

CROSSING EXPERIMENTS WITH *PHILAENUS SPUMARIUS* (HOMOPTERA)

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INTRODUCTION

POLYMORPHISM in field populations of *Philaenus spumarius* has been studied by a number of authors, and the frequencies of the different colour forms have been reported in about 20 publications. Although, from results obtained with natural populations, it has been inferred that the colour polymorphism is genically determined, crucial proof from crossing experiments has been wanting. In the USA, C. R. WEAVER has succeeded in obtaining offspring from known females with unknown mating partners, and has found that the forms tend to reproduce themselves (see OWEN and WIEGERT, 1962). Similar experiments were performed by the present authors in the autumn of 1964. In these experiments, females representing the colour forms *marginella* and *leucophthalma* were found to produce among their offspring daughters like their mother, in addition to males and females belonging to the colour form *typica*. Pictures of the colour forms as distinguished by us, have been published by HALKKA (1964).

Material and methods

All the females and most of the males used for crosses were isolated as larvae. The isolation work was done in the forest and garden area surrounding the "Nurmijärvi A" field, which harbours one of the natural populations studied by the senior author (HALKKA, 1964). The frothy spittle masses produced by the larvae usually contained a single specimen only. Altogether, 49 species of plants were used as food sources by *Philaenus* larvae in the Nurmijärvi area. A detailed report on the distri-

bution of the different colour forms on these plants will be published elsewhere. Here it will suffice to say that the distribution appears to be perfectly random.

In July 1965, 60 pairs were placed in cages containing potted *Trifolium pratense* ("Tammisto" product) and *Avena sativa* (Swedish "Sol"). The clover was intended to serve as food, the oats mainly as a site for oviposition. The flowerpots were sunk into the soil up to the rim, the lower part of the nylon cage net surrounding the pot. Apart from occasional watering, the cages were untended, until 15 of them were moved into a greenhouse on December 31, 1965, and the remaining 45 on February 26, 1966. The cages were maintained at the Agricultural Research Centre.

The winter of 1965—1966 was unusually severe in Finland. At Tikurila, the mean temperatures of January and February were -13.9°C and -13.5°C , respectively. The cages, however, were fairly well protected by about 110 cm of snow on the ground. In the greenhouse, a temperature range between 18 and 25 degrees centigrade was maintained and humidity was kept around 75 %. High humidity is essential for successful rearing of *Philaenus*.

Of the 60 pairs, 24 produced at least one offspring, while 36 were failures. Most of the failures were not due to inability of *Philaenus* eggs to hibernate but to high larval mortality resulting from poor hibernation of the *Trifolium* used as food plant. Spittle masses were observed in many of the cages which later failed to produce adults. A total of 234 F_1 offspring were obtained, 118 females and 116 males.

RESULTS

The crosses, together with their F_1 offspring, are reported in Table 1. The form *trilineata* is rare at Nurmijärvi, and the isolations did not produce enough males belonging to this form. In fact, all the *trilineata* male parents of the successful crosses originated from field collections. The *typica* and *populi* males and the different female forms were isolated from altogether 12 species of food plants. At the time of isolation, the male and female parents of crosses 9, 41, 57 and 59 were living together as larvae in a common spittle mass. In Table 1 and in the following report, the crosses are grouped into three sections according to the form of the male parent:

(1) *With typica as the male parent:*

In crosses 9 and 57 the female, too, belonged to *typica*, and in both cases both parents were isolated from the same spittle mass. Only *typica* offspring were produced.

In crosses 5, 23 and 59 the female belonged to *trilineata*. The pooled result of these crosses is 18 *trilineata* and 16 *typica* specimens.

In crosses 1 and 30, the female belonged to *marginella*. In addition to *marginella* and *typica* specimens, cross 30 includes in the F₁ generation four *leucophthalma* females, two of which bear small white spots on their elytrae and may better be assigned to the form *albomaculata*. The male parent of this cross obviously bore a gene responsible for the *leucophthalma* phenotype in one of his chromosomes, although this gene was not expressed in his phenotype. The two *albomaculata* specimens may have resulted from variation in the expressivity of this gene in the female sex. If this explanation is correct, crosses 1 and 30 demonstrate the existence of two major genes, which are here provisionally named *mar* and *lop*.

Cross 32, with *lateralis* as the female parent, produced a female like her mother and a male like his father.

In crosses 27 and 50, the female belonged to *flavicollis*. Altogether 17 F₁ offspring were obtained, all *typica*. The failure of these crosses to produce *flavicollis* may be due to chance, since only 7 females were found in the F₁ of the two crosses combined.

In cross 31, the female belonged to *leucocephala*. Two females, one orthodox *leucocephala* and one with two small white spots on both elytrae, were obtained. This latter specimen differs from a true *flavicollis* phenotypically and is best interpreted as resulting from variation in the expressivity of a major gene responsible for the *leucocephala* phenotype. This gene is here provisionally named *lce*.

In crosses 20, 25 and 41, the female belonged to *leucophthalma*. Only *leucophthalma* and *typica* offspring were found in the F₁ generation. The gene responsible for the totally black *leucophthalma* phenotype is obviously expressed only in the female sex, a result indicated by the combined information from crosses 30, 20, 25 and 41. If the *lop* gene really is sex-controlled, the black males encountered primarily in northern Finland have a different genotype. It is, of course, also possible that the same gene is sex-controlled only in some parts of its distribution area.

TABLE 1. *The results of crosses between Philaenus colour forms.*

Cross no.	Female parent	Male parent	F ₁ females	F ₁ males	Total F ₁ offspring
9	typ	typ	typ 2	typ 1	3
57	typ	typ	typ 16	typ 28	44
5	tri	typ	tri 2 typ 2	tri 3	7
23	tri	typ	—	tri 2	2
59	tri	typ	tri 7 typ 6	tri 4 typ 8	25
1	mar	typ	mar 1	—	1
30	mar	typ	mar 6 lop 4 typ 1	typ 3	14
32	lat	typ	lat 1	typ 1	2
27	fla	typ	typ 2	typ 6	8
50	fla	typ	typ 5	typ 4	9
31	lce	typ	lce 1 "fla" 1	—	2
20	lop	typ	lop 1	—	1
25	lop	typ	—	typ 1	1
41	lop	typ	lop 5 typ 1	typ 5	11
7	typ	pop	typ 2	typ 1	3
54	typ	tri	typ 4 tri 3	typ 2 tri 5	14
48	tri	tri	tri 2 typ 1	tri 3 typ 1	7
39	mar	tri	mar 1	tri 3 typ 1	5
40	mar	tri	—	typ 1	1
47	lat	tri	lat 3 tri 7 typ 7	tri 4 typ 6	27
49	lat	tri	lat 2 tri 8	tri 2 typ 9	21
19	fla	tri	tri 3 typ 1	tri 1 typ 2	7
44	fla	tri	fla 3 tri 2 typ 2	tri 6 typ 1	14
46	fla	tri	tri 2 typ 1	tri 1 typ 1	5
24			118	116	234

(2) *With populi as the male parent:*

The offspring from the single cross (cross number 7) with *populi* as the male parent and *typica* as the female contained only *typica* specimens.

(3) *With trilineata as the male parent:*

Cross 54, with *typica* as the female parent, is reciprocal to crosses 5, 23 and 59. Again, as in these crosses, *typica* and *trilineata* phenotypes are equally common in both males and females. This fact proves, that *tri* is an autosomal gene, since *Philaenus* males are XO as regards sex determination. The combined information from crosses 5, 23, 59, 54 and

48 shows, that the *tri* gene is dominant in both sexes. In cross 48, *trilineata* parents produced both *trilineata* and *typica* offspring.

The offspring of crosses 39 and 40, between a *marginella* female and a *trilineata* male, shows the recessiveness of the *typica* condition towards the effect of the *mar* and *tri* genes.

The two crosses, 47 and 49, both produced frequent offspring, including, in addition to the parental types *lateralis* and *trilineata*, a number of *typica* specimens. The offspring from crosses 47 and 49, together with the result of cross 32, prove the existence of a gene, *lat*, responsible for the *lateralis* phenotype.

Crosses 19, 44 and 46 all produced *trilineata* (paternal phenotype) and *typica* females and males. The maternal phenotype *flavicollis* was obtained in cross 44 only. The existence of a gene for this phenotype, *fla*, seems unquestionable, but either the gene in question has a low penetrance or the *flavicollis* phenotype may also be caused by the action of other genes.

DISCUSSION

The results of the crosses described above show convincingly that each of the distinctly coloured and readily distinguished phenotypes is determined by a particular major gene. Most of the genes are regularly expressed in the females but practically never in the males. This is true of the expression of the major genes *mar*, *lat*, *lce* and *lop*. The *tri* gene, in contrast, is expressed in both sexes. A fifth sex-controlled gene, the expression of which is confined to the female sex, *fla*, also exists. However, the dependence of the *flavicollis* phenotype on the presence of the *fla* gene is perhaps not so clear-cut as in the case of the other four genes and their respective phenotypes.

Samples collected from the field reveal the extreme variability and obvious heterogeneity of the basic colour form, *typica*. Obviously a great number of modifying colour genes, the effect of some of which is probably further modifiable by external factors, combine to create the long array of phenotypes from uniformly light to uniformly dark forms. Many of these modifying genes are not allelomorphs of the major colour pattern genes. Although it is likely that the *tri*, *mar*, *lat*, *fla*, *lce* and *lop* genes are allelomorphs or closely linked loci, and that they are all autosomal, even this has not been conclusively established. For this reason, neutral and noncommittal symbols for these loci or genes are used throughout this paper.

Provided that the penetrance of the colour genes is complete, the dominance of the *tri*, *mar*, *lat*, *fla*, *lce* and *lop* genes makes estimation of 2pq frequencies fairly easy in populations in which the phenotypes are sharply demarcated. For these genes, the 2pq frequencies vary between 1 % and 7 % in most populations in Finland. The homozygotes thus contribute insignificantly to the gene frequencies in these populations. At the present phase of genetical work on *Philaenus* it is not known whether some of the genes are possibly lethal when homozygous. We also know nothing of the order of dominance or epistasis in this array of colour genes. The experimental program for obtaining F_1 is being extended by us and steps for producing F_2 have been taken.

In many respects, colour polymorphism in *Philaenus* affords an interesting comparison with what is known of balanced polymorphisms in the Lepidoptera. As with the butterflies, polymorphism in the meadow spittlebug is largely sex-controlled. It seems that many of the rules governing polymorphism in the Lepidoptera may also be valid in this Homopteran. After a long period of persistent and painstaking work, both the genetics and ecology of certain butterflies are now being investigated at a rather advanced stage (e.g. SHEPPARD, 1961). With *Philaenus*, ecological work synchronized and parallel with genetic analysis has been barely begun by us. *Philaenus* affords favourable material for studies on sex-controlled inheritance and also for investigations on the evolution of dominance.¹

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SUMMARY

The mode of inheritance of the different genes determining the colour forms of *Philaenus spumarius* was studied at the F_1 generation level.

Six major genes, each one responsible for a distinct colour pattern, were observed in this work. In a single dose, five of these genes, here provisionally named *mar*, *lat*, *fla*, *lce* and *lop*, always manifest their

¹ *Note added in proof:* The first adult specimens belonging to the F_2 generation emerged in November 1966. They show dominant transmission of the genes *tri* and *lop* and strengthen the view that in the females *typ* is the bottom recessive in a dominance hierarchy series. A detailed account of the F_2 progeny will be published in another context.

effects in the females but never in the males. The sixth gene, *tri*, has a dominant mode of inheritance in both sexes.

The *flavicollis* phenotype may perhaps have three alternative genetic backgrounds: a) the *fla* gene, b) action of darkening modifiers on the *typica* genetic condition and c) action of genes nonallelic with *fla* or *lce* and epistatically causing the appearance of two white spots on both elytrae of an individual with a *lce* gene in her genotype.

The six major genes may be allelomorphic with each other.

The expressivity of the six major genes is remarkably independent of external factors, including food plants of the P and F₁ generations.

The possibility that the six major genes constitute a dominance hierarchy is being further studied, together with the ecological aspects of the sex-controlled inheritance of the colour genes.

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