

“Cut-bridles”: a sex-limited mutant phenotype of male orbital bristles of *Ceratitis capitata*

C Lemaître, JP Carante

*Université C Bernard, Lyon 1 et Institut Universitaire de Technologie A,
URA 243 Biométrie, Génétique et Biologie des Populations,
43 Bd du 11 Novembre 1918, 69622 Villeurbanne, France*

(Received 15 July 1991; accepted 15 May 1992)

Summary – The “cut-bridles” phenotype, a new mutant of *Ceratitis capitata*, is characterised by the disappearance of the lancet-shape bristles, a male sex-limited trait. This mutation seems to be under the control of 2 major recessive genes, probably with several additional minor ones, which account for incomplete and variable penetrance. Two models are compatible with our data: 2 autosomal syntenic, but distant genes (no recombination in males) or 1 sex-linked and another autosomal gene.

Mediterranean fruit fly / *Ceratitis capitata* / “cut-bridles” mutant

Résumé – Étude génétique d’une mutation morphologique de *Ceratitis capitata*: «cut-bridles». Le phénotype «cut-bridles» correspond à la disparition des soies orbitales en forme de lance, attributs caractéristiques du sexe mâle. Ce phénotype se révèle être sous le contrôle de 2 gènes majeurs récessifs et sans doute de plusieurs autres gènes mineurs, permettant d’expliquer la pénétrance variable et incomplète observée pour ce caractère. Deux modèles sont compatibles avec nos résultats: 2 gènes autosomiques, synténiques mais éloignés l’un de l’autre (pas de recombinaison chez le mâle) ou un gène lié au sexe et un autre autosomique.

mouche méditerranéenne des fruits / *Ceratitis capitata* / mutant «cut-bridles»

INTRODUCTION

Over the past decade, genetic knowledge of the medfly increased rapidly as the need for pest control programs was recognized (see Saul, 1986, for review). There are, however, still only few genetically marked strains available for ecological studies, and the list established by Rössler (1989) constitutes a poor set compared with other pests. It is thus necessary to consider any kind of morphological mutants, even if their mode of inheritance is still unclear.

Four orbital bristle mutants are mentioned in the literature, but only 2 of them have been analyzed. The first mutation, found by Cavicchi (1973), involved chaetae shape and stem colour and was recessive, monogenic and autosomal. The second mutant, "double-chaetae", isolated by Rössler and Koltin (1976) concerned the number of bristles; its underlying genetic system was not clear and did not conform well to the segregation ratios expected from a single gene model. From further studies, this deviation was attributed to incomplete penetrance and variable expressivity of the character (Rössler, 1982a; Saul and Rössler, 1984). Here we present an additional morphological mutation we named "cut-bristles". This variant concerns the characteristic lancet shape bristles of *Ceratitis capitata* males, an ornament described as "subarticulate horns, planted between the eyes..." by Mac Leay (1829).

MATERIALS AND METHODS

We used 2 strains: a wild strain, donated by a French laboratory in 1968 (Avignon) and a mutant strain, isolated from another strain (inbred because of a selection carried out on a nutritional criterion 1 year ago) itself originated from a strain (Antibes) kept in the laboratory since 1978. Both had been caught in Tunisia, but at different geographical sites and times. These strains were reared under laboratory conditions for several years (see Carante and Lemaître, 1990 for rearing conditions).

Virgin females were isolated in separate containers within 24 h of emergence, and were mated within the following 5 d. For transfer and scoring adults were immobilized by chilling at 10°C.

In each cross, the flies were mated (at least 5 pairs of flies) or in single pairs (single pairs were always less fecund than mass-mated flies and were often sterile). Two repetitions were carried out, and their results, found not to be statistically different by χ^2 test, were pooled.

RESULTS AND DISCUSSION

Description of the phenotype

The "cut-bristles" trait corresponds to the absence of anterior frontal orbital bristles, exactly as if they were cut at their base shaft. Some males bear only one orbital bristle, and they are referred to as "uni-bristle" type (see fig 1). We used this name to distinguish our mutation from previous "chaetaeless" traits (Anonymous,

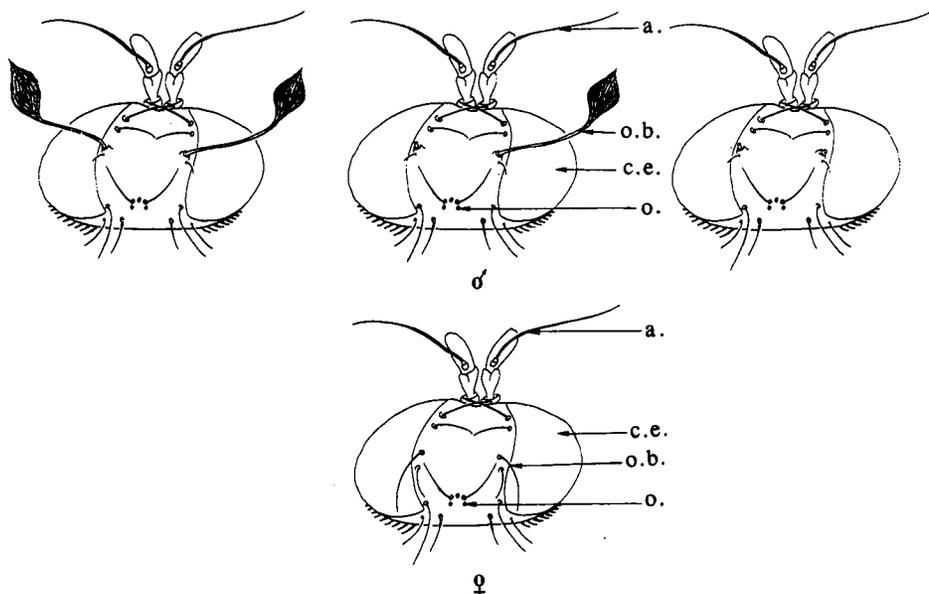


Fig 1. Diagrams of the heads of the wild and "cut-bristles" phenotypes. Upper diagram corresponds to males. From left to right: wild type, uni-bristle type, "cut-bristles" type. Lower diagram corresponds to females: all of them have 2 classic shape orbital bristles, instead of the enlarged type carried by wild type males. a = antenna; ce = compound eye; o = ocellus; ob = orbital bristle.

1980 and Lifschitz, 1985) which are simply mentioned as autosomal incomplete dominant traits, without any analysis.

The "cut-bristles" trait, or even a simple shape change of the analogous bristles, has never been observed in females; the genetic analysis was thus based only on the male progeny phenotypes.

Dissection of pharate adult puparia 1 day before emergence showed that the orbital bristles of "cut-bristles" males were normally formed but unpigmented, contrary to those of wild males. When a "cut-bristles" male was removed from its nymphal wrapping, the bristle shaft broke most of the time; this never happened with wild males. The mutant phenotype, therefore, came from the break of the orbital bristle shaft at emergence time because of its brittleness.

Selection and establishment of a "cut-bristles" strain

The first "cut-bristles" male was found in 1980, in an inbred line. This male was mated to its sisters, "cut-bristles" males were sib-crossed each generation, and a "cut-bristles" strain was established from that time. The fraction of "cut-bristles" males reached its maximum (*ie* 92%) within 6 generations, but the selection pressure was maintained until the 17th generation before being stopped. This relaxation of selection did not cause a decrease of mutant-type frequency in the 4 subsequent

generations, so we considered our selected strain as a true-breeding one. Despite the fact that mutant females were indistinguishable from wild-type, we assumed that females of the selected strain were of mutant genotype, and they will be referred to as "mutant females" in the text.

The persistence of a few wild-type (about 2%) and uni-bristle type (about 6%) males throughout the generations of selection is troublesome. It demonstrates some variability in the expression and penetrance of the phenotype.

No "uni-bristle" male has ever been found in the wild strain.

Inheritance of the "cut-bristles" trait

Male and female mutants were crossed to the wild-type laboratory strain (Avignon). These crosses yielded wild-type progeny, with a very small proportion of males with 1 or 0 bristle (table I). The mutation is thus recessive. The appearance of a few mutants may result either from the presence of individuals heterozygous for the mutation in the wild strain, from non-virgin mutant females, or the occurrence of phenocopies resulting from particular environmental conditions. To check some of these points the uni-bristle F_1 males were pair-mated to virgin "mutant females". These crosses produced 85 wild-type, 38 uni-bristle and 144 cut-bristles males. This phenotypic distribution was similar to that produced by the F_1 wild-type males crossed with "mutant females" (F_{2am} and F_{2bm} in table I; $\chi^2 = 6.13$, for 4 *df*, $P > 0.05$). From these results we infer that F_1 uni-bristle males had the same

Table I. Progeny numbers from various crosses involving wild-type and "cut-bristles flies."

<i>Mating type</i>	<i>Progeny type</i>	<i>No of male progeny</i>		<i>Ratio of "cut-bristles" males</i>	χ^2
		<i>Cut bristles</i>	<i>Uni-bristle + wild-type</i>		
<i>Male × female</i>					
[m] × [+]	F_{1a}	2	8 + 368	0.005	} 0.188 (NS)
[+] × [m]	F_{1b}	1	20 + 298	0.003	
$F_{1a} \times F_{1a}$	F_{2aa}	187	119 + 1 397	0.110	} 2.028 (NS)
$F_{1b} \times F_{1b}$	F_{2bb}	57	36 + 331	0.134	
$F_{1a} \times [m]$	F_{2am}	124	34 + 101	0.479	} 0.195 (NS)
$F_{1b} \times [m]$	F_{2bm}	14	8 + 10	0.438	
[m] × F_{1a}	F_{2ma}	180	91 + 444	0.252	} 0.729 (NS)
[m] × F_{1b}	F_{2mb}	113	46 + 252	0.275	

[m] and [+] symbolize "cut-bristles" and wild types, respectively. F_{1a} , F_{1b} , F_{2aa} , F_{2bb} , F_{2am} , F_{2bm} , F_{2ma} and F_{2mb} correspond to the different progenies. NS = not significant at the 0.05 level.

genotype as F_1 wild type males. Consequently we postulate that they were heterozygous for the studied genetic system; the frequencies of these 2 phenotypes were therefore pooled in statistical analyses. The fact that the same genotype can lead to different phenotypes means that the wild type allele involved was incompletely dominant.

The phenotypic distribution of the progenies of F_1 sib-crosses (F_{2aa} and F_{2bb} and F_1 reciprocal test-crosses (F_{2am} and F_{2bm} and F_{2ma} and F_{2mb}) are not consistent with a single locus model. Moreover, the test-crosses yielded segregation ratios which differed according to the sex of the heterozygous parent. We consider that the genetic system was thus either sex-linked, or at least partly sex influenced. Reciprocal homologous crosses yielded statistically similar progeny distributions (see χ^2 in table I), and the ratio values of "cut-bristles" males to males with 1 and 2 bristles suggest a 2-loci model for the genetic control of the mutation.

According to Carante's proposals (1983) these 2 loci can be symbolized by *obn2* and *obn3* (from orbital number) and the respective mutant and wild type alleles by *obn2(ct)*, *obn2(+)* and *obn3(br)*, *obn3(+)*; for brevity, we will use only *ct*, *br* or *+* (the parenthetical part of the locus symbols), so the following allelic associations (+,+), (ct,+) and (+,br) correspond to the wild phenotype and (ct,br) to the mutant one.

Two models are compatible with the results:

Model 1: 2 autosomal loci

According to the F_{1a} and F_{1b} progenies, the 2 loci are closely linked, but according to the F_{2ma} and F_{2mb} progenies the 2 loci are independent. To resolve this discrepancy we postulate that: 1) the 2 loci belong to the same syntenic group; 2) they are far enough from each other to yield a recombination level in females so high as to seem independent; 3) the recombination level is very low in males as in some other Dipteran species.

According to this hypothesis, all the F_1 genotypes are similar, whatever the direction of the cross. The mean frequency value of F_{2ma} and F_{2mb} "cut-bristles" males is 0.26. These males occur from 1 of the 2 non-recombinant female gamete-types produced by heterozygous F_1 females. We may thus assume a non-recombination level of 0.52 (and therefore a recombination level of 0.48) in females. This high value indicates that the 2 loci are very distant. Accurate location would need further studies using intermediate markers. The mean frequency of F_{2am} and F_{2bm} "cut-bristles" males is 0.46. These males occur from non-recombinant gametes provided by heterozygous F_1 males. We estimate the recombination level to be 0.04 in males. Such values conform with previous estimates of Robinson and Van Heemert (1982), Rössler (1982a,b; 1985) and Rössler and Rosenthal (1988). The frequency of F_{2aa} and F_{2bb} "cut-bristles" males, resulting from 2 non-recombinant F_1 parental gametes would then be equal to 0.125. This expected value is not statistically different from the observed results (0.110 for F_{2aa} : $\chi^2 = 3.594$, 1 *df*, $P > 0.05$ and 0.134 for F_{2bb} : $\chi^2 = 0.345$, 1 *df*, $P > 0.05$).

Model 2: one sex-linked and one autosomal loci

In medfly the female sex is homogametic and the male is heterogametic (Radu *et al.*, 1975; Saul, 1985; Lifschitz and Cladera, 1989). All the F_1 males were thus heterozygous for the autosomal locus, but they differed in the allelic form borne by the X-chromosome: F_{1a} males presented the wild form and the F_{1b} males the mutant one. The similar phenotypic distributions of these 2 F_1 males (see table I: $\chi^2 = 0.188$, 1 *df*, $P > 0.05$), suggest that the 2 alleles involved acted in a similar way and had no additive action upon the trait.

At the F_2 generation, the genotypes of the offspring depend only on the mating type, and the expected value of the "cut-bristles" male relative frequency is 1/2 for F_{2am} and F_{2bm} , 1/4 for F_{2ma} and F_{2mb} , 1/8 for F_{2aa} and F_{2bb} . The observed results are consistent with these expected values ($\chi^2 = 0.467$, 0.500, 0.012, 1.026, 3.594 and 0.345 for F_{2am} , F_{2bm} , F_{2ma} , F_{2mb} , F_{2aa} ; and F_{2bb} , respectively, with 1 *df* and $P > 0.05$).

Like Rössler and Koltin (1976) with their double chaetae mutation, we were confronted in our analyses with the imperfect correspondence between phenotypes and genotypes. F_1 "uni-bristle" males incontestably behave like F_1 wild type males when they are crossed with "cut-bristle" females; however, when persistent "uni-bristle" males of the selected strain are equally crossed with their sisters, they yield a progeny similar to that of "cut-bristles" males. This ambiguity, which would require particular study, could be imputed to modifying minor genes, whose association is removed throughout the selection, interfering with major gene expressivity.

Beside the 2 major genes involved in the genetic determinism of the "cut-bristles" phenotype, we must thus consider minor ones, scattered throughout the chromosomes. They may account for incomplete penetrance and variable expressivity of this trait, as well as incomplete dominance of wild-type of F_1 males.

To set up any population biology or ecology study, markers are essential. At present a maximum of 38 adult morphological mutants of *Ceratitis capitata* are known, a number which is very likely much smaller because of the high degree of redundancy among them (Rössler, 1989); furthermore, 27 of them are color variants whose stability throughout time is unknown. So for such studies the potential "good mutants" are probably very scarce and we consider that the "cut-bristle" trait, with its easy identification and persistence through ageing and death, could be an available marker.

ACKNOWLEDGMENTS

The authors thank C Biémont for his judicious advice in the editing of this text and A Heizmann for her valuable technical assistance with breeding.

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