | 9                       | 19             | 10               | 31.0  |
| 34                         | 1390         | 120                    | 101                    | 19                      | 64             | 56               | 15.8  |
| 35                         | 1420         | 67                     | 55                     | 12                      | 35             | 32               | 17.9  |
| 36                         | 1420         | 57                     | 47                     | 10                      | 25             | 32               | 17.5  |
| 37                         | 1530         | 171                    | 38                     | 133                     | 99             | 72               | 77.8  |

|    |      |     |     |     |     |     |      |
|----|------|-----|-----|-----|-----|-----|------|
| 38 | 1680 | 75  | 22  | 53  | 41  | 34  | 70.7 |
| 39 | 1320 | 42  | 11  | 31  | 24  | 18  | 73.8 |
| 40 | 2000 | 120 | 32  | 88  | 52  | 68  | 73.3 |
| 41 | 1470 | 87  | 26  | 61  | 46  | 41  | 70.1 |
| 42 | 2000 | 241 | 49  | 192 | 110 | 131 | 79.7 |
| 43 | 870  | 47  | 36  | 11  | 23  | 24  | 23.4 |
| 44 | 910  | 35  | 19  | 16  | 22  | 13  | 45.7 |
| 45 | 1750 | 52  | 14  | 38  | 29  | 23  | 73.1 |
| 46 | 1710 | 167 | 33  | 134 | 79  | 88  | 80.2 |
| 47 | 1590 | 127 | 32  | 95  | 55  | 72  | 74.8 |
| 48 | 1000 | 138 | 38  | 100 | 71  | 67  | 72.5 |
| 49 | 920  | 29  | 9   | 20  | 14  | 15  | 69.0 |
| 50 | 1000 | 249 | 67  | 182 | 119 | 130 | 73.1 |
| 51 | 910  | 41  | 33  | 8   | 22  | 19  | 19.5 |
| 52 | 880  | 30  | 7   | 23  | 17  | 13  | 76.7 |
| 53 | 820  | 109 | 101 | 8   | 54  | 55  | 7.3  |
| 54 | 810  | 58  | 52  | 6   | 28  | 30  | 10.3 |
| 55 | 800  | 237 | 231 | 6   | 134 | 103 | 2.5  |
| 56 | 830  | 31  | 29  | 2   | 18  | 13  | 6.5  |
| 57 | 970  | 28  | 7   | 21  | 16  | 12  | 70.5 |
| 58 | 870  | 24  | 7   | 17  | 11  | 13  | 70.8 |
| 59 | 650  | 24  | 23  | 1   | 12  | 12  | 4.2  |
| 60 | 640  | 26  | 26  | 0   | 11  | 15  | 0.0  |
| 61 | 660  | 87  | 76  | 11  | 51  | 36  | 12.6 |
| 62 | 640  | 41  | 38  | 3   | 20  | 21  | 7.3  |
| 63 | 700  | 151 | 145 | 6   | 89  | 62  | 4.0  |
| 64 | 1000 | 59  | 46  | 13  | 26  | 33  | 22.0 |
| 65 | 1100 | 44  | 11  | 33  | 23  | 21  | 75.0 |
| 66 | 700  | 105 | 93  | 12  | 60  | 45  | 11.4 |
| 67 | 730  | 66  | 63  | 3   | 35  | 31  | 4.5  |
| 68 | 770  | 105 | 95  | 10  | 50  | 55  | 9.5  |
| 69 | 920  | 25  | 11  | 14  | 10  | 15  | 56.0 |
| 70 | 990  | 71  | 41  | 30  | 34  | 37  | 42.3 |
| 71 | 860  | 24  | 24  | 0   | 17  | 7   | 0.0  |
| 72 | 940  | 74  | 68  | 6   | 40  | 34  | 8.2  |
| 73 | 1600 | 35  | 22  | 13  | 19  | 16  | 37.2 |
| 74 | 1900 | 108 | 31  | 77  | 52  | 56  | 71.3 |

\* The localities where the populations were collected refer to Fig. 1.

\*\* This ratio corresponds to the "cupreous index" proposed by SUZUKI and SAKURAI (1979).

was not detected. The results clearly showed that the cyaneus-form was dominant over the cupreous-form (Table 1). Consequently, the inheritance pattern of colour forms followed Mendel's law.

Table 3 Mating preference test by the male choice method between two colour forms in *Chrysolina aurichalcea*.

| Female \ Male | Cyaneus-form | Cupreous-form | Isolation index |
|---------------|--------------|---------------|-----------------|
| Cyaneus-form  | 15           | 18            | -0.09*          |
| Cupreous-form | 15           | 18            | 0.09*           |

\*  $X^2=0.545$  ( $0.5 > P > 0.3$ )

#### *Mate choice*

The occurrence of random mating between the two colour forms is necessitated to estimate the gene frequency of the two colour forms by applying the Hardy-Weinberg theorem. Therefore, it was examined by the preference test. Results of mating preference by a male between two females of the different colour forms are shown in Table 3. The results showed that the male mated at random between the two. The fact that no significant differences were detected between an observed ratio of the mating frequencies and an expected ratio of those based on the assumption of random mating in colour forms under natural conditions (FUJIYAMA, unpublished data), supported the occurrence of random mating there.

#### *Frequency of two colour forms in field populations*

The number of both forms collected and the phenotypic frequency of cupreous-form in field populations are shown in Table 2. This table shows that the phenotypic frequency of cupreous-form varies from 0% to 80%, is rather low (at most 22.8%) at low altitude (below alt. 800m) and rather high (at least 70.7%) at high altitude (above alt. 1600m). The sex ratio of female to total number in these samples was 48.1% near to 50%.

Since the inheritance accorded to Mendel's law and mating preference test suggested the occurrence of random mating on colour forms, the gene frequencies of natural populations were calculated by applying the Hardy-Weinberg theorem. The frequency of the two colour forms for 74 populations around the Utsukushigahara Heights, in 1979 is shown in Fig. 1. Since most of host plants distributed along the road and sampling was usually carried out there, the roads were shown by thick lines. In Fig. 1, the central part is the mountain area with the basins on both sides, the Matsumoto and the Ueda basins on the left and right, respectively. It should be noticed in Fig. 1 that the values of gene frequencies of cyaneus-form are much lower than those of phenotypic frequencies expressed in a previous paper (FUJIYAMA, 1979).

The gene frequency of cyaneus-form dominates at both basins (e. g. sites

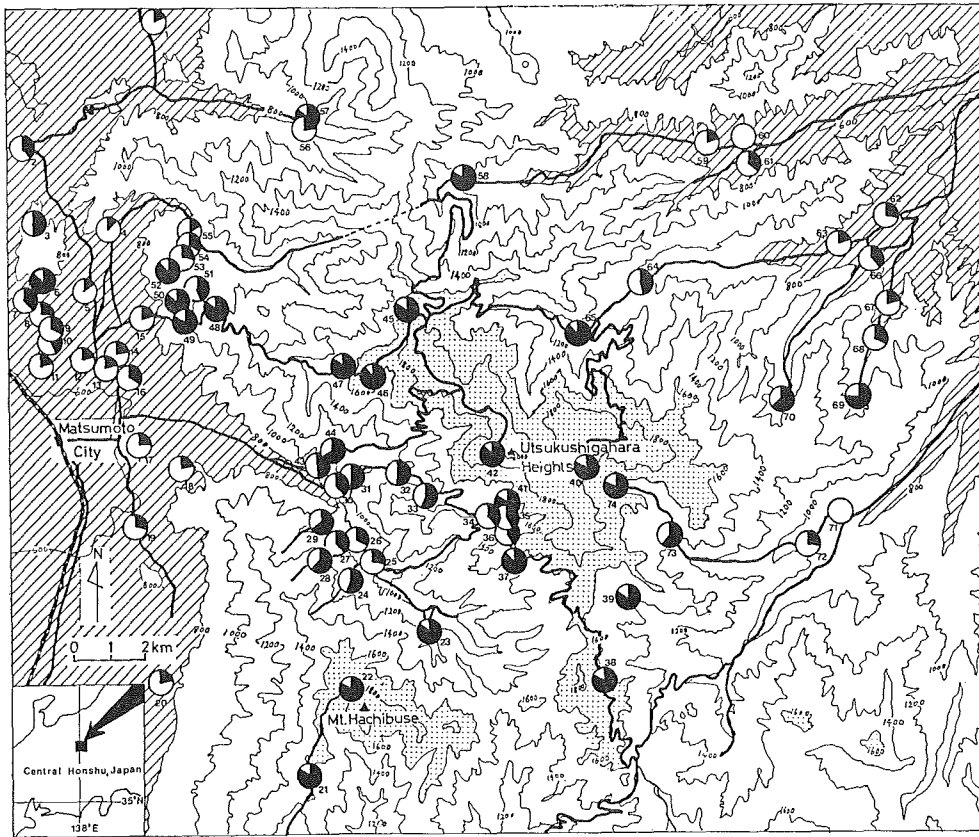


Fig. 1 Map showing the collecting stations and the estimated gene frequency of the two colour forms (black segments: cupreous-form; open segments: cyaneus-form) in *C. aurichalcea* at the Utsukushigahara Heights and in their vicinity. Stations (populations) are designated by the numeral coding. Thick lines show the roads and fine ones, contours. Hatched parts show the basins up to alt. 800m and dotted parts, mountains higher than 1600m.

7 to 20 at the Matsumoto Basin, and sites 59 to 63 and sites 66 to 68 at the Ueda basin) while that of cupreous-form, at the mountain areas (sites 21 to 23, 37 to 42, 45 to 50, 52, 57, 58, 65, 69, 70, 73 and 74). The boundary of both groups lies from alt. 800m to 1400m. The sites neighboring to the boundary have the populations with various gene frequency. And the frequency changed abruptly in many cases but in some cases gradually. At the area of sites 24 to 36, majority of which is used as open lands such as a village and vegetable fields, the frequency of cupreous-form seems to show intermediate rates. These might connect with the environment and the altitude.



There exists a great difference between present paper and a previous paper (FUJIYAMA, 1979). At site 6 in Fig. 1, the frequency of cupreous-form is high, about 80% in spite of low altitude of 800m, and this is in contrast to neighboring populations e. g. sites 5 and 7. This fact was not found when these areas were surveyed in 1977.

In most cases, frequencies are similar in neighboring populations. In some cases, however, they change drastically between neighboring populations (in Fig. 1, sites 15 and 49 or 50; sites 34 or 35 and 41; sites 36 and 37; sites 53 and 52; sites 7 and 6; sites 56 and 57). These seem to connect with the difference of environment, as discussed below.

#### *Yearly changes of the frequencies of two colour forms*

Since the degree of stability in frequency of the two colour forms in time had not been confirmed, it was checked for these populations. Results of major populations and the conspicuous ones are shown in Fig. 2. Considering the sample size of from twenty to several hundreds, we could say that the frequencies of major populations except ones at site 48' (near to site 48) shown in Fig. 2, are fairly stable.

At site 48', which located at about 300m west of site 48, the frequency fluctuated from year to year. Fig. 1 shows that the site is located to the cupreous-

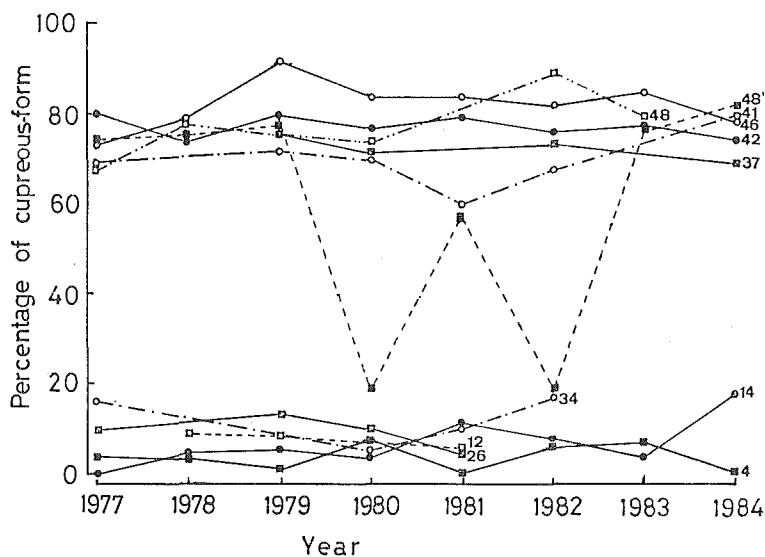


Fig. 2 Yearly changes of the phenotypic frequency of two colour forms among major populations in *C. aurichalcea*. Numerals in the figure refer to the code number in Fig. 1 and Table 2. Station no. 48' locates at about 300m west of no. 48.

form dominant side by only several hundreds meters away from the boundary, which lies between sites 50 and 51 or 48 and 51. As a female mates with many males and this continues for a long period (more than one month) in the breeding season (SHIMIZU and FUJIYAMA, 1986), it seems unlikely that the cause of fluctuation can be explained by the genetic drift. It may be due to immigration from adjacent populations with dominance of cyaneus-form or cupreous-form.

### Discussion

The colour dimorphism in *C. aurichalcea* accorded to Mendel's law and the cyaneus-form is dominant over the cupreous-form. This means that the gene frequency of cyaneus-form is much lower than that of phenotypic frequency. Therefore, the interpretation that the frequency of cyaneus-form was dominant over cupreous-form in most areas and the cupreous-form disappeared in some localities (BABA and KATO, 1978) should be reevaluated by that based on gene frequency.

We discuss in these points. For example, although the phenotypic frequency of cupreous-form in the populations from sites 30 to 36 is rather low (14.5% to 31.0%), the gene frequency is somewhat high (38.1% to 55.7%) as shown in Fig.1. All the studies that concerned with phenotypic frequency of two colour forms in this species treated only the samples of at most 600 individuals for each population. Under the Hardy-Weinberg theorem the gene frequency of 1% in recessive phenotype equals to the phenotypic frequency of 0.01%, namely, one to ten thousands individuals. Thus, we cannot deny the coexistence of both genes in *C. aurichalcea* by such small number of sampling.

In some populations, the gene frequency of cupreous-form showed 0% due to sampling error by small numbers (24-26) as shown in Table 1. Both genes of the colour forms coexisted in virtually all populations around the Utsukushigahara Heights. The gene frequency in a great majority of populations is fairly stable in time (Fig.2) and space (Fig.1). Therefore, the coexistence of both genes must result from a balanced polymorphism. This suggests that the coexistence of both genes is kept by some factors.

What maintains the coexistence of both genes? FUTUYMA (1979) mentioned that the genetic variations can be maintained by following five mechanisms: the balance of selection and recurrent mutation, selection balanced by gene flow, heterozygous advantage, frequency dependent selection and variable selection in time and space. Besides these possibilities, considering that the survey was a short term of 8 years and that SAKANOUÉ and FUJIYAMA (1987) re-

ported a rather high genetic distance with 0.059 between the Matsumoto and the Utsukushigahara populations, the following two possibilities should be taken into account: transient polymorphism and the two strains of low lands and of mountains.

Fig. 3 shows the histogram of frequency distribution of 74 populations on the estimated gene frequency of cupreous-form. The frequency distribution shows a bimodal curve, and both genes coexist in most populations. In addition to these facts, the high stability of gene frequency in time, and the small dispersal ability might suggest the existence of heterozygous advantage. Since the histogram of the populations in gene frequency made by this mechanism would show a unimodal curve, some other selection pressures must also take part in making the bimodal curve shown in Fig. 3.

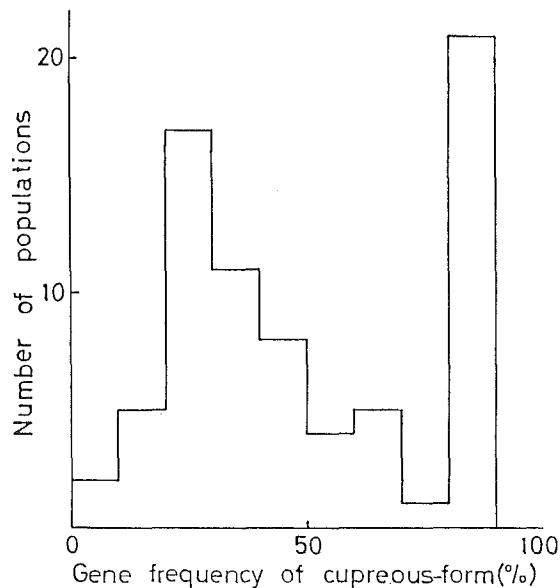


Fig. 3 Histogram of estimated gene frequency of cupreous-form in *C. aurichalcea* among 74 populations.

Fig. 4 shows the relation between the ratio of woody plant cover of the habitat and phenotypic frequency of cupreous-form. When the ratios are lower than 20%, the gene frequencies are as low as 20% or less except the ones collected from the sites of top of mountain and the Utsukushigahara Heights shown by the squares. Beyond the rates, the frequency of cupreous-form increases and reaches a dominant rate (about 70%) at 30 or 40% of woody plant cover.

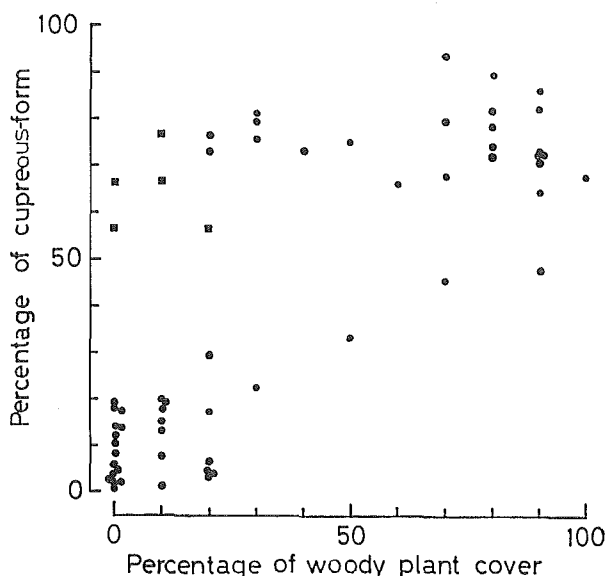


Fig. 4 Relation between the ratio of woody plant cover of the habitat and phenotypic frequency of cupreous-form. The ratio of woody plant cover was calculated with aerophotography in 1976 by counting the fractions which were occupied by the trees higher than 2m in 1 hectare around the sampling stations.

This suggests that the environment plays an important role for determining the gene frequency. BABA and KATO (1978) reported that some populations inhabiting at a small forest (less than 1km<sup>2</sup>) surrounded by open habitats in the Niigata Plain (Japan sea coast, in central Honshu) showed very high frequency of cupreous-form (70-100% in phenotypic frequency).

PESCHKEN (1972) reported in *Chrysolina quadrigemina* that the frequency of cupreous-form positively correlated with minimum temperature in winter. Up to present studies, such physiological difference that may be tied with the difference of fitness between the individuals of different genotypes has not been detected. As the dispersal and migration of adults seemed to be confined by the slight flying ability and the patchy distribution of the host plants, these stabilities must be maintained by some selection pressures. Since the low frequent gene also occupies the ratio higher than 1%, at least two possibilities, the balance selection and recurrent mutation, and selection balanced by gene flow must be eliminated.

The frequency-dependent selection is sometimes derived from the effect of biotic factor. EHRMAN (1967) reported that the female *Drosophila*, when offered a choice of males of two genotypes, often mated with rarer. In *C.*

*aurichalcea*, such tendency was not detected (Table 1). Although there are several well known studies for frequency dependent selection, such as in banding pattern of *Cepaea nemoralis* by predation of birds (CAIN and SHEPPARD, 1950), the possibility in this species must be very low. Because we have not yet seen the individuals which are eaten by birds under natural conditions. Furthermore, it has a peculiar seasonal cycle in which the active period lies in rather cold season such as during early spring and late autumn (FUJIYAMA, 1986). Consequently, the possibility for frequency dependent selection may be low.

According to our studies done so far, two cases seem to be more probable. The first is that two different strains, both of which have the two colour forms, occupy the places with different gene frequencies. This is supported by the following facts. First, the rather high genetic distance (0.059 between the Matsumoto and the Utsukushigahara populations) has been detected (SAKANOUÉ and FUJIYAMA, 1987). Second, there are two tendencies that similar gene frequencies are seen over large areas but great differences of gene frequency are often seen between adjacent populations (Fig.1). Third, great differences of the phenotypic frequency are seen at both sides of a geological border line in the Kii Peninsula (SUZUKI and SAKURAI, 1977), the Island of Shikoku (SUZUKI and SAKURAI, 1979), and the Island of Kyushu (SUZUKI, 1986). However, there is a contradictory result that the populations showing a great difference of the gene frequency have not always the high genetic distance. For example, the genetic distance of Yakushima (Lat. : 30° 20' N, Long. : 130° 30' E) and Matsumoto (Lat. : 36° 20' N, Long. : 138° 0' E) populations is 0.017 but these gene frequencies of cyaneus-form are 2% and 79%, respectively.

The second is that as the seasonal cycle of the host plant, *A. princeps*, which is a weed, is strongly affected by human activities, the seasonal cycle of *C. aurichalcea* is also affected by them through the change of that in the host plant. This might affect the balance of both colour forms.

It will be an important subject to confirm the mechanism explaining this phenomenon.

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