Cream and albinotic – two new mutations affecting body colour in Pyrrhocoris apterus (Heteroptera: Pyrrhocoridae)

RADOMÍR SOCHA

Biology Centre ASCR, Institute of Entomology, Branišovská 31, CZ-370 05 České Budějovice, Czech Republic, e-mail: socha@entu.cas.cz

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Abstract. Two new body-colour mutations, albinotic (alb) and cream (cr) are isolated and described for the flightless wing-polymorphic bug, Pyrrhocoris apterus (L.). The body colour of alb mutants is white and that of cr mutants either a light cream or creamy yellow. Both the alb and cr mutations most probably inhibit the biosynthesis of the red pigment in epidermal cells that is responsible for the red-coloured body of wild-type bugs. Although sometimes the body colour of younger larval instars of cr mutants is a slightly darker creamy yellow and that of last larval instars of the alb mutants a slightly whiter greyish, there were no other important developmental changes in the body colour of alb and cr homozygous bugs associated with metamorphosis and post-metamorphic aging of adults. Standard genetic analyses showed that both these mutations are inherited as autosomal recessives and exhibit complete penetrance and uniform expression in both sexes. The fertility of the alb and cr homozygous females and fertilization success of the alb and cr homozygous males were substantially lower than those of wild-type bugs.

INTRODUCTION

The firebug Pyrrhocoris apterus (L.) is a widely distributed palaeartic species of the heteropteran family Pyrrhocoridae, with the core of its distribution in the Mediterranean area and eastern and central Asia. It is characterized by a non-functional wing-polymorphism (Honěk, 1976; Socha, 1993) since both the long-winged (macropterous) and short-winged (brachypterous) specimens of this bug are flightless (Socha & Zemek, 2000). In central Europe, the bug mostly lives at the foot of lime trees (Tilia cordata Miller, T. platyphlos Scopoli) and mainly feeds on their seeds (Socha, 1993). This bug is a convenient model insect because it is easy to rear in the laboratory. Socha (1993) gives a detailed account of the biology of P. apterus. The popularity of this insect as an experimental model for biological research is reflected in the large number of papers on this species. The firebug was successfully used to study hormonal regulation of mating success in males and maturation of their reproductive accessory glands (Socha, 2004, 2006; Socha & Hodková, 2006), development and histolysis of non-functional flight muscles (Socha & Šula, 2006, 2008) and for identifying the bug’s native adipokinetic hormones and their stimulatory effects on walking activity (Socha et al., 1999; Kodrik et al., 2000, 2002a, b, 2003, 2010) and other important topics of insect physiology and behavioural endocrinology.

Description of chromatin elements in P. apterus by Henking (1891) was in fact the first report of sex chromosomes. Since then P. apterus has been intensively studied by insect physiologists and endocrinologists, but virtually ignored by geneticists. The genetics of P. apterus was not investigated until 1968, when the white (wh) mutation of this bug was first described and shown to cause the white-body colour of mutant specimens and inherited as a single autosomal recessive (Rizki & Sláma, 1968). To date, the following pigment mutations and genetic strains of P. apterus are described and analysed: yellow (yw) (Socha, 1984; Sláma, 1998); reddish lobes (rl 17) (Socha, 1984); melanotic (m) (Socha, 1984; Sláma, 1998); mosaic (mo) (Socha, 1987b); Pale (Pa) (Socha, 1988a); Apricot (Ap) (Socha, 1988b) and yolky body (yb) (Socha, 1997). These mutations affect the biosynthesis and deposition of pterines in epidermal cells or melanine pigments in the cuticle of P. apterus (Socha & Němec, 1992, 1996; Porcar et al., 1996; Bel et al., 1997). The yw, m, and yb mutations are inherited as single autosomal recessives, Pa and Ap as autosomal dominants, and the mo trait behaves as an unstable sex-linked recessive and is probably transposon-mediated (Socha et al., 1993).

The aim of the present study was to elucidate the mode of inheritance of the newly isolated albinotic (alb) and cream (cr) mutations of P. apterus, and to determine the reproductive potential of alb and cr mutant males and females. The results increase the contemporary knowledge of the formal genetics and genetic regulation of body colour in P. apterus and provide new tools for chromatographic studies on the biosynthesis of pteridines.

MATERIAL AND METHODS

Experimental conditions

The wild-type bugs were collected from a population located in České Budějovice, Czech Republic (48°59’ N, 14°28’ E). All stages from egg to adult were reared under a long-day (18L: 6D) photoperiod and a constant temperature of 26 ± 1°C, which allow the bugs to breed continuously. Under these conditions, the wild type and mutant stocks required approximately 1 month to develop from egg to adult. Larvae and adults of all stocks were kept in glass jars (0.5 l) in mass culture (approximately 40
specimens per jar) and supplied with linden seeds (*Tilia cordata*) and water ad libitum, which was replenished twice a week. The water was supplied in small glass tubes plugged with cotton wool. More details of the breeding of the firebug are described elsewhere (Socha & Šula, 1996; Socha et al., 1997). For each genetic cross, several single pairs and mass matings were established. Matings of single pairs and mass matings were made in individual Petri dishes (Ø = 6 cm) and 0.5 l glass jars, respectively, supplied with linden seeds and water in glass tubes plugged with cellulose wool.

**Isolation of alb and cr mutations**

Stocks of the wild-type specimens and the alb and cr mutants of *Pyrrhocoris apterus* were used in the present study. The alb and cr mutant stocks were established in 1997 with 5–7 mutant specimens that occurred spontaneously among the F1 progeny from a wild population of *P. apterus* collected in the vicinity of České Budějovice, Czech Republic (48°59´N, 14°28´E). In succeeding generations, approximately 20–30 pairs of mutant individuals were used for mass mating to establish the alb and cr mutant stocks. Adults of the fifth generation of both wild-type and mutant strains were used for genetic crosses. Freshly ecdysed wild-type and mutant adult bugs were separated according to sex and placed in groups of 10–20 specimens in small glass jars (250 ml) and kept under the same photoperiodic and temperature regime in which they had developed. They were supplied with linden seeds and water. After reaching a required age, adult males and females homozygous for alb and cr genes were used for analysis of reproductive potential and determining the pattern of inheritance of these traits.

**Analysis of reproductive potential of the alb and cr mutants**

For the comparison of the reproductive capacity of the alb and cr homozygous females with that of wild-type females, used as controls, the first five successive egg batches laid by individual mutant and wild-type females of a particular cross were collected and the number of eggs per batch and their hatchability determined. Five days old reproductively active females, i.e. those at their highest attractiveness and receptivity (Zdářek, 1970) were used in the fertility tests. For the comparison of the fertilization success of the alb and cr homozygous males with that of wild-type males, used as controls, the first five successive egg batches laid by wild-type females inseminated individually by one of the 11–14 males of the alb or cr homozygous mutants were collected and their average hatchability assessed.

Adult males and females from all crosses tested were virgins and used only once. Egg laying and mortality of parents were checked daily. Sterile or unfertilised eggs that did not yield larvae were categorized as unhatched.

**Standard genetic analyses**

To determine the mode of inheritance of the alb and cr traits, standard reciprocal genetic crosses between alb and wild-type bugs and between cr and wild-type bugs were performed. Both kinds of matings were done under a long-day (18L : 6D) photoperiod and a constant temperature of 26 ± 1°C. Progenies derived from these crosses (P, F1, and backcrosses) were classified by sex and phenotype in the 4th or 5th larval instars. The alb, cr, and wild-type phenotypes were expressed uniformly in both sexes within each set of crosses. Since there were no significant differences in sex ratio in these crosses, except for a slight tendency for males to prevail in most of crosses, the data obtained for both sexes were combined. The results of phenotypic evaluations obtained for each cross of standard genetic crosses were analysed statistically by chi-square for goodness-of-fit.

**Data presentation and statistical analyses**

Arithmetic means of the results are presented throughout the paper. Statistics were computed using the software GraphPad Prism, version 4.0 (San Diego, CA, U.S.A.). A one-way analysis of variance (ANOVA) followed by the Tukey’s multiple comparison tests was used to compare the numbers of eggs in batches and their hatchability among the experimental groups determined. The results of phenotypic evaluations obtained for each of the standard genetic crosses were analysed statistically by chi-square for goodness-of-fit.

**RESULTS**

**Morphology of the alb and cr mutants of *P. apterus***

The alb and cr individuals of *P. apterus* are clearly distinguishable from the wild-type bugs as they differ in the pattern of their body pigmentation (Fig. 1). Unlike the red-coloured wild-type larvae (Fig. 1C) and adults (Fig. 1F), the colour of the bodies of alb mutant larvae (Fig. 1A) and adults (Fig. 1D) is white and that of cr mutant larvae (Fig. 1B) and adults (Fig. 1E) ranged from a light cream to creamy yellow colour. Thus, larvae and adults with both the alb and cr mutations are characterized by the lack of red pigment in the epidermal cells, and as a consequence the non-melanized parts of their bodies are either white or creamy yellow, respectively, in contrast to the red colour typical of wild-type bugs. No other important developmental changes in body colouration of the alb and cr homozygous bugs associated with metamorphosis and post-metamorphic aging of adults were observed, except sometimes the young larval instars of the cr mutants had a slightly darker creamy yellow colour and the last larval instar of the alb mutant were slightly tinged with a whitish-greyish colour. Both mutant phenotypes were expressed uniformly in both sexes.

**Reproductive potential of alb and cr homozygous males and females**

The reproductive capacity of the alb and cr mutants and that of the wild-type bugs, including the average number of eggs per batch and their average hatchability, is presented in Table 1.

The ANOVA revealed that batches of eggs laid by the alb (cross B) and cr (cross C) mutant pairs contained significantly (*P* < 0.01) fewer eggs than those laid by the wild-type pairs of the same age (cross A), but no significant difference in the average numbers of eggs laid by the alb and cr mutant pairs. This indicates that the reproductive potential of both the alb and cr mutants is substantially lower than that of wild-type bugs. However, no significant differences were found in the average numbers of eggs laid by reciprocal crosses between alb males and wild-type females (cross D), and between the alb females and wild-type males (cross E), which indicates that both sexes are responsible for the lower reproductive capacity of the alb mutants. The average number of eggs per batch produced by both reciprocal crosses between cr males and wild-type females (cross F), and between cr females and wild-type males (cross G) was significantly lower (*P* < 0.01) than in those laid by wild-type pairs (cross A). Despite this fact, the significant differences (*P* < 0.05)
between the average numbers of eggs laid by wild-type females mated with \( cr \) males (cross F) and those laid by \( cr \) females mated with wild-type males (cross G) indicate that it is mainly the homozygous \( cr \) female that is responsible for the lower reproductive potential of \( cr \) mutants.

The ANOVA also revealed that the average hatchability of eggs laid by \( alb \) mutant pairs (cross B) and \( cr \) pairs (cross C) was significantly \((P < 0.01)\) lower than that of eggs laid by wild-type pairs of the same age (cross A). Like the average numbers of eggs, there were no significant differences in the average hatchability of eggs laid by the \( alb \) and \( cr \) mutant pairs (crosses B and C). These results indicate that not only the production but also the hatchability of eggs laid by the \( alb \) and \( cr \) pairs was substantially lower than that of the eggs laid by wild-type pairs. Since no significant differences were found in the average hatchability of eggs laid by reciprocal crosses between \( alb \) males and wild-type females (cross D) and between \( alb \) females and wild-type males (cross E), it is likely that both sexes are responsible for the lower hatch-
ability of these eggs. Both sexes are responsible also for the lower hatchability of eggs obtained from the reciprocal crosses between \( \text{cr} \) males and wild-type females (cross F), and \( \text{cr} \) females and wild-type males (cross G), since there were no significant differences in the average hatchability of eggs obtained from these crosses. Since the fertilization tests showed that the hatchability of eggs laid by wild-type females fertilized by either \( \text{alb} \) or \( \text{cr} \) males was lower than that of wild-type females of the same age fertilized by wild-type males, it is likely that fertilization success of \( \text{alb} \) and \( \text{cr} \) mutant males is lower than that of wild-type males. Thus, the above results revealed that homozygous sets of \( \text{alb} \) and \( \text{cr} \) genes are responsible for both the lower fertility of \( \text{alb} \) and \( \text{cr} \) females and lower fertilization success of \( \text{alb} \) and \( \text{cr} \) males.

**Pattern of inheritance of the \( \text{alb} \) and \( \text{cr} \) traits**

Standard genetic crosses were used to assess the pattern of inheritance of the \( \text{alb} \) and \( \text{cr} \) mutations. The results of mating wild-type pairs and \( \text{alb} \) homozygous mutant pairs (crosses A and B, respectively) are presented in Table 2. Significantly fewer offspring resulted from cross B than cross A. This confirmed the lower reproductive potential of the \( \text{alb} \) mutants recorded in Table 1 (cross A and B). Both reciprocal \( F_1 \) crosses (crosses C and D) resulted in \( F_1 \) progenies containing only individuals with the wild-type phenotype. When \( F_1 \) progenies were intercrossed (crosses E and F), the wild-type and \( \text{alb} \) phenotypes segregated in a ratio of 3 : 1 in favour of wild-type bugs. This indicates that the \( \text{alb} \) trait is under control of a single autosomal gene. The recessive character of the \( \text{alb} \) mutation was confirmed also by the reciprocal backcrosses (crosses G, I and M), giving a 1 : 1 ratio between the wild-type and \( \text{alb} \) bugs, independent of their sex. A marginally significant departure from the expected 1 : 1 ratio in one of the reciprocal backcrosses (cross K) was probably due to the lower viability of the homozygous \( \text{alb} \) individuals. Besides decreased viability of \( \text{alb} \) individuals found in cross K and lowered reproductive potential of the \( \text{alb} \) mutants recorded in Table 1 (cross A and B).
mutants, no other pleiotropic effects of alb locus on the larval development and adult life were observed.

The results of mating wild-type individuals and cr homozygous mutant bugs (crosses A and B, respectively) are presented in Table 3. In comparison to cross A, only a few cr individuals were obtained in cross B, which indicates that the cr mutations have a low reproductive potential. Both reciprocal F1 crosses (crosses C and D) resulted in F1 progenies containing only individuals of the wild-type phenotype. When F1 progenies were intercrossed (crosses E and F), the wild-type and cr phenotypes segregated in a ratio of 3 : 1 in favour of wild-type bugs. These results indicate that the cr trait is controlled by a single autosomal recessive gene. The small, but significant departure from the expected 3 : 1 ratio in one of the crosses (cross E) was most probably due to the lower viability of the homozygous cr individuals. The autosomal recessive character of the cr mutation was confirmed by reciprocal backcross I, resulting in a 1 : 1 ratio of wild-type to cr bugs in the offspring, independent of their sex. The significant departures from the expected 1 : 1 ratio in reciprocal backcrosses K and M was due to the lower viability of the cr homozygous bugs. Thus, in addition to the lower fertilization success of cr homozygous males and the low fertility of cr homozygous females, the cr locus is also associated with the lower viability of the homozygous cr individuals.

**DISCUSSION**

**Pattern of inheritance of alb and cr mutations and their effect on reproductive potential**

The present paper describes the morphology and pattern of inheritance of two newly isolated body-colour mutations (alb and cr) in *P. apterus*. The present study indicates that both of these mutations are inherited as autosomal recessives and exhibit complete penetrance and uniform expression in both sexes. Considering previous studies on the formal genetics of *P. apterus*, the alb and cr mutations are the fifth and sixth autosomal recessive mutations inhibiting the synthesis of red pigment in the body of this heteropteran. Like most of the other body-colour mutations in *P. apterus*, the alb and cr mutations appeared spontaneously in cultures following the transfer of individuals from wild populations to the laboratory. They were probably present in the wild populations and revealed by the inbreeding that occurred in the small laboratory colonies or induced by sudden changes in some environmental cues, e.g. photoperiod or temperature.

The white-coloured body of larvae and adults of *P. apterus* with the alb mutation resembles the body colour of the previously described wh mutant of this species (Rizki & Sláma, 1968). Despite the fact, that both the alb and wh mutations in *P. apterus* cause white colouration of the body, inhibit the synthesis of red pigment in epidermal cells and are inherited as single autosomal recessives, the reciprocal crosses between the alb/wh and wh/wh mutants yielded only red-pigmented bugs in the F1 generation, which indicates that alb and wh are different genes (Socha, unpubl. data).

The cream or creamy yellow colour of freshly hatched first instar larvae of *P. apterus* with the cr mutation is somewhat similar to that of first instar larvae with the Pa mutation (Socha, 1988a), but in the second, third, and fourth larval instars the body colour of Pa mutants is much deeper – usually dark yellow-orange (Socha, 1997). However, the cr and Pa bugs differ mainly in the pigmentation of their eyes, which is a creamy yellow in the cr mutants and not red-pigmented as in the Pa mutant bugs. Unlike the autosomal recessive character of the cr mutation in *P. apterus*, the Pa mutation is inherited as an autosomal dominant and characterized by post-blastokinesis lethality in Pa/Pa homozygous males and females. The
body colour of young larval instars of some \( cr \) mutants are more yellow than cream-coloured and may slightly resemble the larvae of the \( yw \) mutation of \( P. \) \( \text{apterus} \). However, the yellow body of the \( yw \) larvae is usually more intense and much darker. Moreover, preliminary results indicate that the recessive \( yw \) locus is not situated on the same autosome as the \( cr \) locus (data not shown).

The body colour of individuals of \( P. \) \( \text{apterus} \) with the autosomal recessive \( yb \) mutation (Socha, 1997) differ from that of individuals of the \( cr \) mutation as it is a yolk-like colour, except the eyes, which are red as in wild-type bugs. Moreover, the body colour, particularly that of the forewings of homozygous \( yb \) adults changes from yolk-orange to orange-brick-red. Unlike to the \( yb \) mutants, there are no similar developmental changes in body colour associated with metamorphosis and post-metamorphic aging of adults in the \( cr \) and \( alb \) homozygous bugs.

In addition to the body-colour mutants, including the newly described the \( alb \) and \( cr \) mutations, several other mutations and strains of \( P. \) \( \text{apterus} \) are reported, e.g. mutations affecting the course of choriogenesis (Socha, 1988c), embryogenesis (Socha & Matolin, 1985), metamorphosis (Socha, 1987a), wing length (Honěk, 1986; Socha et al., 1993; Socha, 1995), and diapause behaviour (Socha & Hodková, 1994). Despite the fact that the formal genetics of \( P. \) \( \text{apterus} \) is just beginning to be studied and it is necessary to isolate and characterize other visible genetic markers in this species, some of the body-colour mutations have been already successfully used as markers for studying hormone persistence in the body (Sláma & Socha, 1979), genetic control of biosynthesis of pteridines (Socha & Němec, 1992, 1996; Porcar et al., 1996; Bel et al., 1997), determination of sperm utilization in multiple mating of females (Socha, 1993) and sperm competition (Schöfl & Taborsky, 2002; Socha, unpubl. data). The present study is therefore an important contribution to the formal genetics of \( P. \) \( \text{apterus} \) and an increase in the bank of mutations of this bug that can be used as useful tools in future biological studies.

In addition to the lower fertility of \( alb \) and \( cr \) homozygous females, the fertilization success of \( alb \) and \( cr \) homozygous males of newly isolated mutant stocks is also more or less lower, but the reason for this is unknown. It can be speculated that either the transfer of fewer sperm or less viable sperm, or aberrations in the pattern or transfer of seminal fluid might underlie the low fertilization success of the \( alb \) and \( cr \) homozygous males compared to wild-type males of the same age. It is reported that it is the amount of secretion transferred from the male accessory glands to female reproductive tracts and not the sperm load that determines the higher fertilization success of young brachypterous males compared with macropterous males in \( P. \) \( \text{apterus} \) (Socha, 2006; Socha et al., 2004). It cannot be excluded that the same phenomenon might be partly responsible also for the low fertilization success of the \( alb \) and \( cr \) homozygous males. This suggestion accords well with the knowledge that the components of male seminal fluid act in many ways to maximize a male’s reproductive success and are important for the viability and survival of sperm in the reproductive tract of the females (for reviews see e.g. Tram & Wollner, 1999; Wollner, 2002; Gillott, 2003; Kubli, 2003; Chapman & Davies, 2004; Poiani, 2006). Mating behaviour of \( P. \) \( \text{apterus} \) males (Socha, 2006) and those of \( D. \) \( \text{melanogaster} \) (Wilson et al., 2003) is regulated by juvenile hormone, either directly or indirectly, by a component in the secretion of the male accessory gland. In \( P. \) \( \text{apterus} \), the significantly greater amount of juvenile hormone-dependent 53-kDa glycoprotein in the accessory glands of 3-14 days-old brachypterous males than in macropterous males of the same age is thought to result in a more efficient sperm storage and fertilization of females (Socha et al., 2004). It is likely that different amounts of this and/or some other specific protein in the accessory gland secretion transferred by males might be partially responsible for the low fertilization success of \( alb \) and \( cr \) mutant males.

The effects of \( alb \) and \( cr \) mutations on the biosynthesis of pteridines

It is very probable that both the \( alb \) and \( cr \) mutations, described for the first time in the present paper, also negatively affect the biosynthesis and/or deposition of erythropterine and other pteridines in epidermal cells that are responsible for the dark yellow and red colouration of the body of wild-type individuals of this bug, in a similar way to the \( wh \) and \( yw \) mutations of \( P. \) \( \text{apterus} \) (Porcar et al., 1996; Bell et al., 1997). Only a few body-colour mutations are reported for Heteroptera and very little is known about pteridine biosynthesis, since only a few species have been studied in this respect (Berridge, 1965; Merlin & Nasini, 1966; Smith & Forrest, 1969; Lawrence, 1970; Halfenberg & Stein, 1971; Hollweg, 1972; Smith & Forrest, 1976; Melber & Schmidt, 1992, 1994; Shimizu & Kawasaki, 2001). Thus, future chromatographic studies on pteridines present in \( alb \) and \( cr \) mutants of \( P. \) \( \text{apterus} \) should extend our knowledge on the biochemistry and genetics of the pteridine pathway in insects.

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