"Cut-bristles": a sex-limited mutant phenotype of male orbital bristles of Ceratitis capitata

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Summary – The "cut-bristles" 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.

Med. fruit fly / Ceratitis capitata / "cut-bristles" mutant

Résumé – Étude génétique d'une mutation morphologique de Ceratitis capitata: "cut-bristles". Le phénotype "cut-bristles" 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, synteniques 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-bristles"
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 $\chi^2$ 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,
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 F₁ 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 F₁ wild-type males crossed with “mutant females” (*F₂am* and *F₂bm* in table I; $\chi^2 = 6.13$, for 4 df, $P > 0.05$). From these results we infer that F₁ uni-bristle males had the same

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

| Mating type   | Progeny type | No of male progeny | Ratio of “cut-bristles” males | $\chi^2$ |
|---------------|--------------|---------------------|-----------------------------|---------|
| Male × female |              |                      |                             |         |
| [m] × [+]     | F₁a          | 2                   | 8 + 368                     | 0.005   |
| [+ ] × [m]    | F₁b          | 1                   | 20 + 298                    | 0.003   |
| F₁a × F₁a     | F₂aa         | 187                 | 119 + 1397                 | 0.110   |
| F₁b × F₁b     | F₂bb         | 57                  | 36 + 331                   | 0.134   |
| F₁a × [m]     | F₂am         | 124                 | 34 + 101                   | 0.479   |
| F₁b × [m]     | F₂bm         | 14                  | 8 + 10                     | 0.438   |
| [m] × F₁a     | F₂ma         | 180                 | 91 + 444                   | 0.252   |
| [m] × F₁b     | F₂mb         | 113                 | 46 + 252                   | 0.275   |

[m] and [+ ] symbolize “cut-bristles” and wild types, respectively. F₁a, F₁b, F₂aa, F₂bb, F₂am, F₂bm, F₂ma and F₂mb correspond to the different progenies. NS = not significant at the 0.05 level.
genotype as F1 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 F1 sib-crosses (F2aa and F2bb and F1 reciprocal test-crosses (F2am and F2bm and F2ma and F2mb) 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 \( \chi^2 \) 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 F1a and F1b progenies, the 2 loci are closely linked, but according to the F2ma and F2mb 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 F1 genotypes are similar, whatever the direction of the cross. The mean frequency value of F2ma and F2mb “cut-bristles” males is 0.26. These males occur from 1 of the 2 non-recombinant female gamete-types produced by heterozygous F1 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 F2am and F2bm “cut-bristles” males is 0.46. These males occur from non-recombinant gametes provided by heterozygous F1 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 F2aa and F2bb “cut-bristles” males, resulting from 2 non-recombinant F1 parental gametes would then be equal to 0.125. This expected value is not statistically different from the observed results (0.110 for F2aa : \( \chi^2 = 3.594, 1 \text{ df}, P > 0.05 \) and 0.134 for F2bb : \( \chi^2 = 0.345, 1 \text{ df}, P > 0.05 \)).
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 F₁ males were thus heterozygous for the autosomal locus, but they differed in the allelic form borne by the X-chromosome: F₁ₐ males presented the wild form and the F₁ₐb males the mutant one. The similar phenotypic distributions of these 2 F₁ 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 F₂ 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 F₂ᵧm and F₂ᵧb, 1/4 for F₂ma and F₂mb, 1/8 for F₂aa and F₂bb. 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 F₂ᵧm, F₂ᵧb, F₂ma, F₂mb, F₂aa; and F₂bb, 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. F₁ “uni-bristle” males incontestably behave like F₁ 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 F₁ 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.

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