Occurrence and inheritance of a colour pattern dimorphism in adults of *Hyalophora euryalus* (Lepidoptera: Saturniidae)

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ABSTRACT

A white prothoracic collar and white abdominal rings are among the characters used to distinguish adults in the genus *Hyalophora* Duncan (Lepidoptera: Saturniidae) from those in the related genera *Callosamia* Packard and *Eupackardia* Cockerell. However, some adults of *H. euryalus* (Boisduval) on southern Vancouver Island, British Columbia, were found to lack these white body markings. Controlled rearing indicated that the "brown" phenotype is produced by a recessive allele at a single autosomal locus, and examination of museum specimens showed that it is fairly common on southern Vancouver Island and has been present there for at least half a century.

INTRODUCTION

Adults in the genus *Hyalophora* Duncan (Lepidoptera: Saturniidae) are large reddish brown moths with white crescent-shaped discal spots, a white prothoracic collar, and white segmental rings on the abdomen; these white markings are the key characters used to separate this genus from the related genera *Callosamia* Packard and *Eupackardia* Cockerell (Ferguson 1972; Lemaire 1978). In the course of other studies (Morewood 1991a, 1991b) an atypical adult phenotype, characterized by the absence of the white prothoracic collar and abdominal rings (Fig. 1), was discovered in *Hyalophora euryalus* (Boisduval) on southern Vancouver Island, British Columbia. This phenotype appears to be unique in the genus *Hyalophora* and has not been reported previously; for example, Tuskes *et al.* (1996) made no mention of the white prothoracic collar or abdominal rings in their discussion of adult variation for any of the taxa within the genus. The objectives of the current study were to document this adult colour pattern dimorphism, determine its pattern of inheritance, and provide preliminary estimates of its prevalence and distribution.

MATERIALS AND METHODS

A small colony of *H. euryalus* was established from eggs laid by wild adult females captured in Saanich, BC, one female on 16 May 1991 and one female on 6 May 1992 (Fig. 2). Larvae were reared indoors on cuttings of Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco ssp. *menziesii*, in large plastic buckets with screened lids. Pupae were overwintered in small cages outdoors, and in the spring, adults were mated using two different methods. In most cases mating cages constructed from coffee cans, as described by Miller and Cooper (1976), were used to mate reared females to wild males to reduce inbreeding. In some cases matings were obtained between reared adults by keeping them together in a larger cage outdoors overnight. Each spring the phenotypes of all reared adults, as well as wild males attracted by the caged females, were recorded as either "normal" (having a white prothoracic collar and abdominal rings) or, for simplicity, "brown" (lacking the "normal" white body markings such that the body appeared brown overall). Twenty-two

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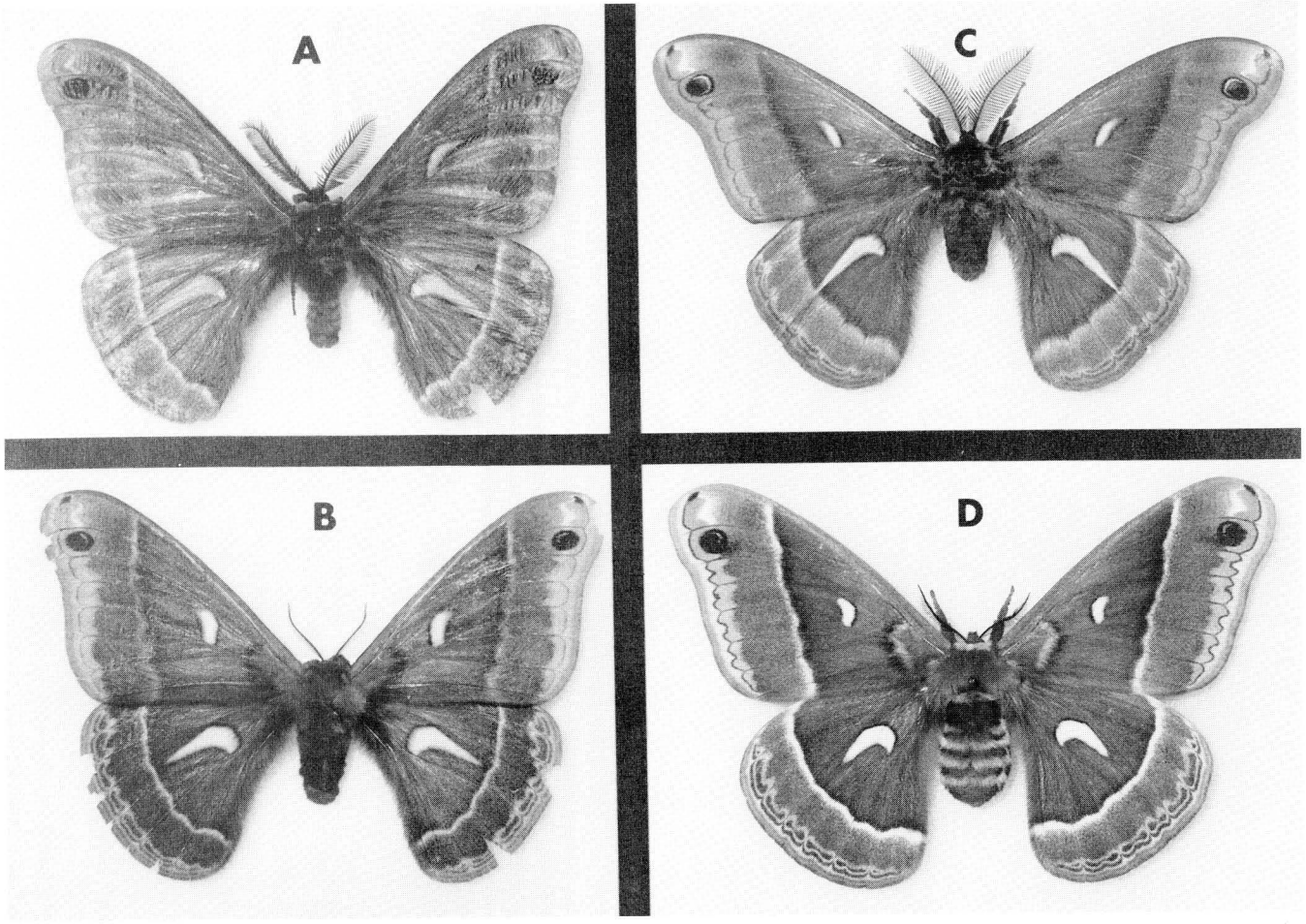

Figure 1. Phenotypically "normal" and "brown" adults of *Hyalophora euryalus* illustrated by specimens from Vancouver Island in the Royal British Columbia Museum collection [catalogue number is given for each specimen]. (A) Normal (very greasy) male, Nanoose, 24 May 1995 [ENT996-006508]. (B) Brown female, Nanoose, 24 May 1995 [ENT996-006511]. (C) Brown male, Saanich, 1959 [ENT991-154531]. (D) Normal female, Victoria, 1958 [ENT991-154526].

different broods were reared between 1991 and 1999, and the study was terminated when in the summer of 1999 all larvae were lost to disease. Due to logistic constraints, only small numbers of larvae were reared from most broods, making analysis of the distribution of adult phenotypes within individual broods unlikely to yield meaningful results. Therefore, broods representing crosses of apparently equivalent genotypes were grouped together and the overall distribution of phenotypes for each type of cross was used to evaluate the pattern of inheritance.

To provide a broader estimate of the prevalence and distribution of the brown phenotype, all of the adult specimens of *H. euryalus* were examined in three major insect collections in southwestern BC, namely those in the Spencer Entomological Museum at the University of British Columbia in Vancouver and in the Pacific Forestry Centre and the Royal British Columbia Museum in Victoria.

RESULTS

Adults were easily classified as having either the normal or the brown phenotype, with no intermediate forms, and both phenotypes were common in both sexes (Figs. 1, 2). The normal pattern of white body markings, in particular the location of the white prothoracic collar, was often detectable as a faint greyish "smudge" in adults with the brown phenotype; however, in no case was there any difficulty in assigning an individual to one phenotype or the other. Crosses in which both parents were brown produced only brown progeny (Brood numbers 3, 5, 9, 16, and 18 in Fig. 2 and Table 1) but crosses in which both parents were normal had the potential to produce progeny of both phenotypes (Brood number 4 in Fig. 2 and Table 1). Crosses in which one parent was brown and the other was heterozygous normal (Brood numbers 2, 6, 7, 8, 10, 11, 12, 13, 14, and 20) produced progeny in almost exactly the expected 1:1 ratio overall (Table 1), including both phenotypes in progeny of each sex (Fig. 2).

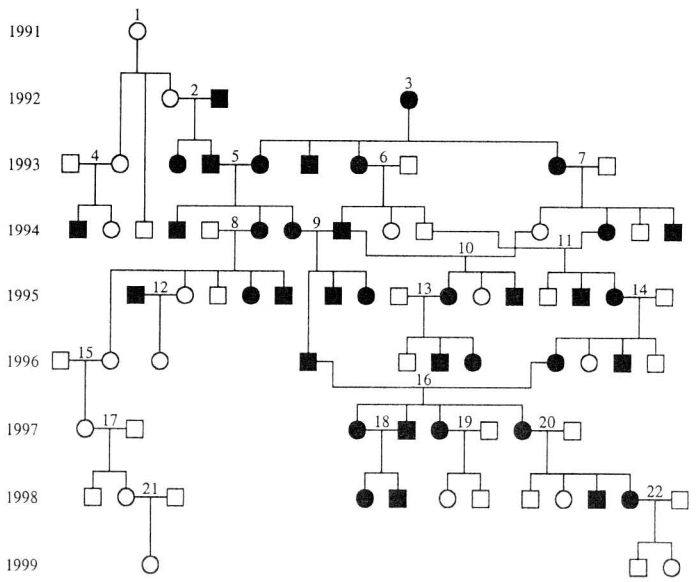

Figure 2. Pedigree for the adults of *Hyalophora euryalus* reared during the course of this study. Circles represent females, squares represent males; open symbols indicate the "normal" phenotype, filled symbols indicate the "brown" phenotype. Numbers above matings, and above the two founding females, are the "Brood numbers" in Table 1.

Table 1

Observed and expected adult phenotypes of *Hyalophora euryalus* reared in Saanich, British Columbia, between 1991 and 1999.

Type of cross (parental genotypes)	Brood numbers[1] (see Fig. 2)	Observed phenotypes Normal	Brown	Expected phenotypes Normal	Brown
Normal x Brown (Bb x bb)	2	0	7		
	6	4	2		
	7	2	4		
	8	11	4		
	10	2	5		
	11	3	3		
	12	1	0		
	13	5	7		
	14	6	4		
	20	6	8		
	Total:	40	44	42	42
Brown x Brown (bb x bb)	3	0	22		
	5	0	5		
	9	0	15		
	16	0	31		
	18	0	4		
	Total:	0	77	0	77
Normal x Normal (Bb x Bb)	4	4	2		
	Total:	4	2	3	1

[1]Excluding broods 1, 15, 17, 19, 21, and 22, for which both parental genotypes could not be conclusively determined.

The phenotypes of 28 wild males attracted by reared females caged in Saanich were recorded during the course of this study; of these, seven (25%) had the brown phenotype. A total of 40 adult specimens of *H. euryalus* were examined at the Spencer Entomological Museum, approximately one-third of these being from southern Vancouver Island and the adjacent Gulf Islands and the remainder from various localities in the southern interior of BC as far north as Riske Creek and as far east as Cranbrook. Only three of these specimens (7.5% of the total) had the brown phenotype, one from Nanaimo (1951), one from Langford (no collection date), and one from Salmon Arm (1961). At the Pacific Forestry Centre 18 adult specimens of *H. euryalus* were examined, over half of these being from southern Vancouver Island and the remainder from the northern Okanagan region of the southern interior, and no brown phenotypes were found. A total of 52 adult specimens of *H. euryalus* were examined at the Royal British Columbia Museum, almost half being from southern Vancouver Island and the Gulf Islands and the remainder of those with locality data being from the southern interior of BC as far north as Williams Lake and as far east as Cranbrook. Six of these specimens (11.5% of the total) had the brown phenotype, two from Saanich (1959 and 1990), one from Nanoose (1995), one from Galiano Island (1989), and two with no locality data (and no collection dates). Overall, of 50 museum specimens from southern Vancouver Island and the Gulf Islands, six (12%) had the brown phenotype and of 40 museum specimens from the mainland (all but one from the interior) of BC, only one (2.5%) had the brown phenotype (the remaining 20 museum specimens had no locality data).

DISCUSSION

The overall distribution of phenotypes was consistent with a simple Mendelian pattern of inheritance. Specifically, the presence or absence of the white prothoracic collar and abdominal rings appears to be controlled by a single autosomal gene with two alleles, a dominant allele ("B") producing the normal phenotype and a recessive allele ("b") producing the brown phenotype (Table 1). The distribution of phenotypes among the progeny of crosses in which the parental female was normal and the parental male was brown indicates that the brown phenotype is not sex-linked. Considering that the female is the heterogametic sex in Lepidoptera, such crosses (Brood numbers 2, 10, and 12) should produce only normal males and brown females if the trait is sex-linked, but that was not the case (Fig. 2).

Other atypical phenotypes with similar patterns of inheritance have been reported previously for various Lepidoptera in the context of biochemistry or evolution or both. Waldbauer and Sternburg (1972) reported that a "blue" larval phenotype arose in their laboratory colony of *Hyalophora cecropia* (L.) and was inherited as a simple autosomal recessive. Based on the work of Clark (1971), they suggested that it was produced by a mutation affecting the biochemical pathway that produces cuticular pigments from dietary carotenoids. Stimson and Meyers (1984) reported that a "white" adult phenotype known since 1965 in the Monarch butterfly, *Danaus plexippus* (L.) (Nymphalidae), in Hawaii is inherited as a simple autosomal recessive. They presented evidence that it might be increasing in frequency due to a lack of natural predators in Hawaii compared to North America where predators would selectively remove adults lacking the normal aposematic colour pattern. Adult females of the Eastern Tiger Swallowtail, *Papilio glaucus* L. (Papilionidae), have either the black and yellow phenotype typical of the species or a melanic phenotype that is thought to mimic the distasteful Pipevine Swallowtail, *Battus philenor* (L.) (Papilionidae) (Brower and Brower 1962). Clarke and Sheppard (1962) presented evidence that the melanic phenotype is completely sex-linked and Koch *et al.* (1998) proposed a biochemical mechanism by which it could be controlled through a single locus on the Y chromosome.

The museum specimens and wild individuals of *H. euryalus* observed during the current study indicate that the brown phenotype has existed for at least half a century and is fairly common on southern Vancouver Island, which suggests that the trait arose in this region and that it offers some selective advantage or at least is not deleterious. The single brown specimen from Salmon Arm indicates that this phenotype may also occur more rarely in the interior of BC in *Hyalophora "kasloensis"* - a population of hybrid origin and uncertain taxonomic standing (Tuskes *et al.* 1996; Collins 1997) which formerly was considered to be a subspecies of *H. euryalus* (Morewood 1991a) - and suggests that there might be gene flow between populations of *Hyalophora* in the interior and on the coast.

Among the colour patterns on the wings of various Lepidoptera, including many Saturniidae, are well-developed "eyespots" that are thought to provide some protection from vertebrate predators. The discal spots of *Hyalophora* can hardly be considered to resemble typical eyes, however. The discal spots of *H. euryalus* are white, more-or-less crescent-shaped, and much larger and more elongate on the hindwings than on the forewings (Fig. 1). In the normal posture of living moths (as opposed to spread museum specimens), the discal spots on the forewings and hindwings, respectively, of *H. euryalus* might be imagined to resemble the narrowed eyes and bared canine teeth of a small feline predator. Such a resemblance might provide some protection against predation by birds and perhaps this resemblance is disrupted by the presence of the white body markings typical of adults of *Hyalophora*.

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