TWO NEW GENES, "WHITISH" AND "BLONDE", PRODUCING PALE MALES AND FEMALES OF *COLIAS PHILODICE*

by CHARLES L. REMINGTON

1. INTRODUCTION

Approximately sixty species of *Colias* are recognized, one or more in every major land-mass of the non-Antarctic world except Australia and the East Indies. It is probable that nearly all of these species have populations dimorphic for ground-color of the female, although the "alba" form may be very rare (Remington, 1954). This dimorphism is controlled by a pair of genes; the allele which produces the white form is dominant over the allele for the male-like yellow or orange form (Gerould, 1911, 1923). Collectors in North America are familiar with the abundant "alba" females of *Colias philodice* and *C. eurytheme,* as are European collectors with the white form of *C. croceus,* Japanese lepidopterists with the "alba" female of *C. erate poliographus,* and South Americans with *C. lesbia.*

Hopkins (1894) named the yellow pigment of *Colias* and other Pieridiдæ "Lepidotic acid" and identified the white pierid pigment as true uric acid. However, Wieland & Schöpf (1925, 1926) succeeded in crystallizing both of these substances and found that the white pigment (which they named "Leukopterin") differs from uric acid; they named the yellow pigment "Xanthopterin". It has been shown that leucopterin is merely oxygenated xanthopterin. Species characteristically white, such as various *Pieris,* were used in the earlier studies on leucopterin. It remained for Ford (1947) to show that the white pigment of female *Colias* is also leucopterin, as expected.

In the six species of *Colias* in which the heredity of this female dimorphism is known, the allele for white color is dominant over that for non-white. It seems likely that this will prove to be true for the other species and that these genes are homologous not only among the species of *Colias,* but in *Eurema, Phœbis,* and some other Pieridiдæ.

There are, however, extremely rare males* of *Colias* which are more or less white. I have found references to rare white males of *C. aurorina heldreichi* Stgr. (Reichel, 1950), *C. croceus* Fourcroy (Warrier, 1951), *C. erate poliographus* Motsch. (Komai & Aé, 1953), *C. eurytheme* Bdv. (Hovanitz, 1944), *C. h. hyale* Linné (Metschl, 1922), *C. phicomone* Esper (Verity, 1911), and *C. philodice* Godart (Edwards, 1892; Gerould, 1911). Probably there are others. All of the cases listed above have been reviewed and discussed elsewhere (Remington, 1954).

*Herrera (1952) has recently found for *Colias flaveola* Blanchard, of the Chilean Andes, that the males are nearly as white as the females. Previous investigators had mistaken males for females! Genetic and chemical studies of this unusual situation have not yet been reported.

2. WHITE MALE FROM CONNECTICUT

It was of great interest to discover in August 1950 a very pale male among the F_2 of a wild yellow female *C. philodice* (Stock 58) taken in Woodbridge, Connecticut. This male proved to represent a distinctive hereditary form hereafter called "whitish". The wild female, her twenty-four sons and thirty-four daughters and her five F_2 granddaughters and ten F_2 grandsons (other than the lone "whitish" male) were all pure *philodice*-yellow. This line was one of more than one hundred reared in the course of a long investigation of the relationships of *C. philodice* to *C. eurytheme* and of the genetics of each species.

We immediately set out to pair the "whitish" male with one of his sisters, and on 7 August we confined him in a standard copulation cage in the sun for five hours, with no success. The next day the "whitish" male was placed in the sun with four sisters, three brothers, and four yellow females from other lines. To our delight, the "whitish" male paired after less than 25 minutes with one of the non-sib females and remained *in copulo* for over an hour. The following day the male was confined in the sun with two sisters, three brothers, and three non-sib females. In 8 minutes he had again paired, this time with a sister, and the copulation lasted nearly two hours. On 10 August the male was back in the copulation cage, with four females and two brothers, but no further mating took place. The male died shortly afterward. Three matings were obtained in this cage during the four days, and the only one in which the "whitish" male did not participate commenced after his removal *in copulo* on 8 August. There can be no doubt of the high reproductive viability of this individual, in contrast to that of some other white males recorded by earlier authors (*e.g.,* Komai & Aé, 1953; Hovanitz, 1944).

The first mating had been with a female F_1 of a wild "alba" female of *C. philodice* (Stock 62) from Woodbridge. The F_1 female having been yellow, she must of course have lacked entirely the "alba" allele of the mother; she had eight yellow sisters and eight white sisters. The mate of the "whitish" male laid many ova on potted *Trifolium repens* (White Clover), and from these we reared nine females and fourteen males, all with *philodice*-yellow ground-color. Since none of the twenty-three F_1 of the aberrant male showed the "whitish" character, it could be assumed with some confidence that the character was recessive to the normal type or that it was non-hereditary; lethality would not be expected, in view of the vigor of the known male.

These twenty-three F_1 emerged in September, a season when we had come to expect poor mating frequencies in our cages. On three successive days three to five males and five to eight females were placed in the mating cage in the sun for a total of over twelve hours, but no copulations were obtained. Following a full week of unsuitable weather, on 23 September we again tried five males and eight females, and this time no less than four pairs were found *in copulo* within an hour. Although each of the four females laid some ova, only nine F_2 of the "whitish" male were reared to emergence from the pupal shell, two males and one female from each of three females. One of these F_2 males was partly yellow but had portions of both pairs of wings whitish, with the white areas on the right and left wings symmetrical. His eight sibs were pure *philodice*-yellow. During parts of three days four males and one female F_2 of the "whitish" male were confined in

the mating cage; none copulated. Later (26 November) one of these males paired with a female of *C. philodice* from another Woodbridge line (Stock 70); from this mating one female and six males were reared, all normal *philodice*-yellow. One of these males copulated twice, with his lone sister on 21 January 1951 and earlier, on 13 January, with an "alba" female from still another Woodbridge line (Stock 75). This 75A female was remarkable in being the sister of a new type of pale male (see below for record of progeny). The 21 January mating produced nine "alba" females, 13 yellow females, and sixteen yellow males, none with the "whitish" character. The eight F_2 of this mating included two "alba" females, two yellow females, and four yellow males; again none were "whitish". None of these F_2 mated.

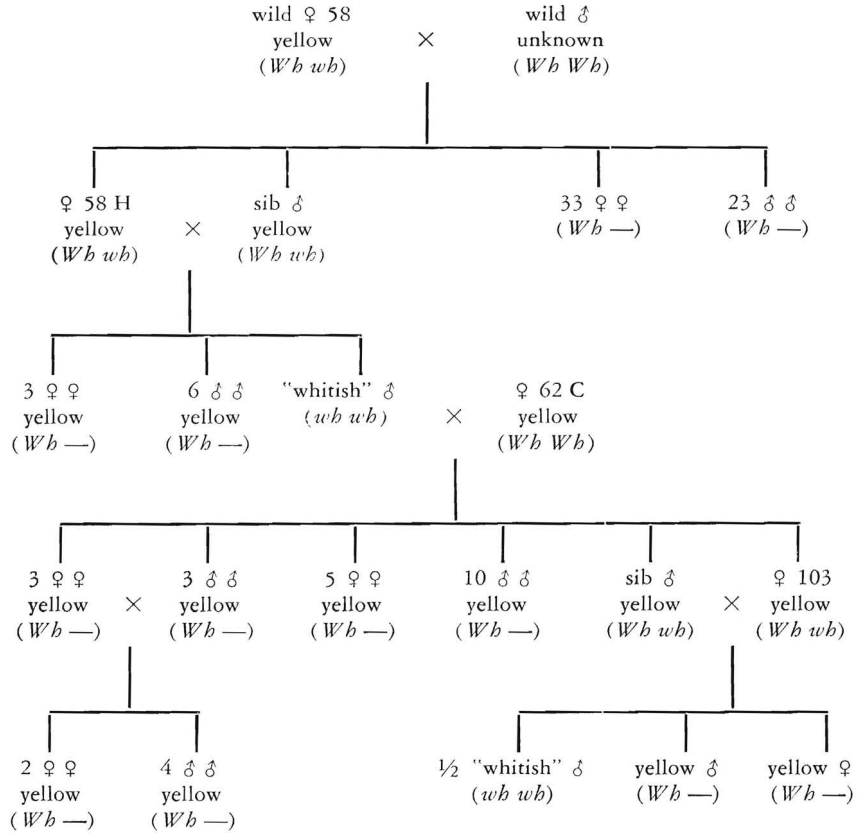

FIG. 1. GENEALOGY OF "WHITISH" LINE OF *COLIAS PHILODICE*

Distinctive characters of the two "whitish" males are contrasted as follows with the characters of two wild-type males selected as average; the color terminology is that of RIDGWAY'S standard system (1912):

FULLY "WHITISH" MALE: Ground-color of upperside and underside Light Greenish Yellow; underside with hindwing and tip of forewing with sprinkling of dark scales; hindwing discal spot on upperside Pinkish Buff; hindwing discal spot on underside silvery, ringed with pale Prout's Brown; head with long, slender, erect hairscales of vertex mostly white; antennal shaft with scales faintly rosy, scales of club white; most wing-fringe scales lost.

The PARTLY "WHITISH" MALE F_2 of the above male did not expand well after eclosion, and part of the left hindwing was destroyed. The extensive pale areas stand out strongly from the adjacent yellow areas and on the upperside are as follows: left and right forewing whitish from slightly anterad of vein M_3 to vein 2A, except basal one-half of second cubital cell; left and right hindwing white from vein Cu_1 to anal margin (in wild-type the scales of the anal and second cubital cells are usually whitish). These "whitish" areas are also visible on the underside. The antennal scales are mostly rosy, but there is a sprinkling of white scales not seen on wild-type *C. philodice*. The erect hair-scales of the vertex and anterior noturn are white, differing from the more or less bright rosy color of these scales in wild-type individuals.

YELLOW MALES: Ground-color of upperside Light Greenish Yellow to Pale Greenish Yellow; underside with hindwing and tip of forewing Wax Yellow, with sprinkling of dark scales; hindwing discal spot on upperside Capucin Yellow in center; hindwing discal spot on underside ringed with Ferruginous; head scales of vertex and antenna Spinel Pink; wing-fringes Spinel Pink.

If the partly white male is considered genetically the same as the fully "whitish" male, the probability is highest that the "whitish" condition is controlled by the recessive allele of a single gene. Somewhat less likely is the possibility that there are two pairs of genes, with double recessives in both necessary to produce "whitish" wings. The genealogy of this line is shown in Figure 1. The genetic notation *wh* is used for the recessive allele for "whitish".

3. Second Pale Form From Connecticut

A wild yellow *C. philodice* female (No. 75) from Woodbridge produced five daughters and one son. The male and one female are pale creamy yellow with the hindwing cell spot whitish. This is a new type, to which I will hereafter refer as "blonde", with the genic notation *bl* for the mutant allele. Three other sib females appear to show the usual "alba" allele for whiteness, and the remaining female is *philodice*-yellow. The "blonde" female and male did not mate. One of their "alba" sibs copulated with a male from the earlier "whitish" male stock (see above). From this female sixty-one F_1 were reared: fourteen "alba" females; twenty yellow females; and twenty-seven yellow males; none with an indication of "blonde". Fifteen brother-sister matings were obtained in early March, and offspring of nine females (two "alba") were reared to maturity, with the following totals recorded: one "blonde" female; eleven "alba" females; thirty-three yellow females; and forty-nine yellow males. The single "blonde" female was one of three offspring reared from one mother, the other two sibs (a male and a female) being *philodice*-yellow. Since neither parent showed the "blonde" character, it is reasonable to assume that the allele for "blondeness" is recessive to the wild-type and that the parents were heterozygous. The pedigree, with suspected genotypes, is shown in Figure 2.

The first "blonde" female and male may be distinguished readily from an "alba" female by the following characters (the last "blonde" female has been lost, so a detailed description is no longer possible, but close comparisons had shown her to be practically identical to the earlier "blonde" female):

"BLONDE" FEMALE: Ground-color of upperside Pale Chalcedony Yellow; underside with ground-color of hindwing and tip of forewing Cream-Buff, remainder of forewing nearly white; hindwing discal spot on upperside Pale Chalcedony Yellow; hindwing discal spot on underside silvery, ringed with Chatenay Pink; hindwing fringe on underside whitish Pale Vinaceous Lilac; forewing fringe darker; head with long, slender, erect hair-scales of vertex white; antennal shaft and tip with scales very faintly rosy.

"BLONDE" MALE: Ground-color of upperside Light Chalcedony Yellow; underside with ground-color of hindwing and tip of forewing pale Honey Yellow, remainder of forewing Light Chalcedony Yellow; hindwing discal spot on upperside white, standing out in contrast to ground-color; hindwing discal spot on underside silvery, ringed with Jasper Pink; hindwing fringe on underside Chatenay Pink; forewing fringe on underside Chatenay Pink; head with long, slender, erect hair-scales of vertex white; antennal shaft and tip with scales only slightly rosy.

"ALBA" FEMALE (selected as average): Ground-color of upperside white; underside with ground-color of hindwing and tip of forewing Cream Color or Naples Yellow, remainder of forewing white; hindwing discal spot on upperside Light Cadmium; hindwing discal spot on underside silvery, ringed with Ferruginous; hindwing fringe on underside Deep Vinaceous; forewing fringe on underside slightly darker; head with long, slender, erect hair-scales of vertex rosy; antennal shaft and tip with rosy scales.

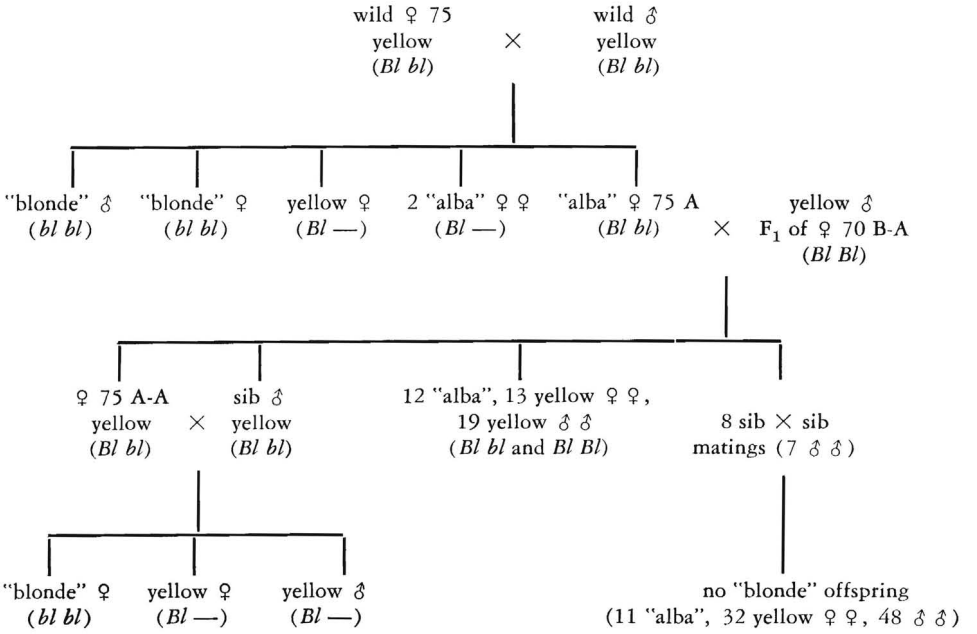

FIG. 2. GENEALOGY OF "BLONDE" LINE OF *COLIAS PHILODICE*

If, as is likely, ♀ 75A was heterozygous for "blonde" and her mate homozygous for wild-type, one in four of the mated pairs of their offspring would be expected to have both the male and female heterozygous. Further, 25% of the expected yield of each such cross would be "blonde", or one in sixteen of all the F_2 of ♀ 75A. Actually, only one in ninety-four was reared. Nothing is known regarding the effects of the "alba" gene in making the

"blonde" gene when an individual *Colias* has both, but it seems likely that "blonde" (as well as "whitish") is expressed in females only in the absence of "alba". If this is true, the eleven "alba" sibs of the "blonde" female are to be ignored in calculating the incidence of "blonde" in the brood of ninety-four, and the observed ratio becomes 1 : 82. The chi-squared test shows that such a deviation from a 1 : 15 ratio is to be expected in just over 5% of trials, and for the present the inheritance of the "blonde" gene can be considered unifactorial.

An attempt was made to find a relationship between scale morphology and ground-color. It was hoped that this might lead to a recognition of specimens heterozygous for the "blonde" gene (*Bl bl*) and perhaps permit separation of a female both "alba" and "blonde" (*A — bl bl*) in phenotype from one solely "alba" (*A — bl —*). The scales were classified according to the number of apical teeth; most specimens have scales with two, three, and four teeth. For uniformity the sample counts were made in the pale spots in the black margin of the forewing of females; four spots were sampled in each specimen: 1) between veins R_5 and M_1; 2) between M_1 and M_2; 3) between M_3 and Cu_1; 4) between Cu_1 and Cu_2. The great observed variability of proportions of the scale types does not seem to be correlated in any simple fashion with ground-color, but a much larger series might reveal some kind of regularity. It was found that the scale-type ratio did not vary significantly among the four spots on each wing; nor was the difference between left and right wings significant. The totals are shown in the following summary, with the notation for each specimen giving scales with two, three, and four points in sequence (*e.g.*, the "blonde" female showed 158 scales with two points, 487 with three points, 2 with four points):

"alba" (*C. philodice*) — 592-195-0; 286-206-0; 124-505-1; 89-293-226
"alba" (*C. eurytheme*) — 903-8-0; 443-386-0; 248-273-6
"blonde" (*C. philodice*) — 158-487-2
yellow (*C. philodice*) — 469-15-0; 364-51-0; 55-562-23; 79-808-92; 8-487-444
orange (*C. eurytheme*) — 385-162-1; 185-298-17.

4. Summary

a. The common *Colias* form with white ground-color is controlled by a dominant sex-limited gene (*i.e.*, carried by both sexes but expressed only in females). This "alba" female form is present in most species of *Colias* and in members of certain related genera. The pigment producing the "alba" coloration is leucopterin; other pterins are present in males and in non-"alba" females.

b. White or very pale ground-color occurs in males of *Colias*, but generally the white males are extremely rare in natural populations. This male dimorphism has been recorded for seven species of *Colias*. Two new cases in *C. philodice* are reported here for the first time. Both appeared in laboratory stocks reared from a single small population in Connecticut.

c. One white male was obtained by inbreeding the offspring of a wild fertile female. This male was outcrossed and its F_1 then inbred. The F_2 included a male with extensive white areas on the wings. This pale form is presumed to be under the control of a single recessive allele. The form is called "whitish" (*wh wh*). There is no reason to consider it a male sex-limited gene, although "whitish" females have not yet been found.

d. A second pale male was among the six F_1 of another wild female *C. philodice.* In this instance one of the female sibs was of the same pale shade, Light or Pale Chalcedony Yellow. The hindwing discal spot lacked completely the reddish or orange pigmentation of wild-type *C. philodice.* The form is called "blonde" (*bl bl*). One of the non-"blonde" sibs of the "blonde" male and female was outcrossed, and among the sixty-one F_2 was one "blonde" female. "Blonde" is probably inherited as a single recessive factor quite distinct from the gene for "whitish". It is not sex-limited. Phenotypically, the "blonde" form differs from the "whitish" form in having deeper, more yellow ground-color and no orange or red pigment in the hindwing discal spot.

e. No correlation was found between scale shape and the ground-color of "blonde", "alba", yellow, or orange females of *C. philodice* and *C. eurytheme.*

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Osborn Zoological Lab., Yale University, New Haven 11, Conn., U.S.A.