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## ANNUAL ADDRESS

### Observations on the Phenomena of Heredity in the Ladybeetle, Coelophora Inaequalis (Fabricius).

BY P. H. TIMBERLAKE.

(Presented at the meeting of December 1, 1921.)

It has long been known that certain species of ladybeetles of the family Coccinellidae exhibit marked colorational dimorphism or even polymorphism. Among North American species *Adalia bipunctata* (Linnaeus) and *Olla abdominalis* (Say) are known to have a predominantly black phase besides the much more abundant paler form. Mr. A. F. Burgess appears to have been the first American entomologist to make observations on the phases of Coccinellidae, and in some breeding experiments that he conducted with *Adalia bipunctata* found that the black and normal phases, when mated together, produced both phases again not only in the first, but in the second generation.*

In experiments carried on at Whittier, California, in 1912, and again in 1915 at Salt Lake City, Utah (using beetles, however, from Brownsville, Texas, collected by Mr. M. M. High), I found a similar set of phenomena in regard to the heredity of the black and normal phases of *Olla abdominalis*. Throughout the experiments a perfect segregation of the phases was obtained, but there were no other evidences of Mendelian inheritance in respect to the dominance of one phase over the other, or in the sequence and proportions of the phases when interbred. It even seemed next to impossible to get pure stock of the black phase by breeding, for when this phase was mated together for two or more successive generations, the offspring was quite as apt to belong to the normal as to the black phase.

In 1912, I had the opportunity of experimenting with an Oriental Coccinellid *Cheilomenes sex-maculata* (Fabricius), which was brought by Mr. R. S. Woglum from India to the United States. Of this species I received originally eight females and one male which were all of the normal *sex-maculata* phase. As soon as they began to lay eggs the females

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* U. S. Dept. Agric. Bur. Ent. Bull. 17, pp. 59, 60, 1898.

were isolated, and the offspring of each were thus kept under observation. Much to my surprise one of the females produced offspring of three types, the normal phase like the parent, a red phase with only the inner margin of the elytra black, and a black phase with a red cross-band behind the humeral angle of the elytra. I experimented with these forms as much as the time at my disposal and the ill-effect of inbreeding would permit, but could discover no evidence of Mendelian inheritance except in the perfect segregation of the phases. A complete set of data obtained from these experiments with *Cheilomenes* was submitted to a well-known student of Mendelian inheritance, Dr. John Detlefsen of the University of Illinois, and he was unwilling to venture any explanation of the phenomena presented, although loath to admit that the inheritance was not amenable to Mendelian laws.

Having seen from my own experiments and from those of other workers, including Mr. A. F. Burgess, Professor R. A. Johnson, and Miss Miriam Palmer, that while the inheritance of ladybeetles is often segregative, its phenomena are not otherwise easily amenable to Mendelian interpretations, I was surprised and delighted to find a case of simple Mendelian inheritance in the Australian ladybeetle, *Coelophora inaequalis* (Fabricius). This inheritance was coupled, moreover, with an example of a segregative but apparently non-Mendelian inheritance in an illuminating manner.

*Coelophora inaequalis,* so well known to Hawaiian entomologists, was an early introduction of Albert Koebele's into these islands from Australia. As found here it exhibits a remarkable uniformity of markings, and shows no trace of the range of variation credited to it elsewhere † and, in fact, it represents the normal and most abundant of the three phases

† The geographical range of this species is reported to extend from Japan and the Philippines through the East Indian Islands to New Caledonia and Queensland, but I am convinced that the Philippine form really represents a similar but quite distinct species from the Australian. The fact that there are two species confused under this name only partially explains the reputed variability of *inaequalis*, as I have reason to believe that both have a similar range of variation, and that similar color phases are common to both.

that I shall discuss later. It is bright red in color, with heavy black markings on the elytra and with the pronotum mostly black except on the anterior margin (Figure 1).

In July, 1919, Dr. F. X. Williams, on returning to Honolulu from Queensland, brought back a few beetles of *Coelophora inaequalis*, which furnished the original stock of beetles with which I experimented during the greater part of the following year. In this stock was included two forms, and a third soon appeared in breeding, which to the uninitiated eye appeared to represent three distinct species, but which by their behavior and ultimately by their heredity were shown to be but one species.

Fig. 1. Normal phase of *Coelophora inaequalis*.

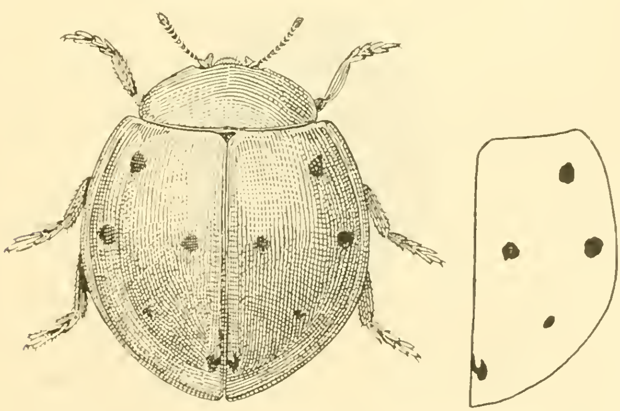

Fig. 2. Eight-spotted phase of *Coelophoria inaequalis*.

Besides the normal phase of *inaequalis* there was a paler red form with four round black dots arranged in an oblique-sided quadrangle on each elytron, a common dot on the elytra near their apex, and usually two similar dots near the middle of the pronotum. This form, represented in Figure 2, may be called the nine-spotted phase from the nine dots on the elytra. The dots vary a little in size, but there has been observed no tendency whatever for this form to intergrade with the normal phase.

The third form was solidly black with only the anterior corners of the pronotum red, and I shall refer to it as the black phase (Fig. 3). It was the rarest from at first, although later obtained in number. It apparently was not included among the original lot of beetles which came from the Herbert River, Queensland, but two specimens were reared in the first generation from the stock supply of beetles, and thus of unknown parentage.

My first experiments were directed towards finding out the behavior of the nine-spotted and normal phases towards each other in heredity. Consequently, on August 26, three nine-spotted females were isolated in vials, and a record kept sep-

Fig. 3. Black phase of *Coelophora inaequalis*.

arately for each beetle of the number and character of the offspring which were reared to maturity. These beetles were from the stock supply, and had mated repeatedly and indiscriminately after the manner of Coccinellidae. The number of offspring reared in these and later experiments may appear trivially small, but this is due to the exacting nature of their voracious appetite and the time and care that has to be bestowed upon each larva to bring it to maturity.

### Heredity of the Nine-spotted Phase.

The offspring obtained from the three nine-spotted females were as follows:

| Female | Offspring reared |
|---|---|
| No. 1. Male parentage composite | 4 nine-spotted and 15 normal |
| No. 2. Male parentage composite | 9 nine-spotted and 15 normal |
| No. 3. Male parentage composite | 20 nine-spotted and 20 normal |
| Totals | 33 nine-spotted and 50 normal |

The breeding together of the nine-spotted offspring gave the following results in the next generation:

| Pair | Parent of female | Parent of male | Offspring reared |
|---|---|---|---|
| No. 6 | No. 1 | No. 3 | 10 nine-spotted and 6 normal |
| No. 7 | No. 3 | No. 2 | 16 nine-spotted and 3 normal |
| No. 9 | No. 1 | No. 2 | 6 nine-spotted and 1 normal |
| No. 10 | No. 2 | No. 3 | 10 nine-spotted and 2 normal |
| Totals | | | 42 12 |

The nine-spotted offspring of the second generation were again mated together, and gave these results:

| Pair | Parent of female | Parent of male | Offspring reared |
|---|---|---|---|
| No. 12 | No. 7 | No. 6 | 13 nine-spotted and 0 normal |
| No. 18 | No. 6 | No. 10 | 12 nine-spotted and 5 normal |
| Totals | | | 25 nine-spotted and 5 normal |

The results of similar matings for the succeeding generation were as follows:

| Pair | Parent of female | Parent of male | Offspring reared |
|---|---|---|---|
| No. 21 | No. 12 | No. 18 | 0 nine-spotted and 2 normal |
| No. 27 | No. 18 | No. 12 | 8 nine-spotted and 5 normal |
| Totals | | | 8 nine-spotted and 7 normal |

It thus appears that into the fourth generation the nine-spotted phase, when bred in a direct line, still produced normal offspring, and, in fact, produced a larger proportion of normal beetles in the fourth than in the third generation. This, however, may have been at least partly due to the smaller number of offspring of the fourth generation reared to maturity. It is possible, but we will not say how probable, if the breeding of this phase in a direct line had been continued longer, that ultimately the production of a practically pure race would have been reached.

### Heredity of the Normal Phase.

No attempt was made to breed the normal phase in the direct line, as we already know that this phase was introduced into the Hawaiian Islands years ago, and that it has bred true to itself here since that time. The heredity of beetles of the normal phase derived from the nine-spotted form, however, may be considered.

Pair No. 8 of normal beetles were reared from the nine-spotted females No. 1 and 2, the character of whose offspring we have already seen. From this pair were reared eleven normal offspring.

Both beetles of the normal pair No. 24 were reared from the nine-spotted pair No. 18, and hence the parentage had been nine-spotted for three preceding generations in the female line, and at least for two generations in the male line of descent. From this pair seven normal beetles were reared.

Although these experiments are not extensive enough to be conclusive they seem to indicate that the normal phase is more stable than the nine-spotted phase. Presumably, however, the production of the nine-spotted phase from such parentage is possible, and at least occasionally takes place, although more rarely than the production of the normal phase from nine-spotted parentage.

### Heredity of the Normal Crossed With the Nine-spotted Phase.

One mating was made to determine whether either the normal or nine-spotted phase were dominant over the other in heredity. Pair No. 31 consisted of a female of the nine-spotted phase reared from pair No. 12 and a normal male of Hawaiian stock. From the account already given of the heredity of the nine-spotted phase it is apparent that pair No. 12 were the nearest approach to a pure race of their kind achieved during the course of the experiments, as their offspring, thirteen in number, were all nine-spotted, and we already know that the Hawaiian beetles are at least presumptively of pure normal stock. The offspring of this interesting pair were seven nine-spotted and eight normal beetles.

There is thus no evidence to show that either the normal or the nine-spotted phase is dominant over the other in heredity, and the results of the various matings are not easily interpreted according to Mendelian laws. It is apparent, however, that the normal phase is considerably more stable than the nine-spotted form, as would be naturally expected from its much greater abundance. That the nine-spotted phase is able to maintain itself when it is both less stable and much less numerous than the normal phase is quite likely due to some other factor of heredity intervening which has not been considered as yet. Otherwise, it would seem inevitable that the nine-spotted phase would be finally swamped and eliminated.

## Heredity of the Black Phase.

The black phase was not represented in the original lot of beetles that reached Honolulu alive, but two specimens appeared about September 1st in the first generation of offspring emerging in the jar in which the mixed lot of nine-spotted and normal beetles were kept. These were left in the breeding jar for a few days after reaching sexual maturity, and mated indiscriminately with each other and the other beetles. On September 12 one of the black beetles was removed and isolated in a vial as female No. 5, and for a mate was given a normal male of Hawaiian stock. But the fact that this female had mated previously with both the black and nine-spotted phases was apparent from the character of its offspring, as 3 black, 3 nine-spotted, and 16 normal beetles were reared from its eggs. Subsequently the offspring of female No. 5 were bred through five generations, and the black phase was found to be recessive to both the normal and nine-spotted forms and recurring in alternate generations in true Mendelian proportions.

## Heredity of the Black Crossed With the Nine-spotted Phase.

In considering these breeding experiments in detail let us first take up the results of the crosses of the black with the nine-spotted phase. Pairs No. 16 and 17 consisted of black females reared from female No. 5 mated with nine-spotted males reared respectively from the nine-spotted pairs Nos. 6 and 9. From female No. 16, 6 nine-spotted beetles, and from female No. 17, 19 beetles of the same form were reared without either normal or black offspring. The reciprocal cross No. 35 had similar ancestry, the female having been reared from the nine-spotted pair No. 27, the black male from the $F_2$ generation of female No. 17. This crossing gave twenty-three nine-spotted beetles.‡ We see, therefore, that the nine-spotted phase is dominant over the black.

If the cross of black and nine-spotted forms is truly Mendelian in inheritance, as indicated by the dominance of the nine-spotted form over the black, the mating together of the nine-spotted offspring of this cross should produce in the following generation both nine-spotted and black forms in the proportion of 3 to 1. Consequently, three matings were made of the offspring of pairs No. 16 and 17, numbered respectively 19, 20, and 22. The results are conveniently given in tabular form.

| Pair No. | Female from | Male from | Phase of parents | Character of offspring |
|---|---|---|---|---|
| 19 | No. 17 | No. 17 | Nine-spotted | 15 nine-spotted, 5 black |
| 20 | No. 17 | No. 17 | Nine-spotted | 3 nine-spotted, 2 black |
| 22 | No. 17 | No. 17 | Nine-spotted | 4 nine-spotted, 1 black |
| | Totals | | | 22 nine-spotted, 8 black |

We see, therefore, that the black phase reappeared in $F_2$ generation approximately in the correct Mendelian proportion.

‡ Towards the end of this breeding experiment one normal and one black beetle issued from two lots of larvae reared to maturity, but there is considerable probability that these had gotten mixed in by mistake. If large numbers of larvae are handled at the same time, a small percentage of mixture of the different lots must be expected to occur occasionally, even if great care is taken to prevent this mishap.

The assumption follows that the nine-spotted and black phases behave as a simple allelomorphic pair.

## Heredity of the Black Crossed With the Normal Phase.

The inheritance of the black crossed with the normal phase was next studied. A black female reared from pair No. 19 was mated with a normal male of Hawaiian stock as pair No. 33. From this mating nineteen normal beetles were reared and no black offspring, thus showing that the normal phase is dominant over the black. A reciprocal cross No. 34 was also made, using a normal female reared from pair No. 27 (parents and grandparents for three generations being nine-spotted) and a black male from pair No. 20. This mating produced twenty-six normal offspring, and thus also showed complete dominance of the normal phase.

The normal offspring of these two crosses were next mated together and the following results were obtained:

| Pair No. | Female from | Male from | Phase of parents | Character of offspring |
|---|---|---|---|---|
| 38 | No. 33 | No. 34 | Normal | 22 normal, 7 black |
| 39 | No. 33 | No. 34 | Normal | 36 normal, 9 black |
| | Totals | | | 58 normal, 16 black |

We thus find an approximate Mendelian proportion of the black offspring to the more numerous normal beetles, and the conclusion is forced upon us that the black and normal phases form a simple allelomorphic pair quite the same as the black and nine-spotted phase. It would seem better, however, to consider the normal and nine-spotted phases taken together as forming one unit of the allelomorphic pair, and the black phase as the other unit.

## The Heterozygous Normal Phase.

In the $F_2$ generation of crosses between the black and normal phases about three-fourths of the offspring are normal, of which one-fourth should be pure normal and one-half heterozygous or carrying both the black and normal factors of inheritance. During the course of my experiments I discovered that the heterozygous normal beetles differ slightly in coloration from the homozygous normals, and that they could be distinguished without fail. The homozygous beetles have the black of the pronotum extending out at the base clear to the lateral margins, whereas in the heterozygous beetles the pronotum is pale at the sides, and the femora and underparts of the thorax are paler (Fig. 4). Pair No. 42 of these normal

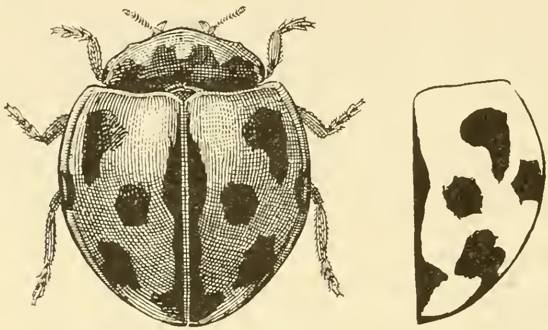

Fig. 4. Heterozygous normal phase of *Coelophora inaequalis*.

but heterozygous beetles selected from the offspring of pairs No. 38 and 39 produced 60 offspring of the $F_3$ generation; of these 47 were normal and 13 belonged to the black phase. On the other hand, no difference was detected between the homozygous nine-spotted beetles and those of the same phase which are heterozygous for black.

## Heredity of Heterozygous Normal and Nine-spotted Beetles in Crosses.

If normal and nine-spotted beetles which are heterozygous for black are mated together we would expect to find segregation of all three phases in the offspring, and that the normal and nine-spotted taken together, outnumber the black offspring about 3 to 1. We would also expect the nine-spotted and normal phases to be produced in about equal numbers, or possibly with a preponderance in favor of the normal as the more stable phase. A cross of this kind was actually made, and we consequently are able to consider the outcome as expressed in the character of the offspring.

The female of pair No. 37 was a heterozygous nine-spotted beetle raised from pair No. 35 (nine-spotted X black), and the male a heterozygous normal beetle from pair No. 34 (normal X black). A total of 59 beetles were raised from this pair, of which 14 were normal, 28 nine-spotted, and 17 black. The blacks were produced, therefore, in true Mendelian proportion, but there was an unexpected preponderance of the nine-spotted over the normal.

If the ancestry of pair No. 37 be examined, the nine-spotted phase will be found greatly in the ascendency, the direct female line of descent having been nine-spotted for five preceding generations, and the direct male line also nine-spotted for the first four generations. This, perhaps, is the true explanation of the predominance of the nine-spotted phase in the offspring of this pair.

### Nine-spotted Phase More Stable When Crossed With the Black.

But, on the other hand, when the nine-spotted phase is crossed with the black, as in pairs Nos. 16, 17 and 35, and in pairs Nos. 19, 20, and 22 of the $F_2$ generation, the nine-spotted phase is evidently stabilized, as from these pairs as many as seventy nine-spotted offspring were reared without the appearance of the normal phase except in one instance which may have been due to a mixture of lots. In crosses between heterozygous, and presumably homozygous nine-spotted beetles, a large percentage of the offspring were nine spotted. The data on these crosses may be conveniently tabulated:

| Pair No. | Female from | | Male from | | Character of parents | Character of offspring | | | |
|---|---|---|---|---|---|---|---|---|---|
| 15 | No. 10 | 9-spotted | No. 5 | black X normal | 9-spotted | 7 | 9-spotted | 4 | normal |
| 23 | No. 17 | black X 9-spotted | No. 18 | black X 9-spotted | 9-spotted | 14 | 9-spotted | 1 | normal |
| 26 | No. 18 | 9-spotted | No. 16 | black X 9-spotted | 9-spotted | 2 | 9-spotted | 0 | normal |
| 28 | No. 12 | 9-spotted | No. 16 | black X 9-spotted | 9-spotted | 19 | 9-spotted | 6 | normal |
| 29 | No. 15 | 9-spotted | No. 17 | black X 9-spotted | 9-spotted | 14 | 9-spotted | 4 | normal |
| 36 | No. 22 | 9-spotted | No. 23 | 9-spotted | 9-spotted | 19 | 9-spotted | 4 | normal |
| | Totals | | | | | 75 | 9-spotted | 19 | normal |

There is some evidence, therefore, that the nine-spotted phase is given greater stability when crossed with the black.

and this may be the explanation of the preponderance of the nine-spotted phase in the offspring of pair No. 37. We have also already seen that in crosses between the nine-spotted and the normal phase there is a distinct tendency for the former to be gradually eliminated, as it is less stable than the normal. If it is true that the nine-spotted phase is constantly stabilized by crosses with the black, the one tendency counteracts the other, and the nine-spotted phase is thus able to maintain itself under natural conditions.

## Heredity of Heterozygous Beetles Crossed With Black.

In crosses where one parent is recessive and the other heterozygous, the offspring should be produced in the ratio of one recessive to every dominant. Two crosses of this nature were made and produced results given here in tabular form:

| Pair No. | Female from | Male from | Character of parents | Character of offspring | |
|---|---|---|---|---|---|
| 40 | No. 35 | No. 20 | Heterozygous 9-spotted female X black male | 33 black | 35 9-spotted |
| 41 | No. 34 | No. 20 | Heterozygous normal female X black male | 26 black | 29 normal |

We thus observe that the expected proportion of the phases was nearly perfectly attained in the offspring.

## Heredity of the Black Phase Crossed With Black.

As the last link in the chain of evidence to prove that the black phase is a simple Mendelian recessive, the cross was made of black with black, using a female reared from pair No. 38, and a male from pair No. 39. From this union (pair No. 43) were reared a total of sixty-two beetles, all of which were black like the parents, showing that the black phase breeds true to itself as a recessive should.

## Conclusions.

From the data now presented to you it is evident that the black phase of *Coelophora inaequalis* is a Mendelian recessive to the dominant normal and nine-spotted phases, that it breeds true to itself, and when crossed with either of the dominant forms is produced in Mendelian proportions in alternate generations.

The relationship of the normal and nine-spotted phases is difficult of Mendelian interpretation, although they are perfectly segregative in inheritance. On the whole the evidence in this species and in other species of Coccinellidae with corresponding forms, would indicate that the inheritance of these forms is not Mendelian and that the laws governing its manifestations are not yet discovered or understood. Taken together, however, the normal and nine-spotted phases form one unit of the allelomorphic pair of which the black phase is the other unit.

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